British Pharmacopoeia 2022

Volume II

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The monographs of the Tenth Edition of the European Pharmacopoeia (2019), as amended by Supplements 10.1 to 10.5, published by the Council of Europe are reproduced either in this edition of the British Pharmacopoeia or in the associated edition of the British Pharmacopoeia (Veterinary).

See General Notices

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Contents

Notices

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

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

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

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

General Notices

CONTENTS OF THE GENERAL NOTICES

Part I

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

European Pharmacopoeia

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

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

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

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

Part II

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

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

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

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

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

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

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

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

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

Definition of Terms

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

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

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

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

Expression of Standards

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

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

Temperature The Celsius thermometric scale is used in expressing temperatures.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

In the graphic formulae the following abbreviations are used:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Freshly and Recently Prepared

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

Methods of Sterilisation

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

Water

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Biological Assays and Tests

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

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

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

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

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

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

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

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

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

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

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

Reference Substances and Reference Preparations

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

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

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

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

Storage

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

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

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

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

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

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

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

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

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

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

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

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

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

Crude Drugs; Traditional Herbal and Complementary Medicines

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

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

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

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

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

(a) macroscopical and microscopical descriptions and chemical/chromatographic tests for identification

(b) tests for absence of any related species

(c) microbial test to assure microbial quality

(d) tests for inorganic impurities and non-specific purity tests, including extractive tests, Sulfated ash and Heavy metals, where appropriate

(e) test for Loss on drying or Water

(f) wherever possible, a method for assaying the active constituent(s) or suitable marker constituent(s).

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

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

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

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

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

Homoeopathic Medicines

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

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

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

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

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

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

Unlicensed Medicines

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

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

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

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

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

Part III

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

GENERAL NOTICES OF THE EUROPEAN PHARMACOPOEIA

1.1. GENERAL STATEMENTS

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

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

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

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

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

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

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

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

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

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

Demonstration of compliance with the Pharmacopoeia

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

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

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

Grade of materials

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

General monographs

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

1.2. OTHER PROVISIONS APPLYING TO GENERAL CHAPTERS AND MONOGRAPHS

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

- per cent *m/m* (percentage, mass in mass) expresses the number of grams of substance in 100 g of final product;
- per cent *V/V* (percentage, volume in volume) expresses the number of millilitres of substance in 100 mL of final product.

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

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

- in a deep-freeze: below −15 °C;
- in a refrigerator: 2 °C to 8 °C;
- cold or cool: 8 °C to 15 °C;
- room temperature: 15 °C to 25 °C.

1.3. GENERAL CHAPTERS

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

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

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

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

1.4. MONOGRAPHS

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

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

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

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

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

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

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

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

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

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

Potential Adulteration

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

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

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

Characters

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

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

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

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

Identification

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

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

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

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

Tests And Assays

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

1.5. ABBREVIATIONS AND SYMBOLS

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

Abbreviations used in the monographs on immunoglobulins, immunosera and vaccines

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

Collections of micro-organisms

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

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

International System Of Units (SI)

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

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

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

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

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

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

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

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

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

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

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

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

Notes

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

$$t = T - T_0$$

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

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

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

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

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

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

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

Monographs

Medicinal and Pharmaceutical Substances (J to Z)

Substances for Pharmaceutical Use

(Ph. Eur. monograph 2034)

Ph Eur

DEFINITION

Substances for pharmaceutical use are any organic or inorganic substances that are used as active substances or excipients for the production of medicinal products for human or veterinary use. They may be obtained from natural sources or produced by extraction from raw materials, fermentation or synthesis.

This general monograph does not apply to herbal drugs, herbal drugs for homoeopathic preparations, herbal drug preparations, herbal drug extracts, or mother tinctures for homoeopathic preparations, which are the subject of separate general monographs (*Herbal drugs (1433)*, *Herbal drugs for homoeopathic preparations (2045)*, *Herbal drug preparations (1434)*, *Herbal drug extracts (0765)*, *Mother tinctures for homoeopathic preparations (2029)*). It does not apply to raw materials for homoeopathic preparations, except where there is an individual monograph for the substance in the non-homoeopathic part of the Pharmacopoeia.

This monograph does not apply to chemical precursors for radiopharmaceutical preparations which are the subject of a separate monograph (*Chemical precursors for radiopharmaceutical preparations (2902)*).

Where a substance for pharmaceutical use not described in an individual monograph of the Pharmacopoeia is used in a medicinal product prepared for the special needs of individual patients, the need for compliance with the present general monograph is decided in the light of a risk assessment that takes account of the available quality of the substance and its intended use.

Where medicinal products are manufactured using substances for pharmaceutical use of human or animal origin, the requirements of chapter *5.1.7. Viral safety* apply.

Substances for pharmaceutical use may be used as such or as starting materials for subsequent formulation to prepare medicinal products. Depending on the formulation, certain substances may be used either as active substances or as excipients. Solid substances may be compacted, coated, granulated, powdered to a certain fineness, or processed in other ways. A monograph is applicable to a substance processed with an excipient only where such processing is mentioned in the definition section of the monograph.

Substance for pharmaceutical use of special grade Unless otherwise indicated or restricted in the individual monographs, a substance for pharmaceutical use is intended for human and veterinary use, and is of appropriate quality for the manufacture of all dosage forms in which it can be used.

Polymorphism Individual monographs do not usually specify crystalline or amorphous forms, unless bioavailability is affected. All forms of a substance for pharmaceutical use comply with the requirements of the monograph, unless otherwise indicated.

PRODUCTION

Substances for pharmaceutical use are manufactured by procedures that are designed to ensure a consistent quality and comply with the requirements of the individual monograph or approved specification.

The manufacture of active substances must take place under conditions of good manufacturing practice.

The provisions of general chapter *5.10* apply to the control of impurities in substances for pharmaceutical use.

Whether or not it is specifically stated in the individual monograph that the substance for pharmaceutical use:

- is a recombinant protein or another substance obtained as a direct gene product based on genetic modification, where applicable, the substance also complies with the requirements of the general monograph *Products of recombinant DNA technology (0784)*;
- is obtained from animals susceptible to transmissible spongiform encephalopathies other than by experimental challenge, where applicable, the substance also complies with the requirements of the general monograph *Products with risk of transmitting agents of animal spongiform encephalopathies (1483)*;
- is a substance derived from a fermentation process, whether or not the micro-organisms involved are modified by traditional procedures or recombinant DNA (rDNA) technology, where applicable, the substance also complies with the requirements of the general monograph *Products of fermentation (1468)*.

If solvents are used during production, they are of suitable quality. In addition, their toxicity and their residual level are taken into consideration (*5.4*). If water is used during production, it is of suitable quality.

The identity of elemental impurities derived from intentionally added catalysts and reagents is known, and strategies for controlling them should be established using the principles of risk management.

If substances are produced or processed to yield a certain form or grade, that specific form or grade of the substance complies with the requirements of the monograph. Certain functionality-related tests may be described to control properties that may influence the suitability of the substance and subsequently the properties of dosage forms prepared from it.

Powdered substances May be processed to obtain a certain degree of fineness (*2.9.35*).

Compacted substances Are processed to increase the particle size or to obtain particles of a specific form and/or to obtain a substance with a higher bulk density.

Coated active substances Consist of particles of the active substance coated with one or more suitable excipients.

Granulated active substances Are particles of a specified size and/or form produced from the active substance by granulation directly or with one or more suitable excipients.

If substances are processed with excipients, these excipients comply with the requirements of the relevant monograph or, where no such monograph exists, the approved specification.

Where active substances have been processed with excipients to produce, for example, coated or granulated substances, the processing is carried out under conditions of good manufacturing practice and the processed substances are regarded as intermediates in the manufacture of a medicinal product.

CHARACTERS

The statements under the heading Characters (e.g. statements about the solubility or a decomposition point) are not to be interpreted in a strict sense and are not requirements. They are given for information.

Where a substance may show polymorphism, this may be stated under Characters in order to draw this to the attention

of the user who may have to take this characteristic into consideration during formulation of a preparation.

IDENTIFICATION

Where under Identification an individual monograph contains subdivisions entitled 'First identification' and 'Second identification', the test or tests that constitute the 'First identification' may be used in all circumstances. The test or tests that constitute the 'Second identification' may be used in pharmacies only, provided it can be demonstrated that the substance or preparation is fully traceable to a batch certified to comply with all the other requirements of the monograph. The implementation of the tests under the second identification is subject to national regulation.

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

TESTS

Polymorphism (*5.9*)

If the nature of a crystalline or amorphous form imposes restrictions on its use in preparations, the nature of the specific crystalline or amorphous form is identified, its morphology is adequately controlled and its identity is stated on the label.

Related substances

Unless otherwise prescribed or justified and authorised, organic impurities in active substances are to be reported, identified wherever possible, and qualified as indicated in Table 2034.-1 or in Table 2034.-2 for peptides obtained by chemical synthesis.

Table 2034.-1. – *Reporting, identification and qualification of organic impurities in active substances*

Use	Maximum daily dose	Reporting threshold	Identification threshold	Quallfication threshold
Human use or human and veterinary use	≤ 2 g/day	> 0.05 per cent	> 0.10 per cent or a daily intake of > 1.0 mg (whichever is the lower)	> 0.15 per cent or a daily intake of > 1.0 mg (whichever is the lower)
Human use or human and veterinary use	> 2 g/day	> 0.03 per cent	> 0.05 per cent	> 0.05 per cent
Veterinary use only	Not applicable	> 0.10 per cent	> 0.20 per cent	> 0.50 per cent

Table 2034.-2. – *Reporting, identification and qualification of organic impurities in peptides obtained by chemical synthesis*

Reporting threshold	Identification threshold	Qualification threshold
> 0.1 per cent	> 0.5 per cent	> 1.0 per cent

Specific thresholds may be applied for impurities known to be unusually potent or to produce toxic or unexpected pharmacological effects.

For DNA reactive impurities, the requirements of ICH Guideline M7 *Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk* must be complied with for active substances to be used in medicinal products for human use, in cases defined in the scope of the guideline.

If the individual monograph does not provide suitable control for a new impurity, a suitable test for control must be developed and included in the specification for the substance.

The requirements above do not apply to biological and biotechnological products, oligonucleotides, products of fermentation and semi-synthetic products derived therefrom, to crude products of animal or plant origin or herbal products.

Elemental impurities

Permitted daily exposures for elemental impurities (e.g. as included in the ICH Q3D guideline, the principles of which are reproduced in general chapter *5.20. Elemental impurities*) apply to the medicinal product. Individual monographs on substances for pharmaceutical use therefore do not contain specifications for elemental impurities unless otherwise prescribed.

Residual solvents

Are limited according to the principles defined in chapter *5.4*, using general method *2.4.24* or another suitable method. Where a quantitative determination of a residual solvent is carried out and a test for loss on drying is not carried out, the content of residual solvent is taken into account for calculation of the assay content of the substance, the specific optical rotation and the specific absorbance.

Microbiological quality

Individual monographs give acceptance criteria for microbiological quality wherever such control is necessary. Table 5.1.4.-2. – *Acceptance criteria for microbiological quality of non-sterile substances for pharmaceutical use* in chapter *5.1.4. Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use* gives recommendations on microbiological quality that are of general relevance for substances subject to microbial contamination. Depending on the nature of the substance and its intended use, different acceptance criteria may be justified.

Sterility (*2.6.1*)

If intended for use in the manufacture of sterile dosage forms without a further appropriate sterilisation procedure, or if offered as sterile grade, the substance for pharmaceutical use complies with the test for sterility.

Bacterial endotoxins (*2.6.14*)

The substance for pharmaceutical use complies with the test for bacterial endotoxins if it is labelled as a bacterial endotoxin-free grade or if it is intended for use in the manufacture of parenteral preparations or preparations for irrigation without a further appropriate procedure for the removal of bacterial endotoxins. The limit, when not indicated in the individual monograph, is determined in accordance with the recommendations of general chapter *5.1.10. Guidelines for using the test for bacterial endotoxins.*

Pyrogens (*2.6.8*)

If the test for pyrogens is justified rather than the test for bacterial endotoxins and if a pyrogen-free grade is offered, the substance for pharmaceutical use complies with the test for pyrogens. The limit and test method are stated in the individual monograph or approved by the competent authority. Based on appropriate test validation for bacterial

endotoxins and pyrogens, the test for bacterial endotoxins may replace the test for pyrogens.

Additional properties
Control of additional properties (e.g. physical characteristics, functionality-related characteristics) may be necessary for individual manufacturing processes or formulations. Grades (such as sterile, endotoxin-free, pyrogen-free) may be produced with a view to manufacture of preparations for parenteral administration or other dosage forms and appropriate requirements may be specified in an individual monograph.

ASSAY
Unless justified and authorised, contents of substances for pharmaceutical use are determined. Suitable methods are used.

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

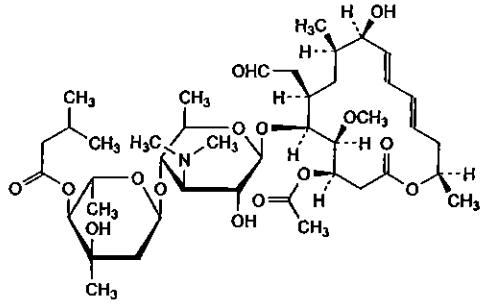
Where appropriate, the label states that the substance is:
— intended for a specific use;
— of a distinct crystalline form;
— of a specific degree of fineness;
— compacted;
— coated;
— granulated;
— sterile;
— free from bacterial endotoxins;
— free from pyrogens;
— containing gliding agents.

Where applicable, the label states:
— the degree of hydration;
— the name and concentration of any excipient.

Ph Eur

Josamycin

(Ph. Eur. monograph 1983)



$C_{42}H_{69}NO_{15}$ 828 *16846-24-5*

Action and use
Antibacterial.

Ph Eur

DEFINITION
Josamycin is a macrolide antibiotic obtained by fermentation using, for example, certain strains of *Streptomyces narbonensis* var. *josamyceticus* var. *nova*. The main component is (4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-4-(acetyloxy)-6-[[3,6-dideoxy-4-*O*-[2,6-dideoxy-3-*C*-methyl-4-*O*-(3-methylbutanoyl)-α-L-*ribo*-hexopyranosyl]-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-10-hydroxy-5-methoxy-9,16-dimethyl-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-2-one.

Content
Minimum 900 Ph. Eur. U./mg (dried substance).

CHARACTERS

Appearance
White or slightly yellowish powder, slightly hygroscopic.

Solubility
Very slightly soluble in water, freely soluble in methanol and in methylene chloride, soluble in acetone.

IDENTIFICATION
First identification: A, C.
Second identification: A, B.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 0.10 g in *methanol R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of this solution to 50.0 mL with *methanol R*.

Spectral range 220-350 nm.

Absorption maximum At 232 nm.

Specific absorbance at the absorption maximum 330 to 370.

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in 2.5 mL of *methanol R*.

Reference solution (a) Dissolve 10 mg of *josamycin CRS* in 2.5 mL of *methanol R*.

Reference solution (b) Dissolve 10 mg of *josamycin propionate CRS* in 2.5 mL of *methanol R*.

Plate *TLC silica gel GF_{254} plate R.*

Mobile phase *methanol R, acetone R, ethyl acetate R, toluene R, hexane R* (8:10:20:25:30 *V/V/V/V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying At 100 °C for 10 min.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a) and different in position from the principal spot in the chromatogram obtained with reference solution (b).

C. Examine the chromatograms obtained in the test for related substances.

Results The principal peak in the chromatogram obtained with the test solution is similar in position and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_4 (*2.2.2, Method II*).

Dissolve 2.0 g in *methanol R* and dilute to 20 mL with the same solvent.

Specific optical rotation (*2.2.7*)

−65 to −75 (dried substance).

Dissolve 1.000 g in *methanol R* and dilute to 100.0 mL with the same solvent. Allow to stand for 30 min before measuring the angle of rotation.

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R, water R* (30:70 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 20.0 mL with the solvent mixture.

Reference solution (a) Dissolve 25.0 mg of *josamycin CRS* in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 20.0 mL with the solvent mixture.

Reference solution (c) Dilute 1.0 mL of reference solution (b) to 50.0 mL with the solvent mixture.

Reference solution (d) To 10 mL of the test solution add 0.1 mL of *strong hydrogen peroxide solution R* and heat in a water-bath for 10 min. Mix 1.0 mL of this solution and 1.0 mL of the test solution.

Reference solution (e) Dissolve 12.5 mg of *josamycin for peak identification CRS* (containing impurities A, B, C, D and E) in 5 mL of the solvent mixture.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 45 °C.

Mobile phase:
- *mobile phase A*: mix 3 volumes of a 67.9 g/L solution of *tetrabutylammonium hydrogen sulfate R*, 5 volumes of a 27.6 g/L solution of *sodium dihydrogen phosphate monohydrate R* adjusted to pH 3.0 with *dilute phosphoric acid R*, and 21 volumes of *acetonitrile R*, and dilute to 100 volumes with *water R*;
- *mobile phase B*: mix 5 volumes of a 27.6 g/L solution of *sodium dihydrogen phosphate monohydrate R* adjusted to pH 3.0 with *dilute phosphoric acid R*, and 50 volumes of *acetonitrile R*, and dilute to 100 volumes with *water R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 38	100	0
38 - 55	100 → 0	0 → 100

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 232 nm.

Injection 10 µL of the test solution and reference solutions (b), (c), (d) and (e).

Identification of impurities Use the chromatogram supplied with *josamycin for peak identification CRS* and the chromatogram obtained with reference solution (e) to identify the peaks due to impurities A, B, C, D and E.

Relative retention With reference to josamycin (retention time = about 35 min): impurity A = about 0.5; impurity B = about 0.8; impurity C = about 0.9; impurity D = about 1.2; impurity E = about 1.4.

System suitability Reference solution (d):
- *resolution*: minimum 1.7 between the 2 peaks due to josamycin and the peak eluted with a relative retention with reference to josamycin of about 1.1;
- *retention time of josamycin*: between 32 min and 38 min.

If necessary, adjust the concentration of acetonitrile in the mobile phases.

Limits:
- *impurities A, B, C, D, E* (any shoulder observed on the peak due to impurity A and/or the peak due to impurity B is not to be integrated separately): for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (5.0 per cent);
- *any other impurity*: not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent);
- *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (20.0 per cent);
- *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 3 h.

Sulfated ash (*2.4.14*)

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Dissolve 30.0 mg in 5 mL of *methanol R* and dilute to 100.0 mL with *water R*.

Carry out the microbiological assay of antibiotics (*2.7.2*).Use *josamycin CRS* as the chemical reference substance.

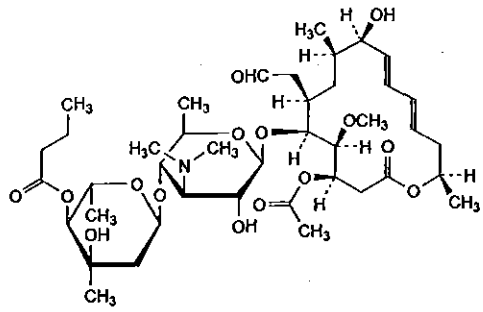
STORAGE

In an airtight container.

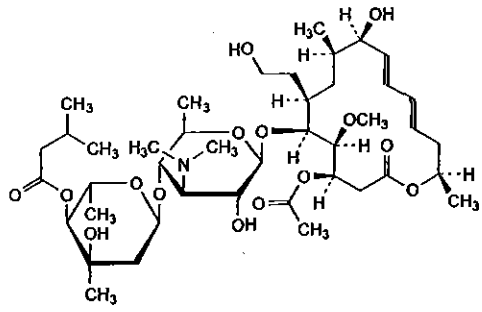
IMPURITIES

Specified impurities *A, B, C, D, E.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *F, G, H, I, J, K.*

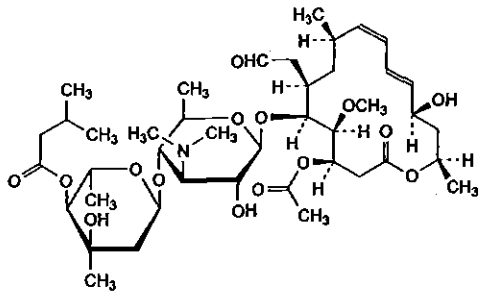


A. (4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-4-(acetyloxy)-6-[[3,6-dideoxy-4-*O*-(2,6-dideoxy-4-*O*-butanoyl-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-10-hydroxy-5-methoxy-9,16-dimethyl-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-2-one,

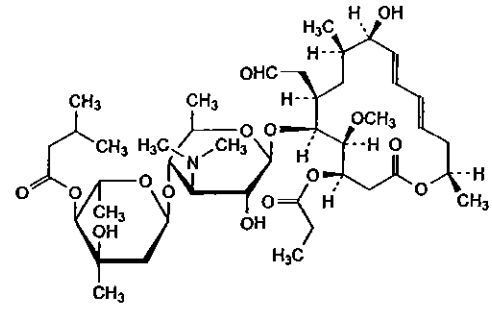


B. (4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-4-(acetyloxy)-6-[[3,6-dideoxy-4-*O*-[2,6-dideoxy-3-*C*-methyl-4-*O*-(3-methylbutanoyl)-α-L-*ribo*-hexopyranosyl]-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-10-hydroxy-7-(2-hydroxyethyl)-5-methoxy-9,16-dimethyloxacyclohexadeca-11,13-dien-2-one,

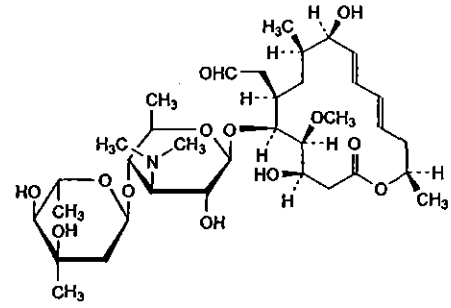
C. unknown structure,



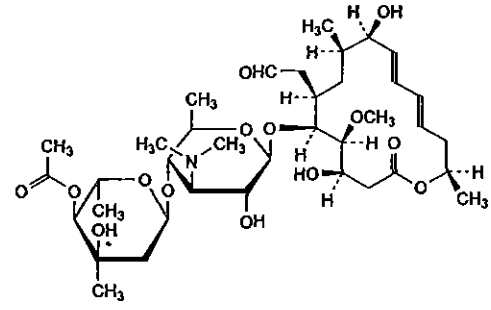
D. (4*R*,5*S*,6*S*,7*R*,9*R*,10*Z*,12*E*,14*R*,16*R*)-4-(acetyloxy)-6-[[3,6-dideoxy-4-*O*-[2,6-dideoxy-3-*C*-methyl-4-*O*-(3-methylbutanoyl)-α-L-*ribo*-hexopyranosyl]-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-14-hydroxy-5-methoxy-9,16-dimethyl-7-(2-oxoethyl)oxacyclohexadeca-10,12-dien-2-one (isojosamycin),



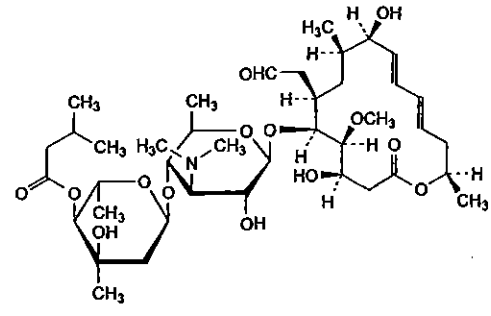
E. (4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-6-[[3,6-dideoxy-4-*O*-[2,6-dideoxy-3-*C*-methyl-4-*O*-(3-methylbutanoyl)-α-L-*ribo*-hexopyranosyl]-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-10-hydroxy-5-methoxy-9,16-dimethyl-7-(2-oxoethyl)-4-(propanoyloxy)oxacyclohexadeca-11,13-dien-2-one,



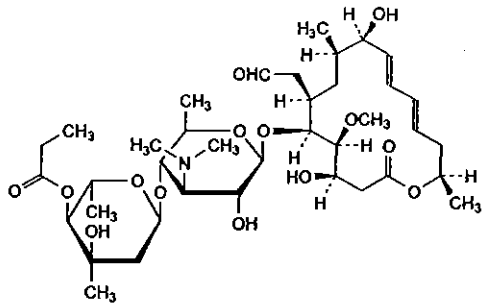
F. (4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-6-[[3,6-dideoxy-4-*O*-(2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-4,10-dihydroxy-5-methoxy-9,16-dimethyl-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-2-one,



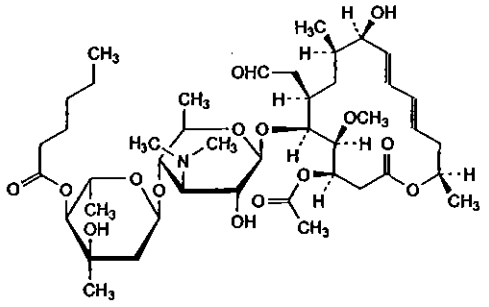
G. (4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-6-[[4-*O*-(4-*O*-acetyl-2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-3,6-dideoxy-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-4,10-dihydroxy-5-methoxy-9,16-dimethyl-7-(2-oxoethyl) oxacyclohexadeca-11,13-dien-2-one,



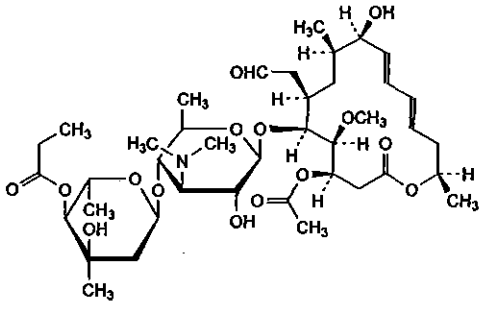
H. (4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-6-[[3,6-dideoxy-4-*O*-[2,6-dideoxy-3-*C*-methyl-4-*O*-(3-methylbutanoyl)-α-L-*ribo*-hexopyranosyl]-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-4,10-dihydroxy-5-methoxy-9,16-dimethyl-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-2-one,



I. (4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-6-[[3,6-dideoxy-4-*O*-(2,6-dideoxy-3-*C*-methyl-4-*O*-propanoyl-α-L-*ribo*-hexopyranosyl)-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-4,10-dihydroxy-5-methoxy-9,16-dimethyl-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-2-one,



J. (4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-4-(acetyloxy)-6-[[3,6-dideoxy-4-*O*-(2,6-dideoxy-4-*O*-hexanoyl-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-10-hydroxy-5-methoxy-9,16-dimethyl-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-2-one,

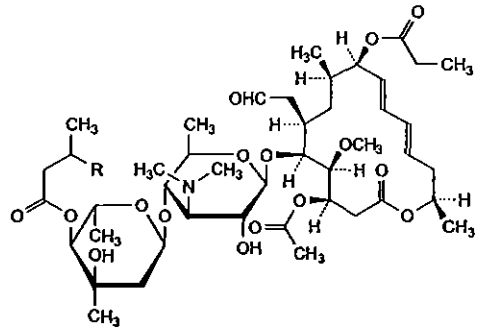


K. (4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-4-(acetyloxy)-6-[[3,6-dideoxy-4-*O*-(2,6-dideoxy-3-*C*-methyl-4-*O*-propanoyl-α-L-*ribo*-hexopyranosyl)-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-10-hydroxy-5-methoxy-9,16-dimethyl-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-2-one.

Ph Eur

Josamycin Propionate

(Ph. Eur. monograph 1982)



Leucomycin propionate	R	Mol. Formula	M_r
A3	CH_3	$C_{45}H_{73}NO_{16}$	884
A4	H	$C_{44}H_{71}NO_{16}$	870

Action and use
Antibacterial.

Ph Eur

DEFINITION

Propionyl ester of a macrolide antibiotic produced by certain strains of *Streptomyces narbonensis* var. *josamyceticus* var. *nova*, or obtained by any other means. The main component is (4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-4-(acetyloxy)-6-[[3,6-dideoxy-4-*O*-[2,6-dideoxy-3-*C*-methyl-4-*O*-(3-methylbutanoyl)-α-L-*ribo*-hexopyranosyl]-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-5-methoxy-9,16-dimethyl-7-(2-oxoethyl)-10-(propanoyloxy)oxacyclohexadeca-11,13-dien-2-one propionate (leucomycin A3 propionate).

Semi-synthetic product derived from a fermentation product.

Content
— minimum 843 Ph. Eur. U./mg (dried substance).

CHARACTERS

Appearance
White or slightly yellowish, crystalline, slightly hygroscopic powder.

Solubility
Practically insoluble in water, freely soluble in methanol and in methylene chloride, soluble in acetone.

IDENTIFICATION

First identification: A, B.

Second identification: B, C.

Prepare solutions in methanol immediately before use.

A. Dissolve 0.10 g in *methanol R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of the solution to 50.0 mL with *methanol R*. Examined between 220 nm and 350 nm (*2.2.25*), the solution shows an absorption maximum at 231 nm. The specific absorbance at the absorption maximum is 310 to 350.

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 1 mL with the same solvent.

Reference solution (a) Dissolve 10 mg of *josamycin propionate CRS* in *methanol R* and dilute to 1 mL with the same solvent.

Reference solution (b) Dissolve 10 mg of *josamycin CRS* in *methanol R* and dilute to 1 mL with the same solvent.

Reference solution (c) Dissolve 10 mg of *spiramycin CRS* in *methylene chloride R* and dilute to 1 mL with the same solvent.

Reference solution (d) Mix 0.5 mL of reference solution (a) with 0.5 mL of reference solution (b).

Plate *TLC silica gel G plate R.*

Mobile phase *methanol R, acetone R, ethyl acetate R, toluene R, hexane R* (8:10:20:25:30 *V/V/V/V/V*).

Application 10 µL.

Development Over 2/3 of the plate.

Drying At 100 °C for 10 min.

Detection Spray with *dilute sulfuric acid R* and heat at 100 °C for 10 min.

System suitability The chromatogram obtained with reference solution (d) shows 2 clearly separated principal spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a) and its position is different from that of the principal spot in the chromatograms obtained with reference solutions (b) and (c).

C. Dissolve about 10 mg in 5 mL of *hydrochloric acid R1* and allow to stand for 10-20 min. A pink colour develops, turning brown.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_4 (*2.2.2, Method II*).

Dissolve 1 g in *methanol R* and dilute to 10 mL with the same solvent.

Specific optical rotation (*2.2.7*)

−65 to −75 (dried substance).

Dissolve 1.000 g in *methanol R* and dilute to 100.0 mL with the same solvent. Allow to stand for 30 min before measuring the angle of rotation.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in *acetonitrile for chromatography R* and dilute to 100.0 mL with the same solvent.

Reference solution (a) Dissolve 50.0 mg of *josamycin propionate CRS* in *acetonitrile for chromatography R* and dilute to 100.0 mL with the same solvent.

Reference solution (b) Dissolve 5 mg of the substance to be examined in 10 mL of *methanol R* and add 40 µL of *dilute phosphoric acid R*. Mix, allow to stand for 5 min and inject.

Reference solution (c) Dilute 2.0 mL of reference solution (a) to 100.0 mL with *acetonitrile for chromatography R.*

Column:
- *size*: *l* = 0.15 m, Ø = 3.9 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 30 °C.

Mobile phase *acetonitrile R*, a 15.4 g/L solution of *ammonium acetate R* previously adjusted to pH 6.0 with *dilute phosphoric acid R* (60:40 *V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 232 nm.

Injection 20 µL of the test solution and reference solutions (b) and (c).

Run time 3 times the retention time of leucomycin A3 propionate.

Relative retention With reference to leucomycin A3 propionate (retention time = about 18 min): impurity E = about 0.2; impurity A = about 0.3; impurity B = about 0.5; leucomycin A4 propionate = about 0.7; impurity C = about 1.4; impurity D = about 2.0.

System suitability Reference solution (b):
- *resolution*: minimum 2.0 between the 2 peaks eluting with a relative retention with reference to leucomycin A3 propionate of about 0.5 and 0.7 respectively.

Limits:
- *impurity D*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (c);
- *impurities A, B, C, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c);
- *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c);
- *total*: not more than 7 times the area of the principal peak in the chromatogram obtained with reference solution (c);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c).

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven *in vacuo* at 60 °C for 3 h.

Sulfated ash (*2.4.14*)

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Dissolve 40.0 mg in 20 mL of *methanol R* and dilute to 100.0 mL with *phosphate buffer solution pH 5.6 R.*

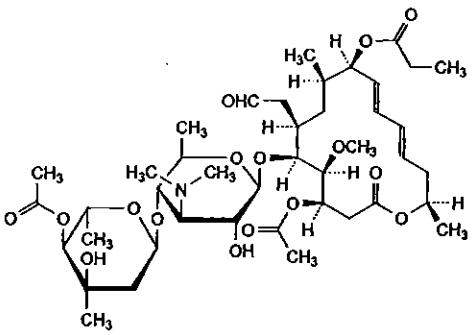
Carry out the microbiological assay of antibiotics (*2.7.2*). Use *josamycin propionate CRS* as the chemical reference substance.

STORAGE

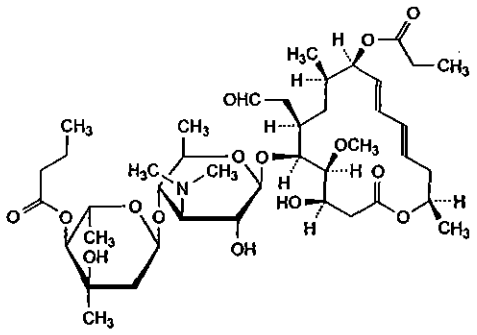
In an airtight container.

IMPURITIES

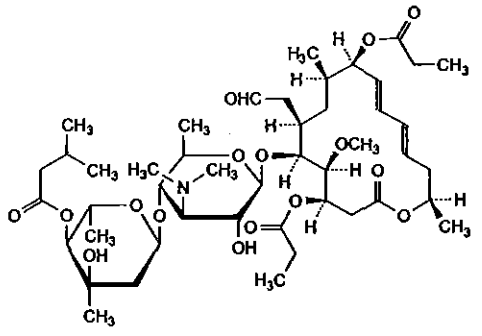
Specified impurities *A, B, C, D, E.*



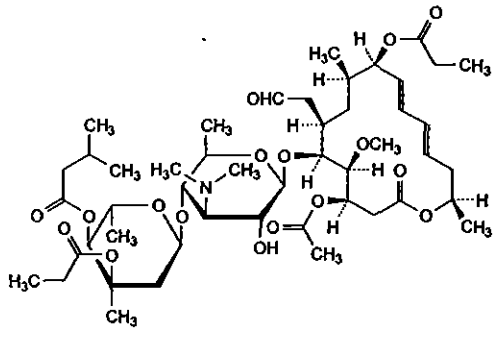
A. leucomycin A8 9-propionate,



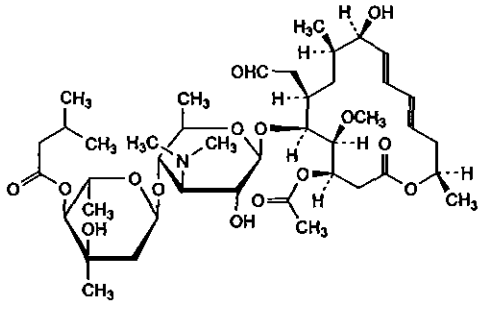
B. leucomycin A5 9-propionate,



C. platenomycin A1 9-propionate,



D. leucomycin A3 3″,9-dipropionate,



E. (4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-4-(acetyloxy)-6-[[3,6-dideoxy-4-*O*-[2,6-dideoxy-3-*C*-methyl-4-*O*-(3-methylbutanoyl)-α-L-*ribo*-hexopyranosyl]-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-10-hydroxy-5-methoxy-9,16-dimethyl-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-2-one (josamycin).

Ph Eur

Kanamycin Acid Sulfate

Kanamycin Acid Sulphate

(Ph. Eur. monograph 0033)

Action and use
Aminoglycoside antibacterial.

Ph Eur

DEFINITION

Kanamycin acid sulfate is a form of kanamycin sulfate prepared by adding sulfuric acid to a solution of kanamycin monosulfate and drying by a suitable method. The potency is not less than 670 IU/mg, calculated with reference to the dried substance.

Fermentation product.

PRODUCTION

It is produced by methods of manufacture designed to eliminate or minimise substances lowering blood pressure.

CHARACTERS

A white or almost white powder, hygroscopic, soluble in about 1 part of water, practically insoluble in acetone and in ethanol (96 per cent).

IDENTIFICATION

A. Examine by thin-layer chromatography (*2.2.27*), using a plate coated with a 0.75 mm layer of the following mixture: mix 0.3 g of *carbomer R* with 240 mL of *water R* and allow to stand, with moderate shaking, for 1 h; adjust to pH 7 by the gradual addition, with continuous shaking, of *dilute sodium hydroxide solution R* and add 30 g of *silica gel H R*.

Heat the plate at 110 °C for 1 h, allow to cool and use immediately.

Test solution Dissolve 10 mg of the substance to be examined in *water R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dissolve 10 mg of *kanamycin monosulfate CRS* in *water R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 10 mg of *kanamycin monosulfate CRS*, 10 mg of *neomycin sulfate CRS* and 10 mg of *streptomycin sulfate for identification CRS* in *water R* and dilute to 10 mL with the same solvent.

Apply separately to the plate 10 µL of each solution. Develop over a path of 12 cm using a 70 g/L solution of *potassium dihydrogen phosphate R*. Dry the plate in a current of warm air and spray with a mixture of equal volumes of a 2 g/L solution of *1,3-dihydroxynaphthalene R* in *ethanol (96 per cent) R* and a 460 g/L solution of *sulfuric acid R*. Heat at 150 °C for 5 min to 10 min. The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows 3 clearly separated spots.

B. Dissolve 0.5 g in 10 mL of *water R*. Add 10 mL of *picric acid solution R*. Initiate crystallisation if necessary by scratching the wall of the tube with a glass rod and allow to stand. Collect the crystals, wash with 20 mL of *water R* and filter. Dry at 100 °C. The crystals melt (*2.2.14*) at about 235 °C, with decomposition.

C. Dissolve about 50 mg in 2 mL of *water R*. Add 1 mL of a 10 g/L solution of *ninhydrin R* and heat for a few minutes on a water-bath. A violet colour develops.

D. It gives the reactions of sulfates (*2.3.1*).

TESTS

Solution S
Dissolve 0.20 g in *carbon dioxide-free water R* and dilute to 20.0 mL with the same solvent.

pH (*2.2.3*)
The pH of solution S is 5.5 to 7.5.

Specific optical rotation (*2.2.7*)
+ 103 to + 115, determined on solution S and calculated with reference to the dried substance.

Kanamycin B
Examine by thin-layer chromatography (*2.2.27*), using a plate prepared as prescribed under identification test A.

Heat the plate at 110 °C for 1 h, allow to cool and use immediately.

Test solution Dissolve 0.11 g of the substance to be examined in *water R* and dilute to 20 mL with the same solvent.

Reference solution Dissolve 4 mg of *kanamycin B sulfate CRS* in *water R* and dilute to 20 mL with the same solvent.

Apply separately to the plate 4 µL of each solution. Develop over a path of 12 cm using a 70 g/L solution of *potassium dihydrogen phosphate R*. Dry the plate in a current of warm air and spray with *ninhydrin and stannous chloride reagent R*. Heat the plate at 110 °C for 15 min. Any spot corresponding to kanamycin B in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (4.0 per cent).

Loss on drying (*2.2.32*)
Not more than 5.0 per cent, determined on 1.00 g by drying at 60 °C at a pressure not exceeding 670 Pa for 3 h.

Sulfated ash (*2.4.14*)
Not more than 0.5 per cent, determined on 1.0 g.

Sulfate
23.0 per cent to 26.0 per cent of sulfate (SO_4), calculated with reference to the dried substance. Dissolve 0.175 g in 100 mL of *water R* and adjust the solution to pH 11 using *concentrated ammonia R*. Add 10.0 mL of *0.1 M barium chloride* and about 0.5 mg of *phthalein purple R*. Titrate with *0.1 M sodium edetate* adding 50 mL of *ethanol (96 per cent) R* when the colour of the solution begins to change and continue the titration until the violet-blue colour disappears.

1 mL of *0.1 M barium chloride* is equivalent to 9.606 mg of sulfate (SO_4).

Pyrogens (*2.6.8*)
If intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of pyrogens, it complies with the test for pyrogens. Inject per kilogram of the rabbit's mass 1 mL of a solution in *water for injections R* containing 10 mg per millilitre of the substance to be examined.

ASSAY
Carry out the microbiological assay of antibiotics (*2.7.2*). Use *kanamycin monosulfate CRS* as the reference substance.

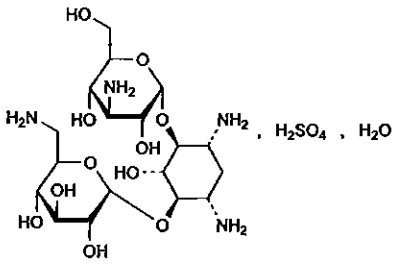
STORAGE
In an airtight container. If the substance is sterile, the container is also sterile and tamper-evident.

Ph Eur

Kanamycin Sulfate

Kanamycin Sulphate

(Kanamycin Monosulfate, Ph. Eur. monograph 0032)



$C_{18}H_{38}N_4O_{15}S,H_2O$ 601 *5965-95-7*

Action and use
Aminoglycoside antibacterial.

Ph Eur

DEFINITION
6-*O*-(3-Amino-3-deoxy-α-D-glucopyranosyl)-4-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-2-deoxy-D-streptamine sulfate monohydrate.

Antimicrobial substance produced by the growth of certain strains of *Streptomyces kanamyceticus*.

Content
Minimum 750 IU/mg (dried substance).

PRODUCTION
It is produced by methods of manufacture designed to eliminate or minimise substances lowering blood pressure.

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water, practically insoluble in acetone and in ethanol (96 per cent).

IDENTIFICATION
A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *water R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dissolve 10 mg of *kanamycin monosulfate CRS* in *water R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 10 mg of *kanamycin monosulfate CRS*, 10 mg of *neomycin sulfate CRS* and 10 mg of *streptomycin sulfate for identification CRS* in *water R* and dilute to 10 mL with the same solvent.

Plate Suitable plate coated with a 0.75 mm layer of a mixture prepared as follows: mix 0.3 g of *carbomer R* with 240 mL of *water R* and allow to stand, with moderate shaking, for 1 h; adjust to pH 7 by the gradual addition, with continuous shaking, of *dilute sodium hydroxide solution R* and add 30 g of *silica gel H R*.

Pretreatment Heat the plate at 110 °C for 1 h, allow to cool and use immediately.

Mobile phase 70 g/L solution of *potassium dihydrogen phosphate R*.

Application 10 µL.

Development Over a path of 12 cm.

Drying In a current of warm air.

Detection Spray with a mixture of equal volumes of a 2 g/L solution of *1,3-dihydroxynaphthalene R* in *ethanol (96 per cent) R* and a 460 g/L solution of *sulfuric acid R*. Heat at 150 °C for 5 min to 10 min.

System suitability Reference solution (b):
— the chromatogram shows 3 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

B. Dissolve 0.5 g in 10 mL of *water R*. Add 10 mL of *picric acid solution R*. Initiate crystallisation if necessary by scratching the wall of the tube with a glass rod and allow to stand. Collect the crystals, wash with 20 mL of *water R* and filter. Dry at 100 °C. The crystals melt (*2.2.14*) at about 235 °C, with decomposition.

C. Dissolve about 50 mg in 2 mL of *water R*. Add 1 mL of a 10 g/L solution of *ninhydrin R* and heat for a few minutes on a water-bath. A violet colour develops.

D. It gives the reactions of sulfates (*2.3.1*).

TESTS

Solution S

Dissolve 0.20 g in *carbon dioxide-free water R* and dilute to 20.0 mL with the same solvent.

pH (*2.2.3*)

6.5 to 8.5 for solution S.

Specific optical rotation (*2.2.7*)

+ 112 to + 123 (dried substance), determined on solution S.

Kanamycin B

Thin-layer chromatography (*2.2.27*) as described under Identification A with the following modifications.

Test solution Dissolve 0.1 g of the substance to be examined in *water R* and dilute to 20 mL with the same solvent.

Reference solution Dissolve 4 mg of *kanamycin B sulfate CRS* in *water R* and dilute to 20 mL with the same solvent.

Application 4 µL.

Detection Spray with *ninhydrin and stannous chloride reagent R*. Heat at 110 °C for 15 min.

Limit:
— *kanamycin B*: any spot corresponding to kanamycin B in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (4.0 per cent).

Loss on drying (*2.2.32*)

Maximum 1.5 per cent, determined on 1.000 g by drying at 60 °C at a pressure not exceeding 670 Pa for 3 h.

Sulfated ash (*2.4.14*)

Maximum 0.5 per cent, determined on 1.0 g.

Sulfate

15.0 per cent to 17.0 per cent of sulfate (dried substance).

Dissolve 0.250 g in 100 mL of *water R* and adjust the solution to pH 11 with *concentrated ammonia R*.
Add 10.0 mL of *0.1 M barium chloride* and about 0.5 mg of *phthalein purple R*. Titrate with *0.1 M sodium edetate* adding 50 mL of *ethanol (96 per cent) R* when the colour of the solution begins to change and continue the titration until the violet-blue colour disappears.

1 mL of *0.1 M barium chloride* is equivalent to 9.606 mg of SO_4.

Pyrogens (*2.6.8*)

If intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of pyrogens, it complies with the test for pyrogens. Inject per kilogram of the rabbit's mass 1 mL of a 10 mg/mL solution of the substance to be examined in *water for injections R*.

ASSAY

Carry out the microbiological assay of antibiotics (*2.7.2*). Use *kanamycin monosulfate CRS* as the reference substance.

STORAGE

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

Ph Eur

Heavy Kaolin

(Ph. Eur. monograph 0503)

Preparation

Kaolin Poultice

When kaolin is prescribed or demanded, Light Kaolin shall be dispensed or supplied, unless it is ascertained that Light Kaolin (Natural) is required.

Ph Eur

DEFINITION

Purified, natural, hydrated aluminium silicate of variable composition.

CHARACTERS

Appearance

Fine, white or greyish-white, unctuous powder.

Solubility

Practically insoluble in water and in organic solvents.

IDENTIFICATION

A. To 0.5 g in a metal crucible add 1 g of *potassium nitrate R* and 3 g of *sodium carbonate R* and heat until the mixture melts. Allow to cool. To the residue add 20 mL of boiling *water R*, mix and filter. Wash the residue with 50 mL of *water R*. To the residue add 1 mL of *hydrochloric acid R* and 5 mL of *water R*. Filter. To the filtrate add 1 mL of *strong sodium hydroxide solution R* and filter. To the filtrate add 3 mL of *ammonium chloride solution R*. A gelatinous white precipitate is formed.

B. Add 2.0 g in 20 portions to 100 mL of a 10 g/L solution of *sodium laurilsulfate R* in a 100 mL graduated cylinder about 30 mm in diameter. Allow 2 min between additions for each portion to settle. Allow to stand for 2 h.
The apparent volume of the sediment is not greater than 5 mL.

C. 0.25 g gives the reaction of silicates (*2.3.1*).

TESTS

Solution S

To 4 g add a mixture of 6 mL of *acetic acid R* and 34 mL of *distilled water R*, shake for 1 min and filter.

Acidity or alkalinity

To 1.0 g add 20 mL of *carbon dioxide-free water R*, shake for 2 min and filter. To 10 mL of the filtrate add 0.1 mL of *phenolphthalein solution R*. The solution is colourless.
Not more than 0.25 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink.

Organic impurities
Heat 0.3 g to redness in a calcination tube. The residue is only slightly more coloured than the original substance.

Adsorption power
To 1.0 g in a ground-glass-stoppered test-tube add 10.0 mL of a 3.7 g/L solution of *methylene blue R* and shake for 2 min. Allow to settle. Centrifuge and dilute the solution 1 to 100 with *water R*. The solution is not more intensely coloured than a 0.03 g/L solution of *methylene blue R*.

Swelling power
Triturate 2 g with 2 mL of *water R*. The mixture does not flow.

Substances soluble in dilute hydrochloric acid
Maximum 1 per cent.

To 5.0 g add 7.5 mL of *dilute hydrochloric acid R* and 27.5 mL of *water R* and boil for 5 min. Filter, wash the residue on the filter with *water R* and dilute the combined filtrate and washings to 50.0 mL with *water R*. To 10.0 mL of the solution add 1.5 mL of *dilute sulfuric acid R*, evaporate to dryness on a water-bath and ignite. The residue weighs a maximum of 10 mg.

Chlorides (*2.4.4*)
Maximum 250 ppm.

Dilute 2 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 0.1 per cent.

Dilute 1.5 mL of solution S to 15 mL with *distilled water R*.

Calcium (*2.4.3*)
Maximum 250 ppm.

Dilute 4 mL of solution S to 15 mL with *distilled water R*.

Microbial contamination
TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*).

TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

LABELLING
The label states, where applicable, that the substance is suitable for internal use.

Ph Eur

Light Kaolin

Action and use
Antidiarrhoeal.

Preparations
Kaolin Mixture

Kaolin and Morphine Mixture

When Kaolin or Light Kaolin is prescribed or demanded, Light Kaolin shall be dispensed or supplied unless it is ascertained that Light Kaolin (Natural) is required.

DEFINITION
Light Kaolin is a native hydrated aluminium silicate, freed from most of its impurities by elutriation and dried.
It contains a suitable dispersing agent.

CHARACTERISTICS
A light, white powder free from gritty particles; odourless or almost odourless; unctuous.

Practically insoluble in *water* and in mineral acids.

IDENTIFICATION
A. To 0.5 g in a metal crucible add 1 g of *potassium nitrate* and 3 g of *sodium carbonate* and heat until the mixture melts. Allow to cool. To the residue add 20 mL of boiling *water*, mix and filter. Wash the residue with 50 mL of *water*, add to the residue 1 mL of *hydrochloric acid* and 5 mL of *water*, mix and filter. To the filtrate add 1 mL of *strong sodium hydroxide solution*, filter and add to the filtrate 3 mL of *ammonium chloride solution*. A gelatinous white precipitate is produced.

B. 0.25 g yields the reaction characteristic of *silicates*, Appendix VI.

C. Triturate 2 g with 2 mL of *water*. The resulting mixture flows.

TESTS
Coarse particles
Transfer 5 g to a stoppered cylinder (about 16 cm × 35 mm), add 60 mL of a 1% w/v solution of *sodium pyrophosphate*, shake thoroughly and allow to stand for 5 minutes. Using a pipette, withdraw 50 mL from a point about 5 cm below the surface of the liquid. To the remaining liquid add 50 mL of *water*, shake, allow to stand for 5 minutes and withdraw 50 mL in the same manner as before. Repeat the operation until a total of 400 mL of suspension has been withdrawn under the prescribed conditions. Transfer the remainder to an evaporating dish and evaporate to dryness on a water bath. The residue, after drying at 105°, weighs not more than 25 mg.

Fine particles
Disperse 5 g in 250 mL of *water* by shaking vigorously for 2 minutes in a stoppered flask, pour immediately into a glass cylinder 5 cm in diameter and transfer 20 mL to a glass dish using a pipette. Evaporate to dryness and dry to constant weight at 105°. Allow the remainder of the suspension to stand for 4 hours at 20° and withdraw a second 20 mL portion using a pipette with its tip exactly 5 cm below the surface and without disturbing the sediment. Transfer the second portion to a glass dish, evaporate to dryness and dry to constant weight at 105°. The weight of the residue from the second portion is not less than 70% of the weight of the residue from the first portion.

Arsenic
0.50 g dispersed in 25 mL of *water* complies with the *limit test for arsenic*, Appendix VII (2 ppm).

Chloride
Boil 1.0 g with 80 mL of *water* and 20 mL of 2M *nitric acid* under a reflux condenser for 5 minutes, cool and filter. 15 mL of the filtrate complies with the *limit test for chlorides*, Appendix VII (330 ppm).

Loss on drying
When dried to constant weight at 105°, loses not more than 1.5% of its weight. Use 1 g.

Loss on ignition
When ignited at 600°, loses not more than 15.0% of its weight. Use 1 g.

Soluble matter
Boil 2 g with 100 mL of 0.2M *hydrochloric acid* under a reflux condenser for 5 minutes, cool, filter and evaporate 50 mL of the filtrate to dryness. The residue, after ignition at about 600° for 30 minutes, weighs not more than 10 mg.

Light Kaolin (Natural)

Action and use
Antidiarrhoeal.

Preparations
Kaolin Mixture

Kaolin and Morphine Mixture

When Kaolin or Light Kaolin is prescribed or demanded, Light Kaolin shall be dispensed or supplied unless it is ascertained that Light Kaolin (Natural) is required.

DEFINITION
Light Kaolin (Natural) is a native hydrated aluminium silicate, freed from most of its impurities by elutriation and dried. It does not contain a dispersing agent.

CHARACTERISTICS
A light, white powder free from gritty particles; odourless or almost odourless; unctuous.

Practically insoluble in *water* and in mineral acids.

IDENTIFICATION
A. To 0.5 g in a metal crucible add 1 g of *potassium nitrate* and 3 g of *sodium carbonate* and heat until the mixture melts. Allow to cool. To the residue add 20 mL of boiling *water*, mix and filter. Wash the residue with 50 mL of *water*, add to the residue 1 mL of *hydrochloric acid* and 5 mL of *water*, mix and filter. To the filtrate add 1 mL of *strong sodium hydroxide solution*, filter and add to the filtrate 3 mL of *ammonium chloride solution*. A gelatinous white precipitate is produced.

B. 0.25 g yields the reaction characteristic of *silicates*, Appendix VI.

C. Triturate 2 g with 2 mL of *water*. The resulting mixture does not flow.

TESTS
Coarse particles
Transfer 5 g to a stoppered cylinder (about 16 cm × 35 mm), add 60 mL of a 1% w/v solution of *sodium pyrophosphate*, shake thoroughly and allow to stand for 5 minutes. Using a pipette, withdraw 50 mL from a point about 5 cm below the surface of the liquid. To the remaining liquid add 50 mL of *water*, shake, allow to stand for 5 minutes and withdraw 50 mL in the same manner as before. Repeat the operation until a total of 400 mL of suspension has been withdrawn under the prescribed conditions. Transfer the remainder to an evaporating dish and evaporate to dryness on a water bath. The residue, after drying at 105°, weighs not more than 25 mg.

Fine particles
Disperse 5 g in 250 mL of *water* containing 50 mg of *sodium pyrophosphate* by shaking vigorously for 2 minutes in a stoppered flask, pour immediately into a glass cylinder 5 cm in diameter and transfer 20 mL to a glass dish using a pipette. Evaporate to dryness and dry to constant weight at 105°. Allow the remainder of the suspension to stand for 4 hours at 20° and withdraw a second 20 mL portion using a pipette with its tip exactly 5 cm below the surface and without disturbing the sediment. Transfer the second portion to a glass dish, evaporate to dryness and dry to constant weight at 105°. The weight of the residue from the second portion is not less than 70% of the weight of the residue from the first portion.

Arsenic
0.50 g dispersed in 25 mL of *water* complies with the *limit test for arsenic*, Appendix VII (2 ppm).

Chloride
Boil 1.0 g with 80 mL of *water* and 20 mL of 2M *nitric acid* under a reflux condenser for 5 minutes, cool and filter. 15 mL of the filtrate complies with the *limit test for chlorides*, Appendix VII (330 ppm).

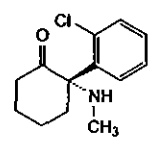
Loss on drying
When dried to constant weight at 105°, loses not more than 1.5% of its weight. Use 1 g.

Loss on ignition
When ignited at 600°, loses not more than 15.0% of its weight. Use 1 g.

Soluble matter
Boil 2 g with 100 mL of 0.2M *hydrochloric acid* under a reflux condenser for 5 minutes, cool, filter and evaporate 50 mL of the filtrate to dryness. The residue, after ignition at about 600° for 30 minutes, weighs not more than 10 mg.

Ketamine Hydrochloride

(Ph. Eur. monograph 1020)



and enantiomer . HCl

$C_{13}H_{17}Cl_2NO$ 274.2 *1867-66-9*

Action and use
Intravenous general anaesthetic

Preparations
Ketamine Injection

Ketamine Nasal Spray

Ketamine Oral Solution

Ph Eur

DEFINITION
(2*RS*)-2-(2-Chlorophenyl)-2-(methylamino)cyclohexanone hydrochloride.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water and in methanol, soluble in ethanol (96 per cent).

mp
About 260 °C, with decomposition.

IDENTIFICATION
A. Optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison ketamine hydrochloride CRS.

C. It gives reaction (a) of chlorides (*2.3.1*).

TESTS
Solution S
Dissolve 10.0 g in *carbon dioxide-free water R* and dilute to 50.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)
3.5 to 4.1.

Dilute 10 mL of solution S to 20 mL with *carbon dioxide-free water R*.

Optical rotation (*2.2.7*)
−0.2° to + 0.2°.

Dilute 2.5 mL of solution S to 25.0 mL with *water R*.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 50 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 5 mg of *ketamine impurity A CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase (using ultrasound if necessary). To 1.0 mL of the solution, add 0.5 mL of the test solution and dilute to 100.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 10.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 50.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.125 m, Ø = 4.0 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 0.95 g of *sodium hexanesulfonate R* in 1 L of a mixture of 25 volumes of *acetonitrile R1* and 75 volumes of *water R* and add 4 mL of *acetic acid R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µL.

Run time 10 times the retention time of ketamine.

Relative retention With reference to ketamine (retention time = about 3 min): impurity A = about 1.6; impurity B = about 3.3; impurity C = about 4.6.

System suitability Reference solution (a):
— *resolution*: minimum 1.5 between the peaks due to ketamine and impurity A.

Limits:
— *impurities A, B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

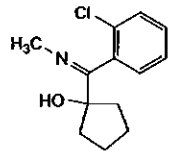
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.200 g in 50 mL of *methanol R* and add 1.0 mL of *0.1 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

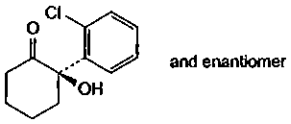
1 mL of *0.1 M sodium hydroxide* is equivalent to 27.42 mg of $C_{13}H_{17}Cl_2NO$.

STORAGE
Protected from light.

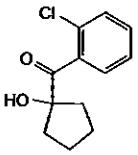
IMPURITIES
Specified impurities *A, B, C.*



A. 1-(2-chloro-*N*-methylbenzimidoyl)cyclopentanol,



B. (2*RS*)-2-(2-chlorophenyl)-2-hydroxycyclohexanone,

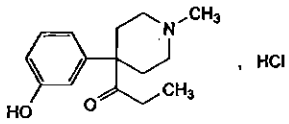


C. (2-chlorophenyl)(1-hydroxycyclopentyl)methanone.

Ph Eur

Ketobemidone Hydrochloride

(Ph. Eur. monograph 1746)



$C_{15}H_{22}ClNO_2$ 283.8 *5965-49-1*

Action and use
Opioid receptor agonist; analgesic.

Ph Eur

DEFINITION
1-[4-(3-Hydroxyphenyl)-1-methylpiperidin-4-yl]propan-1-one hydrochloride.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water, soluble in ethanol (96 per cent), very slightly soluble in methylene chloride.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of ketobemidone hydrochloride.*

B. Solution S (see Tests) gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 0.250 g in *carbon dioxide-free water R* and dilute to 25.0 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_8 (*2.2.2, Method II*).

pH (*2.2.3*)

4.5 to 5.5 for solution S.

Related substances

Liquid chromatography (*2.2.29*).

Solution A 1.54 g/L solution of *ammonium acetate R* adjusted to pH 8.0 with *dilute ammonia R1*.

Test solution Dissolve 50.0 mg of the substance to be examined in solution A and dilute to 25.0 mL with the same solution.

Reference solution (a) Dissolve 1 mg of *ketobemidone impurity B CRS* and 1 mg of *ketobemidone impurity C CRS* in solution A and dilute to 25 mL with the same solution.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with solution A. Dilute 20.0 mL of this solution to 100.0 mL with solution A.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *phenylhexylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase *acetonitrile R*, solution A (20:80 *V/V*).

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 278 nm.

Injection 20 µL.

Run time 4.5 times the retention time of ketobemidone.

Relative retention With reference to ketobemidone (retention time = about 10 min): impurity A = about 0.4; impurity B = about 0.6; impurity C = about 0.7; impurity D = about 3.5.

System suitability Reference solution (a):
- *resolution*: minimum 4.0 between the peaks due to impurity B and impurity C.

Limits:
- *impurities A, B, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- *total*: not more than 3.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.7 per cent);
- *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.12*)

Maximum 1.0 per cent, determined on 0.50 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

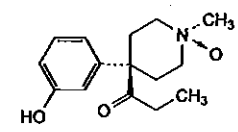
Dissolve 0.200 g in a mixture of 5.0 mL of *0.01 M hydrochloric acid* and 50 mL of *ethanol (96 per cent) R*. Carry out a potentiometric titration (*2.2.20*) using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 28.38 mg of $C_{15}H_{22}ClNO_2$.

IMPURITIES

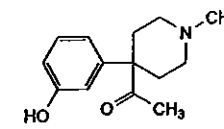
Specified impurities *A, B, C, D.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *E.*

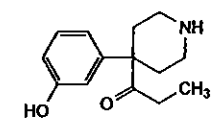


and *trans* isomer

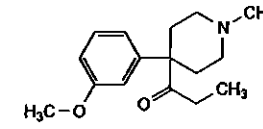
A. 1-[4-(3-hydroxyphenyl)-1-methyl-1-oxidopiperidin-4-yl] propan-1-one (*cis* and *trans* isomers),



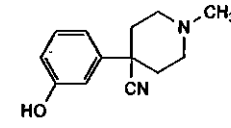
B. 1-[4-(3-hydroxyphenyl)-1-methylpiperidin-4-yl]ethanone,



C. 1-[4-(3-hydroxyphenyl)piperidin-4-yl]propan-1-one,



D. 1-[4-(3-methoxyphenyl)-1-methylpiperidin-4-yl]propan-1-one,

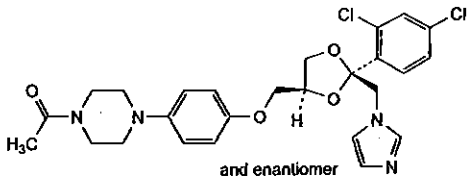


E. 4-(3-hydroxyphenyl)-1-methylpiperidin-4-carbonitrile.

Ph Eur

Ketoconazole

(Ph. Eur. monograph 0921)



$C_{26}H_{28}Cl_2N_4O_4$ 531.4 *65277-42-1*

Action and use
Antifungal.

Preparations
Ketoconazole Cream
Ketoconazole Shampoo

Ph Eur

DEFINITION

1-[4-[4-[[(2*RS*,4*SR*)-2-(2,4-Dichlorophenyl)-2-[(1*H*-imidazol-1-yl)methyl]-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]ethan-1-one.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Practically insoluble in water, freely soluble in methylene chloride, soluble in methanol, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: B.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 148 °C to 152 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *ketoconazole CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 30 mg of the substance to be examined in the mobile phase and dilute to 5 mL with the mobile phase.

Reference solution (a) Dissolve 30 mg of *ketoconazole CRS* in the mobile phase and dilute to 5 mL with the mobile phase.

Reference solution (b) Dissolve 30 mg of *ketoconazole CRS* and 30 mg of *econazole nitrate CRS* in the mobile phase, then dilute to 5 mL with the mobile phase.

Plate *TLC octadecylsilyl silica gel plate R.*

Mobile phase *ammonium acetate solution R, dioxan R, methanol R* (20:40:40 *V/V/V*).

Application 5 µL.

Development Over 3/4 of the plate.

Drying In a current of warm air for 15 min.

Detection Expose to iodine vapour until the spots appear and examine in daylight.

System suitability Reference solution (b):
— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To about 30 mg in a porcelain crucible add 0.3 g of *anhydrous sodium carbonate R.* Heat over an open flame for 10 min. Allow to cool. Take up the residue with 5 mL of *dilute nitric acid R* and filter. To 1 mL of the filtrate add 1 mL of *water R.* The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S
Dissolve 1.0 g in *methylene chloride R* and dilute to 10.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_4 (*2.2.2, Method II*).

Optical rotation (*2.2.7*)
−0.10° to + 0.10°, determined on solution S.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dissolve the contents of a vial of *ketoconazole impurity mixture CRS* (impurities C and D) in 1 mL of *methanol R.*

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *methanol R.* Dilute 1.0 mL of this solution to 10.0 mL with *methanol R.*

Column:
— *size: l* = 0.10 m, Ø = 4.6 mm;
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
— *mobile phase A: acetonitrile for chromatography R,* 3.4 g/L solution of *tetrabutylammonium hydrogen sulfate R* (5:95 *V/V*);
— *mobile phase B: acetonitrile for chromatography R,* 3.4 g/L solution of *tetrabutylammonium hydrogen sulfate R* (50:50 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	100 → 0	0 → 100
10 - 15	0	100

Flow rate 2 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µL; inject *methanol R* as a blank.

Identification of impurities Use the chromatogram supplied with *ketoconazole impurity mixture CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities C and D.

Relative retention With reference to ketoconazole (retention time = about 6 min): impurity D = about 0.8; impurity C = about 0.9.

System suitability Reference solution (a):
— *resolution:* minimum 1.5 between the peaks due to impurities D and C.

Calculation of percentage contents:
— *correction factor:* multiply the peak area of impurity D by 1.4;

— for each impurity, use the concentration of ketoconazole in reference solution (b).

Limits:
— *impurity D*: maximum 0.2 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.3 per cent;
— *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

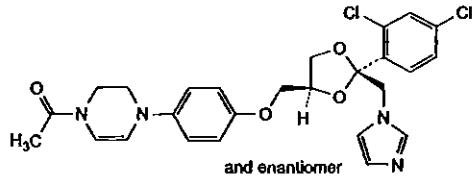
ASSAY
Dissolve 0.200 g in 70 mL of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 26.57 mg of $C_{26}H_{28}Cl_2N_4O_4$.

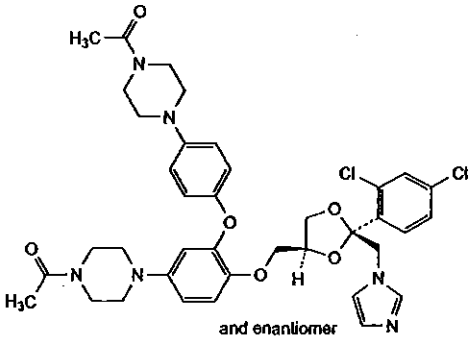
STORAGE
Protected from light.

IMPURITIES
Specified impurities *D.*

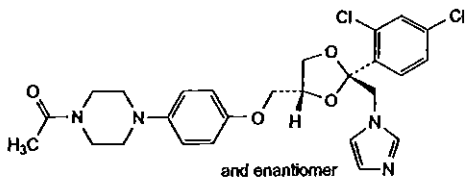
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, E.*



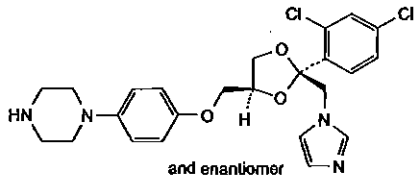
A. 1-[4-[4-[[(2*RS*,4*SR*)-2-(2,4-dichlorophenyl)-2-[(1*H*-imidazol-1-yl)methyl]-1,3-dioxolan-4-yl]methoxy]phenyl]-1,2,3,4-tetrahydropyrazin-1-yl]ethan-1-one,



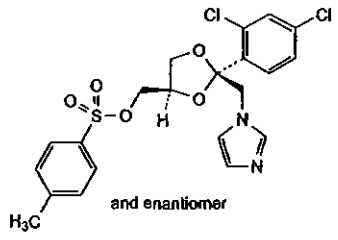
B. 1-[4-[4-[5-(4-acetylpiperazin-1-yl)-2-[[(2*RS*,4*SR*)-2-(2,4-dichlorophenyl)-2-[(1*H*-imidazol-1-yl)methyl]-1,3-dioxolan-4-yl]methoxy]phenoxy]phenyl]piperazin-1-yl]ethan-1-one,



C. 1-[4-[4-[[(2*RS*,4*RS*)-2-(2,4-dichlorophenyl)-2-[(1*H*-imidazol-1-yl)methyl]-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]ethan-1-one,



D. 1-[4-[[(2*RS*,4*SR*)-2-(2,4-dichlorophenyl)-2-[(1*H*-imidazol-1-yl)methyl]-1,3-dioxolan-4-yl]methoxy]phenyl]piperazine,

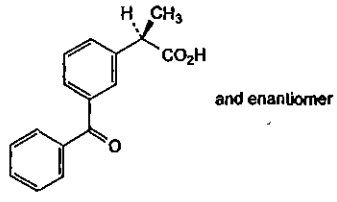


E. [(2*RS*,4*SR*)-2-(2,4-dichlorophenyl)-2-[(1*H*-imidazol-1-yl)methyl]-1,3-dioxolan-4-yl]methyl 4-methylbenzene-1-sulfonate.

Ph Eur

Ketoprofen

(Ph. Eur. monograph 0922)



$C_{16}H_{14}O_3$ 254.3 *22071-15-4*

Action and use
Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Preparations
Ketoprofen Capsules
Ketoprofen Gel

Ph Eur

DEFINITION
(2*RS*)-2-(3-Benzoylphenyl)propanoic acid.

Content
99.0 per cent to 100.5 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, freely soluble in acetone, in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

First identification: C.

Second identification: A, B, D.

A. Melting point (*2.2.14*): 94 °C to 97 °C.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 50.0 mg in *ethanol (96 per cent) R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of this solution to 50.0 mL with *ethanol (96 per cent) R.*

Spectral range 230-350 nm.

Absorption maximum At 255 nm.

Specific absorbance at the absorption maximum 615 to 680.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *ketoprofen CRS.*

D. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *acetone R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dissolve 10 mg of *ketoprofen CRS* in *acetone R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 10 mg of *indometacin CRS* in *acetone R* and dilute to 10 mL with the same solvent. To 1 mL of this solution add 1 mL of reference solution (a).

Plate *TLC silica gel GF_{254} plate R.*

Mobile phase *glacial acetic acid R, methylene chloride R, acetone R* (1:49:50 *V/V/V*).

Application 10 µL.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):
— the chromatogram shows 2 clearly separated principal spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Dissolve 1.0 g in *acetone R* and dilute to 10 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 20.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 50.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *ketoprofen impurity A CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution (c) Dissolve 5.0 mg of *ketoprofen impurity C CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution (d) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. To 1.0 mL of this solution add 1.0 mL of reference solution (b).

Column:
— *size: l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: spherical *octadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 350 m^2/g and a pore size of 10 nm.

Mobile phase Mix 2 volumes of freshly prepared phosphate buffer solution pH 3.5 R, 43 volumes of *acetonitrile R* and 55 volumes of *water R.*

Flow rate 1 mL/min.

Detection Spectrophotometer at 233 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity A; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity C.

Run time 7 times the retention time of ketoprofen.

Relative retention With reference to ketoprofen (retention time = about 7 min): impurity C = about 0.3; impurity E = about 0.69; impurity B = about 0.73; impurity D = about 1.35; impurity A = about 1.5; impurity F = about 2.0.

System suitability Reference solution (d):
— *resolution*: minimum 7.0 between the peaks due to ketoprofen and impurity A.

Limits:
— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
— *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);
— *impurities B, D, E, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *sum of impurities other than A and C*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);
— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying at 60 °C at a pressure not exceeding 0.67 kPa.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

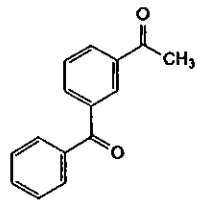
Dissolve 0.200 g in 25 mL of *ethanol (96 per cent) R.* Add 25 mL of *water R.* Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M sodium hydroxide* is equivalent to 25.43 mg of $C_{16}H_{14}O_3$.

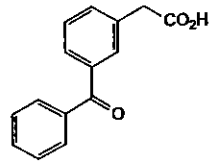
IMPURITIES

Specified impurities *A, B, C, D, E, F.*

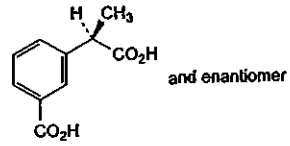
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *G, H, I, J, K, L.*



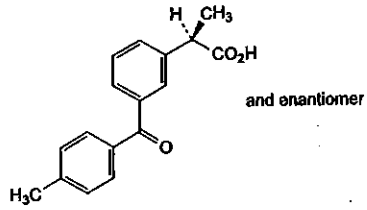
A. 1-(3-benzoylphenyl)ethanone,



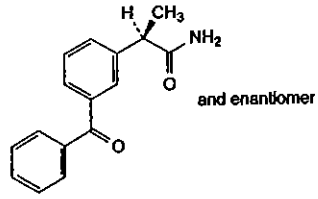
B. (3-benzoylphenyl)acetic acid,



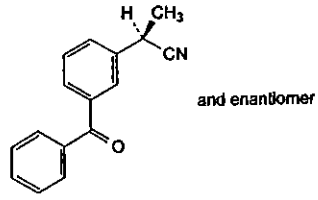
C. 3-[(1*RS*)-1-carboxyethyl]benzoic acid,



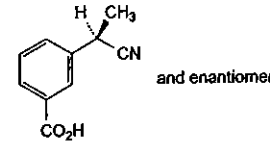
D. (2*RS*)-2-[3-(4-methylbenzoyl)phenyl]propanoic acid,



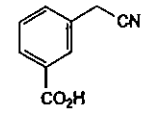
E. (2*RS*)-2-(3-benzoylphenyl)propanamide,



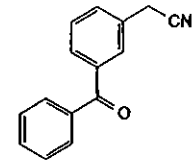
F. (2*RS*)-2-(3-benzoylphenyl)propanenitrile,



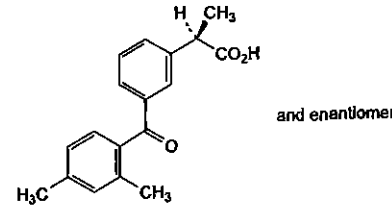
G. 3-[(1*RS*)-1-cyanoethyl]benzoic acid,



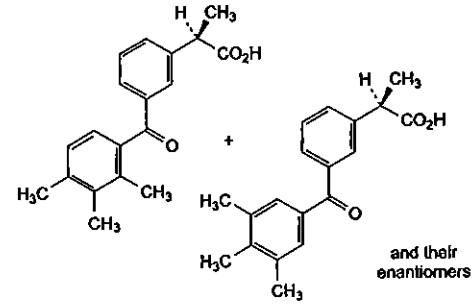
H. 3-(cyanomethyl)benzoic acid,



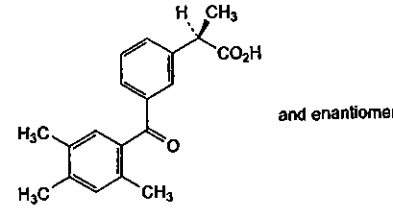
I. (3-benzoylphenyl)ethanenitrile,



J. (2*RS*)-2-[3-(2,4-dimethylbenzoyl)phenyl]propanoic acid,



K. mixture of (2*RS*)-2-[3-(2,3,4-trimethylbenzoyl) phenyl]propanoic acid and (2*RS*)-2-[3-(3,4,5-trimethylbenzoyl)phenyl]propanoic acid,

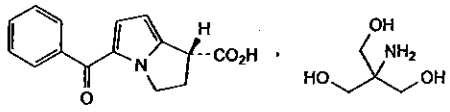


L. (2*RS*)-2-[3-(2,4,5-trimethylbenzoyl)phenyl]propanoic acid.

Ph Eur

Ketorolac Trometamol

(Ph. Eur. monograph 1755)



and enantiomer

$C_{19}H_{24}N_2O_6$ 376.4 *74103-07-4*

Action and use
Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Ph Eur

DEFINITION
2-Amino-2-(hydroxymethyl)propane-1,3-diol (1*RS*)-5-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylate.

Content
98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water and in methanol, slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *ketorolac trometamol CRS.*

TESTS

Solution S
Dissolve 0.75 g in *carbon dioxide-free water R* and dilute to 25.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*).

pH (*2.2.3*)
5.7 to 6.7.
Dilute 5 mL of solution S to 15 mL with *carbon dioxide-free water R.*

Absorbance (*2.2.25*)
Maximum 0.10, determined at 430 nm for solution S.

Related substances
Liquid chromatography (*2.2.29*). *Protect the solutions from bright light.*
Solvent mixture *tetrahydrofuran R, water R* (30:70 *V/V*).
Test solution Dissolve 20 mg of the substance to be examined in the solvent mixture and dilute to 50 mL with the solvent mixture.
Reference solution (a) Dilute 1.0 mL of the test solution to 10.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 100.0 mL with the solvent mixture.
Reference solution (b) Dissolve 2 mg of *ketorolac trometamol for peak identification CRS* (containing impurities A, B, C and D) in 5 mL of the solvent mixture.
Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: octylsilyl silica gel for chromatography R* (5 µm);
— *temperature:* 40 °C.
Mobile phase Mix 30 volumes of *tetrahydrofuran R* with 70 volumes of a solution prepared as follows: dissolve 5.75 g of *ammonium dihydrogen phosphate R* in 900 mL of *water R,* adjust to pH 3.0 with *phosphoric acid R* and dilute to 1000 mL with *water R.*
Flow rate 1.5 mL/min.
Detection Spectrophotometer at 313 nm.
Injection 10 µL.
Run time 3 times the retention time of ketorolac.
Identification of impurities Use the chromatogram supplied with *ketorolac trometamol for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C and D.
Relative retention With reference to ketorolac (retention time = about 10 min): impurity C = about 0.5; impurity A = about 0.6; impurity D = about 0.7; impurity B = about 0.9.
System suitability Reference solution (b):
— *resolution*: minimum 1.5 between the peaks due to impurity B and ketorolac.
Limits:
— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.67; impurity B = 0.52; impurity C = 2.2;
— *impurities A, B, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

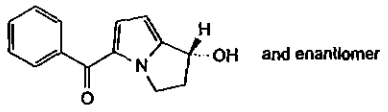
Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 3 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

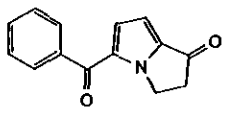
ASSAY
Dissolve 0.300 g in 60 mL of *anhydrous acetic acid R.* Titrate with *0.1 M perchloric acid,* determining the end-point potentiometrically (*2.2.20*).
1 mL of *0.1 M perchloric acid* is equivalent to 37.64 mg of $C_{19}H_{24}N_2O_6$.

STORAGE
Protected from light.

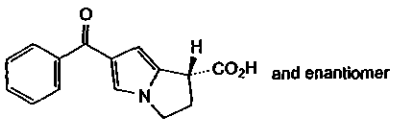
IMPURITIES
Specified impurities *A, B, C, D.*
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *E, F, G, H, I, J.*



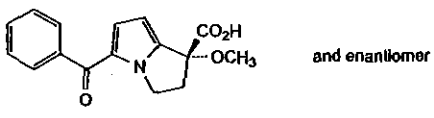
A. (1*RS*)-5-benzoyl-2,3-dihydro-1*H*-pyrrolizin-1-ol,



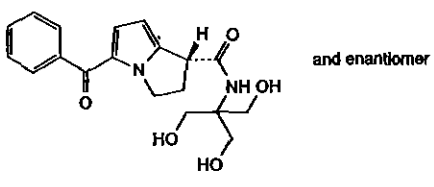
B. 5-benzoyl-2,3-dihydro-1*H*-pyrrolizin-1-one,



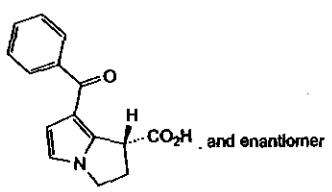
C. (1*RS*)-6-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylic acid,



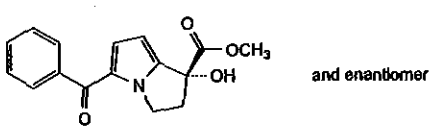
D. (1*RS*)-5-benzoyl-1-methoxy-2,3-dihydro-1*H*-pyrrolizine-1-carboxylic acid,



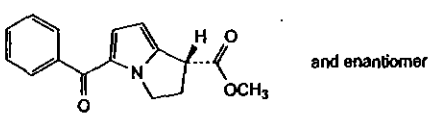
E. (1*RS*)-5-benzoyl-*N*-[2-hydroxy-1,1-bis (hydroxymethyl)ethyl]-2,3-dihydro-1*H*-pyrrolizine-1-carboxamide,



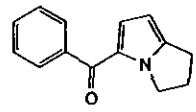
F. (1*RS*)-7-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylic acid,



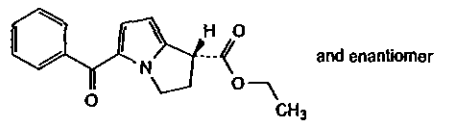
G. methyl (1*RS*)-5-benzoyl-1-hydroxy2,3-dihydro-1*H*-pyrrolizine-1-carboxylate,



H. methyl (1*RS*)-5-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylate,



I. phenyl(2,3-dihydro-1*H*-pyrrolizin-5-yl)methanone,

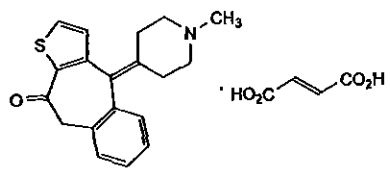


J. ethyl (1*RS*)-5-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylate.

Ph Eur

Ketotifen Fumarate

(Ketotifen Hydrogen Fumarate, Ph. Eur. monograph 1592)



$C_{23}H_{23}NO_5S$ 425.5 *34580-14-8*

Action and use
Histamine H_1 receptor antagonist.

Ph Eur

DEFINITION
4-(1-Methylpiperidin-4-ylidene)-4,9-dihydro-10*H*-benzo [4,5]cyclohepta[1,2-*b*]thiophen-10-one hydrogen (*E*)-butenedioate.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or brownish-yellow, fine, crystalline powder.

Solubility
Sparingly soluble in water, slightly soluble in methanol, practically insoluble in heptane.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *ketotifen hydrogen fumarate CRS.*

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_4, BY_4 or B_4 (*2.2.2, Method II*).
Dissolve 0.2 g in *methanol R* and dilute to 10 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*
Solvent mixture *methanol R, water R* (50:50 *V/V*).
Test solution Dissolve 30.0 mg of the substance to be examined in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve the contents of a vial of *ketotifen impurity G CRS* in 1.0 mL of a solution prepared as follows: mix 1.0 mL of the test solution with 9.0 mL of the solvent mixture. Sonicate until dissolution of impurity G is complete.

Reference solution (c) Dissolve 5 mg of *ketotifen for peak identification CRS* (containing impurity A) in the solvent mixture and dilute to 5.0 mL with the solvent mixture.

Column:
- *size*: *l* = 0.15 m, Ø = 4.0 mm;
- *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (3 µm);
- *temperature*: 40 °C.

Mobile phase:
- *mobile phase A*: mix 175 µL of *triethylamine R* and 500 mL of *water R*;
- *mobile phase B*: mix 175 µL of *triethylamine R* and 500 mL of *methanol R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 12	40	60
12 - 20	40 → 10	60 → 90
20 - 25	10	90

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 297 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram supplied with *ketotifen for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peak due to impurity A; use the chromatogram obtained with reference solution (b) to identify the peak due to impurity G.

Relative retention With reference to ketotifen (retention time = about 11 min): fumaric acid = about 0.1; impurity G = about 0.8; impurity A = about 1.9.

System suitability Reference solution (b):
- *resolution*: minimum 1.5 between the peaks due to impurity G and ketotifen.

Limits:
- *correction factor*: for the calculation of content, multiply the peak area of impurity G by 1.4;
- *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *impurity G*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent); disregard the peak due to fumaric acid.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

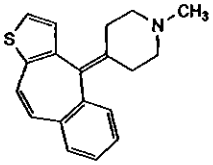
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.350 g in a mixture of 30 mL of *acetic anhydride R* and 30 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

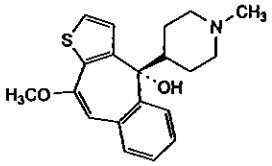
1 mL of *0.1 M perchloric acid* is equivalent to 42.55 mg of $C_{23}H_{23}NO_5S$.

IMPURITIES
Specified impurities *A, G.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D, E, F.*

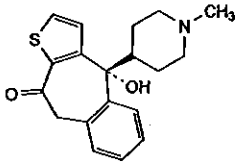


A. 4-(4*H*-benzo[4,5]cyclohepta[1,2-*b*]thiophen-4-ylidene)-1-methylpiperidine,



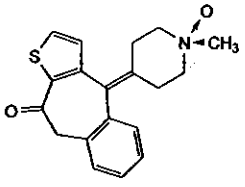
and enantiomer

B. (4*RS*)-10-methoxy-4-(1-methylpiperidin-4-yl)-4*H*-benzo[4,5]cyclohepta[1,2-*b*]thiophen-4-ol,



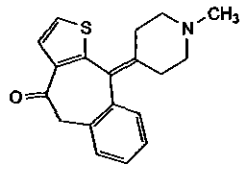
and enantiomer

C. (4*RS*)-4-hydroxy-4-(1-methylpiperidin-4-yl)-4,9-dihydro-10*H*-benzo[4,5]cyclohepta[1,2-*b*]thiophen-10-one,

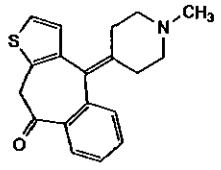


and enantiomer

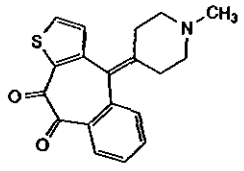
D. 4-[(R_aS_a)-1-methylpiperidin-4-ylidene]-4,9-dihydro-10*H*-benzo[4,5]cyclohepta[1,2-*b*]thiophen-10-one *N*-oxide (ketotifen *N*-oxide),



E. 10-(1-methylpiperidin-4-ylidene)-5,10-dihydro-4*H*-benzo[5,6]cyclohepta[1,2-*b*]thiophen-4-one,



F. 4-(1-methylpiperidin-4-ylidene)-4,10-dihydro-9*H*-benzo[4,5]cyclohepta[1,2-*b*]thiophen-9-one,

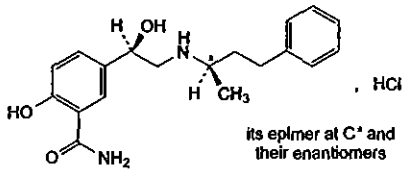


G. 4-(1-methylpiperidin-4-ylidene)-4*H*-benzo[4,5]cyclohepta[1,2-*b*]thiophen-9,10-dione.

Ph Eur

Labetalol Hydrochloride

(Ph. Eur. monograph 0923)



$C_{19}H_{25}ClN_2O_3$ 364.9 *32780-64-6*

Action and use
Alpha-and beta-adrenoceptor antagonist.

Preparations
Labetalol Injection
Labetalol Tablets

Ph Eur

DEFINITION
Mixture of 4 stereoisomers of 2-hydroxy-5-[(1*S*)-1-hydroxy-2-[[(2*S*)-4-phenylbutan-2-yl]amino]ethyl]benzamide hydrochloride.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white powder.

Solubility
Sparingly soluble in water and in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION
First identification: A, C, E.

Second identification: A, B, D, E.

A. Optical rotation (*2.2.7*): −0.05° to + 0.05°, determined on solution S (see Tests).

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 25.0 mg in a 10.3 g/L solution of *hydrochloric acid R* and dilute to 250.0 mL with the same solution.

Spectral range 230-350 nm.

Absorption maximum 302 nm.

Specific absorbance at the absorption maximum 83 to 88.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *labetalol hydrochloride CRS.*

D. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in 1 mL of *ethanol (96 per cent) R.*

Reference solution (a) Dissolve 10 mg of *labetalol hydrochloride CRS* in 1 mL of *ethanol (96 per cent) R.*

Reference solution (b) Dissolve 10 mg of *labetalol hydrochloride CRS* and 10 mg of *propranolol hydrochloride CRS* in *ethanol (96 per cent) R* and dilute to 5 mL with the same solvent.

Plate *TLC octadecylsilyl silica gel F_{254} plate R.*

Mobile phase *perchloric acid R, water R, methanol R* (0.5:50:80 *V/V/V*).

Application 2 µL.

Development Place the plate in a chromatographic tank immediately after the addition of the mobile phase, close the tank and develop over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):
— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

E. It gives reaction (a) of chlorides (*2.3.1*).

TESTS
Solution S
Dissolve 0.50 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent. *Solution S must be freshly prepared.*

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than intensity 6 of the range of reference solutions of the most appropriate colour (*2.2.2, Method II*).

pH (*2.2.3*)
4.0 to 5.0 for solution S.

Diastereoisomer ratio
Gas chromatography (*2.2.28*).

Test solution Dissolve 2.0 mg of the substance to be examined in 1.0 mL of a 12.0 g/L solution of *butylboronic acid R* in *anhydrous pyridine R* and allow to stand for 20 min.

Column:
— *material:* glass;
— *size:* *l* = 1.5 m, Ø = 4 mm;

— *stationary phase*: *silanised diatomaceous earth for gas chromatography R* (125-150 µm) impregnated with 3 per cent *m/m* of *phenyl(50)methyl(50)polysiloxane R*.

Carrier gas *nitrogen for chromatography R*.

Flow rate 40 mL/min.

Temperature:
— *column, injection port and detector*: 300 °C.

Detection Flame ionisation.

Injection 2 µL.

System suitability:
— the height of the trough separating the 2 peaks due to the pairs of diastereoisomers is less than 5 per cent of the full scale of the recorder.

Limit:
— *each pair of diastereoisomers*: for the area of each peak, 45 per cent to 55 per cent of the total area of the 2 peaks.

Related substances
Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 25.0 mg of the substance to be examined in mobile phase A and dilute to 10.0 mL with mobile phase A.

Test solution (b) Dilute 1.0 mL of test solution (a) to 50.0 mL with mobile phase A.

Reference solution (a) Dilute 1.0 mL of test solution (a) to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

Reference solution (b) Dilute 2 mL of test solution (a) to 100 mL with mobile phase A (solution A). Dissolve 5 mg of *labetalol impurity A CRS* in 50 mL of mobile phase B and dilute to 100 mL with solution A.

Reference solution (c) Dissolve 25.0 mg of *labetalol hydrochloride CRS* in mobile phase A and dilute to 10.0 mL with mobile phase A. Dilute 1.0 mL of the solution to 50.0 mL with mobile phase A.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped ethylene-bridged octadecylsilyl silica gel for chromatography (hybrid material) R* (3.5 µm);
— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: *phosphoric acid R, water for chromatography R* (0.1:99.9 *V/V*);
— *mobile phase B*: *acetonitrile for chromatography R*, mobile phase A (50:50 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	100	0
5 - 40	100 → 0	0 → 100
40 - 45	0	100

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µL of test solution (a) and reference solutions (a) and (b).

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity A.

Relative retention With reference to labetalol (retention time = about 22 min): impurity A = about 1.1.

System suitability Reference solution (b):
— *resolution*: minimum 4.5 between the peaks due to labetalol and impurity A.

Calculation of percentage contents:
— for each impurity, use the concentration of labetalol hydrochloride in reference solution (a).

Limits:
— *unspecified impurities*: for each impurity, maximum 0.05 per cent;
— *total*: maximum 0.2 per cent;
— *reporting threshold*: 0.03 per cent.

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C at a pressure not exceeding 0.7 kPa.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Mobile phase Mobile phase A, mobile phase B (45:55 *V/V*).

Injection Test solution (b) and reference solution (c).

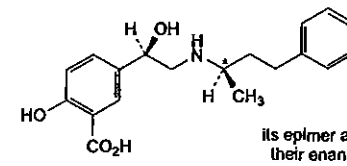
Run time Twice the retention time of labetalol.

Retention time Labetalol = about 2 min.

Calculate the percentage content of $C_{19}H_{25}ClN_2O_3$ taking into account the assigned content of *labetalol hydrochloride CRS*.

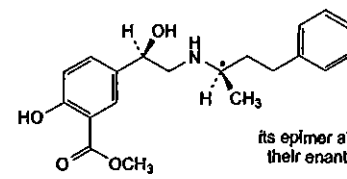
IMPURITIES

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, E, F, G.*



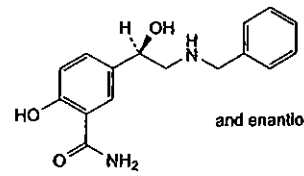
its epimer at C* and their enantiomers

A. mixture of 4 stereoisomers of 2-hydroxy-5-[(1*S*)-1-hydroxy-2-[[(2*S*)-4-phenylbutan-2-yl]amino]ethyl]benzoic acid,

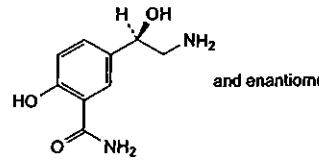


its epimer at C* and their enantiomers

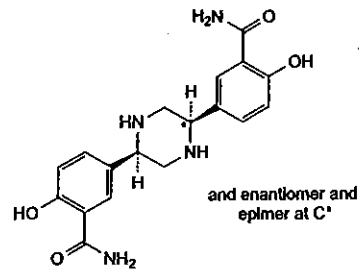
B. mixture of 4 stereoisomers of methyl 2-hydroxy-5-[(1*S*)-1-hydroxy-2-[[(2*S*)-4-phenylbutan-2-yl]amino]ethyl] benzoate,



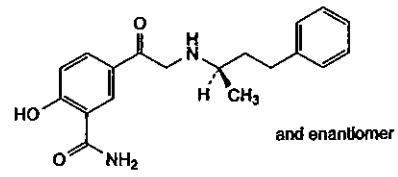
C. 5-[(1*RS*)-2-(benzylamino)-1-hydroxyethyl]-2-hydroxybenzamide,



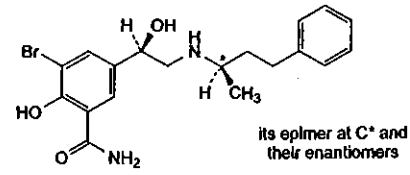
D. 5-[(1*RS*)-2-amino-1-hydroxyethyl]-2-hydroxybenzamide,



E. mixture of 3 stereoisomers of 5,5′-[(2*S*,5*S*)-piperazine-2,5-diyl]bis(2-hydroxybenzamide),



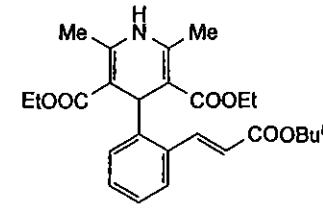
F. 2-hydroxy-5-[2-[[(2*RS*)-4-phenylbutan-2-yl]amino]acetyl] benzamide,



G. mixture of 4 stereoisomers of 3-bromo-2-hydroxy-5-[(1*S*)-1-hydroxy-2-[[(2*S*)-4-phenylbutan-2-yl]amino]ethyl] benzamide.

Ph Eur

Lacidipine



$C_{26}H_{33}NO_6$ 455.6 *103890-78-4*

Action and use
Calcium channel blocker.

Preparation
Lacidipine Tablets

DEFINITION
Lacidipine is diethyl (*E*)-4-{2-[(*tert*-butoxycarbonyl)vinyl] phenyl}-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate. It contains not less than 97.5% and not more than 102.0% of $C_{26}H_{33}NO_6$, calculated with reference to the anhydrous, propan-2-ol–free substance.

CHARACTERISTICS
A white to pale yellow crystalline powder. It melts at about 178°.

Practically insoluble in *water*; freely soluble in *acetone*; sparingly soluble in *absolute ethanol*.

Carry out all of the following procedures protected from light and prepare solutions immediately before use.

IDENTIFICATION
A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of Lacidipine (*RS 407*).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS
Propan-2-ol
Carry out the method for *gas chromatography*, Appendix III B. Prepare a 0.002% v/v solution of *1,4-dioxan* (internal standard) in *dimethylacetamide* (solution A).

(1) 0.002% v/v solution of *propan-2-ol* in solution A.

(2) 2% w/v of the substance being examined in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a glass column (60 m × 0.32 mm) bonded with a film (5 µm) of *polymethylsiloxane* (CP-Sil 5CB is suitable).

(b) Use *helium* as the carrier gas at 1.7 mL per minute.

(c) Use a temperature gradient as described below.

(d) Use an injection temperature of 170°.

(e) Use a detector temperature of 250°.

(f) Inject 1 µL of each solution.

Time (Minutes)	Temperature	Comment
0 - 1	60°	isothermal
1 - 18	60°→110°	linear increase 3°/minute
18 – 20	110°→200°	linear increase 50°/minute
20 – 27	200°	isothermal

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (1) shows two clearly separated peaks. The retention time for propan-2-ol is about 6.2 minutes and that for dioxan is about 15 minutes.

LIMITS

In the chromatogram obtained with solution (2):

the percentage content of propan-2-ol is not more than 0.5% w/w.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute 1 volume of a 0.1% w/v solution of the substance being examined in *absolute ethanol* to 5 volumes with the mobile phase.

(2) Dilute 1 volume of solution (1) to 500 volumes with the mobile phase.

(3) Dilute 1 volume of a 0.1% w/v solution of *lacidipine impurity standard BPCRS* in *absolute ethanol* to 5 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm x 4.6 mm) packed with *cyanosilyl silica gel for chromatography* (5 µm) (Spherisorb CN is suitable).

(b) Use isocratic elution using the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 240 nm.

(f) Inject 20 µL of each solution.

(g) If necessary adjust the composition of the mobile phase so that, in the chromatogram obtained with solution (3), the retention time of the peak due to lacidipine is about 10 minutes.

(h) For solution (1), allow the chromatography to proceed for 2 times the retention time of the principal peak.

MOBILE PHASE

3 volumes of *absolute ethanol* and 97 volumes of n-*hexane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the chromatogram supplied with *lacidipine impurity standard BPCRS*.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to lacidipine impurity B is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%, taking into account the correction factor of 0.5);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the total nominal content of impurities is not greater than 0.5%.

Water

Not more than 0.2% w/w, Appendix IX C. Use 0.5 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute 5 volumes of a 0.1% w/v solution of the substance being examined in *absolute ethanol* to 100 volumes with the mobile phase.

(2) Dilute 5 volumes of a 0.1% w/v solution of *lacidipine BPCRS* in *absolute ethanol* to 100 volumes with the mobile phase.

(3) Dilute 1 volume of a 0.1% w/v solution of *lacidipine impurity standard BPCRS* in *absolute ethanol* to 5 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic procedure described under Related substances may be used.

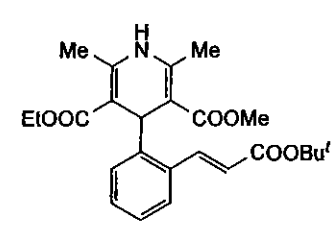
SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) closely resembles the corresponding chromatogram supplied with *lacidipine impurity standard BPCRS*.

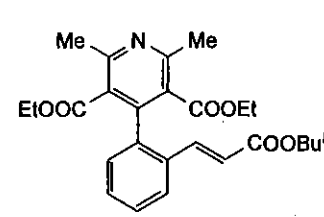
DETERMINATION OF CONTENT

Calculate the content of $C_{26}H_{33}NO_6$ from the chromatograms obtained and using the declared content of $C_{26}H_{33}NO_6$ in *lacidipine BPCRS*.

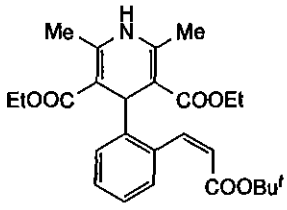
IMPURITIES



A. Ethyl methyl (*E*)-4-{2-[2-(*tert*-butoxycarbonyl)vinyl] phenyl}-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate,



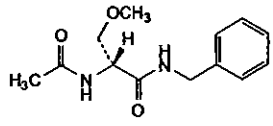
B. Diethyl (*E*)-4-{2-[2-(*tert*-butoxycarbonyl)vinyl]phenyl}-2,6-dimethylpyridine-3,5-dicarboxylate,



C. Diethyl (*Z*)-4-{2-[2-(*tert*-butoxycarbonyl)vinyl]phenyl}-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate.

Lacosamide

(Ph. Eur. monograph 2992)



$C_{13}H_{18}N_2O_3$ 250.3 *175481-36-4*

Action and use
Antiepileptic.

Preparations
Lacosamide Infusion
Lacosamide Oral Solution
Lacosamide Tablets

Ph Eur

DEFINITION
(2*R*)-2-Acetamido-*N*-benzyl-3-methoxypropanamide.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white or light yellow powder.

Solubility
Sparingly soluble in water, freely soluble in methanol, practically insoluble in heptane.

It shows polymorphism (*5.9*).

IDENTIFICATION
Carry out either tests A, B or tests B, C.

A. Specific optical rotation (*2.2.7*): + 14 to + 18 (anhydrous substance), measured at 25 °C.

Dissolve 0.100 g in *methanol R* and dilute to 10.0 mL with the same solvent.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *lacosamide CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

C. Enantiomeric purity (see Tests).

TESTS
Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 0.500 g in *water R* and dilute to 50.0 mL with the same solvent.

Enantiomeric purity
Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 1 mg of *lacosamide impurity A CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 20 mg of the substance to be examined in the mobile phase, add 1.0 mL of reference solution (a) and dilute to 20.0 mL with the mobile phase.

Reference solution (c) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 20.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *amylose derivative of silica gel for chiral separation R* (10 µm).

Mobile phase *water for chromatography R, 2-propanol R, heptane R* (3:100:900 *V/V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µL of the test solution and reference solutions (b) and (c).

Run time 1.7 times the retention time of lacosamide.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity A.

Relative retention With reference to lacosamide (retention time = about 25 min): impurity A = about 0.8.

System suitability Reference solution (b):
— *resolution*: minimum 3.0 between the peaks due to impurity A and lacosamide.

Limit:
— *impurity A*: maximum 0.15 per cent;
— *reporting threshold*: 0.05 per cent (reference solution (c)).

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in 1 mL of *methanol R* and dilute to 10.0 mL with *water R.*

Reference solution (a) Dissolve 50.0 mg of *lacosamide CRS* in 1 mL of *methanol R* and dilute to 10.0 mL with *water R.*

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with a mixture of 10 volumes of *methanol R* and 90 volumes of *water R.* Dilute 1.0 mL of this solution to 10.0 mL with the same mixture of solvents.

Reference solution (c) Dissolve 5 mg of *lacosamide for system suitability CRS* (containing impurities B, C, G and I) in 0.1 mL of *methanol R* and dilute to 1.0 mL with *water R.*

Reference solution (d) Dissolve 1 mg of *lacosamide impurity F CRS* in 2 mL of *methanol R* and dilute to 10.0 mL with *water R.* Dilute 1.0 mL of the solution to 10.0 mL with *water R.*

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped extra-dense bonded octylsilyl silica gel for chromatography R* (3.5 µm).

Mobile phase:
— *mobile phase A*: 0.1 per cent *V/V* solution of *trifluoroacetic acid R;*

— *mobile phase B*: *trifluoroacetic acid R, acetonitrile R, methanol R* (0.3:500:500 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	89	11
2 - 14.2	89 → 69	11 → 31
14.2 - 19.5	69 → 23	31 → 77
19.5 - 20	23 → 0	77 → 100
20 - 21	0	100

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 258 nm.

Injection 20 µL of the test solution and reference solutions (b), (c) and (d).

Identification of impurities Use the chromatogram supplied with *lacosamide for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities B, C, G and I; use the chromatogram obtained with reference solution (d) to identify the peak due to impurity F.

Relative retention With reference to lacosamide (retention time = about 12 min): impurity F = about 0.7; impurity G = about 0.9; impurity B = about 1.2; impurity C = about 1.3; impurity I = about 1.6.

System suitability Reference solution (c):
— *resolution*: minimum 2.0 between the peaks due to impurity G and lacosamide; minimum 4.5 between the peaks due to lacosamide and impurity B.

Calculation of percentage contents:
— *correction factors*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity G = 0.7; impurity I = 0.7;
— for each impurity, use the concentration of lacosamide in reference solution (b).

Limits:
— *impurities B, C, F, G, I*: for each impurity, maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.5 per cent;
— *reporting threshold*: 0.05 per cent.

Water (*2.5.32*)
Maximum 0.2 per cent, determined on 0.150 g by direct sample introduction.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (a).

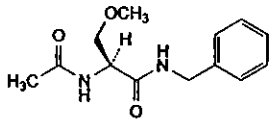
System suitability Reference solution (a):
— *symmetry factor*: maximum 2.4 for the peak due to lacosamide.

Calculate the percentage content of $C_{13}H_{18}N_2O_3$ taking into account the assigned content of *lacosamide CRS*.

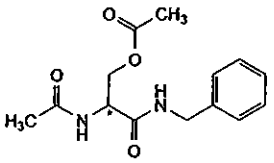
IMPURITIES

Specified impurities *A, B, C, F, G, I.*

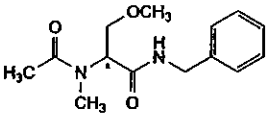
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *D, E, H, J, K.*



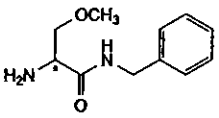
A. (2*S*)-2-acetamido-*N*-benzyl-3-methoxypropanamide,



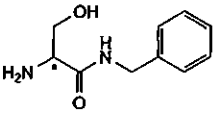
B. (2*S*)-2-acetamido-3-(benzylamino)-3-oxopropyl acetate,



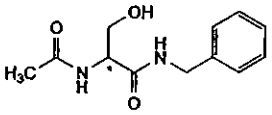
C. (2*S*)-*N*-benzyl-3-methoxy-2-(*N*-methylacetamido)propanamide,



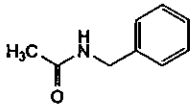
D. (2*S*)-2-amino-*N*-benzyl-3-methoxypropanamide,



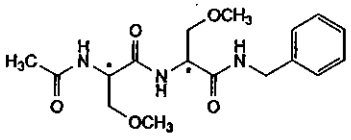
E. (2*S*)-2-amino-*N*-benzyl-3-hydroxypropanamide,



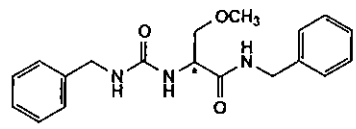
F. (2*S*)-2-acetamido-*N*-benzyl-3-hydroxypropanamide,



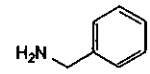
G. *N*-benzylacetamide,



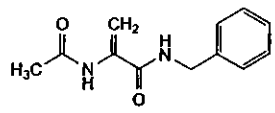
H. (2*S*)-2-acetamido-*N*-[(2*S*)-1-(benzylamino)-3-methoxy-1-oxopropan-2-yl]-3-methoxypropanamide,



I. (2*S*)-*N*-benzyl-2-[(benzylcarbamoyl)amino]-3-methoxypropanamide,



J. phenylmethanamine,

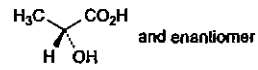


K. 2-acetamido-*N*-benzylprop-2-enamide.

Ph Eur

Lactic Acid

(Ph. Eur. monograph 0458)



$C_3H_6O_3$ 90.1

Preparations
Sodium Lactate Infusion
Compound Sodium Lactate Infusion
Lactic Acid Pessaries

Ph Eur

DEFINITION
Mixture of 2-hydroxypropanoic acid, its condensation products, such as lactoyl-lactic acid and polylactic acids, and water. The equilibrium between lactic acid and polylactic acids depends on the concentration and temperature. It is usually the racemate ((*RS*)-lactic acid).

Content
88.0 per cent *m/m* to 92.0 per cent *m/m* of $C_3H_6O_3$.

CHARACTERS
Appearance
Colourless or slightly yellow, syrupy liquid.

Solubility
Miscible with water and with ethanol (96 per cent).

IDENTIFICATION
A. Dissolve 1 g in 10 mL of *water R*. The solution is strongly acidic (*2.2.4*).

B. Relative density (*2.2.5*): 1.20 to 1.21.

C. It gives the reaction of lactates (*2.3.1*).

TESTS
Solution S
Dissolve 5.0 g in 42 mL of *1 M sodium hydroxide* and dilute to 50 mL with *distilled water R*.

Appearance
The substance to be examined is not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Ether-insoluble substances
Dissolve 1.0 g in 25 mL of *ether R*. The solution is not more opalescent than the solvent used for the test.

Sugars and other reducing substances
To 1 mL of solution S add 1 mL of *1 M hydrochloric acid*, heat to boiling, allow to cool and add 1.5 mL of *1 M sodium hydroxide* and 2 mL of *cupri-tartaric solution R*. Heat to boiling. No red or greenish precipitate is formed.

Methanol (*2.4.24*)
Maximum 50 ppm, if intended for use in the manufacture of parenteral preparations.

Citric, oxalic and phosphoric acids
To 5 mL of solution S add *dilute ammonia R1* until slightly alkaline (*2.2.4*). Add 1 mL of *calcium chloride solution R*. Heat on a water-bath for 5 min. Both before and after heating, any opalescence in the solution is not more intense than that in a mixture of 1 mL of *water R* and 5 mL of solution S.

Sulfates (*2.4.13*)
Maximum 200 ppm.
Dilute 7.5 mL of solution S to 15 mL with *distilled water R*.

Calcium (*2.4.3*)
Maximum 200 ppm.
Dilute 5 mL of solution S to 15 mL with *distilled water R*.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

Bacterial endotoxins (*2.6.14*)
Less than 5 IU/g, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins. Before use, neutralise the test solution to pH 7.0-7.5 with *strong sodium hydroxide solution R* and shake vigorously.

ASSAY
Place 1.000 g in a ground-glass-stoppered flask and add 10 mL of *water R* and 20.0 mL of *1 M sodium hydroxide*. Close the flask and allow to stand for 30 min. Using 0.5 mL of *phenolphthalein solution R* as indicator, titrate with *1 M hydrochloric acid* until the pink colour is discharged.

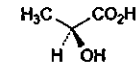
1 mL of *1 M sodium hydroxide* is equivalent to 90.1 mg of $C_3H_6O_3$.

LABELLING
The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

Ph Eur

(*S*)-Lactic Acid

(Ph. Eur. monograph 1771)



$C_3H_6O_3$ 90.1

Ph Eur

DEFINITION
Mixture of (*S*)-2-hydroxypropanoic acid, its condensation products, such as lactoyl-lactic acid and polylactic acids, and water. The equilibrium between lactic acid and polylactic acids depends on the concentration and temperature.

Content
88.0 per cent *m/m* to 92.0 per cent *m/m* of $C_3H_6O_3$, not less than 95.0 per cent of which is the (*S*)-enantiomer.

CHARACTERS

Appearance
Colourless or slightly yellow, syrupy liquid.

Solubility
Miscible with water and with ethanol (96 per cent).

IDENTIFICATION
A. Dissolve 1 g in 10 mL of *water R*. The solution is strongly acidic (*2.2.4*).

B. Relative density (*2.2.5*): 1.20 to 1.21.

C. It gives the reaction of lactates (*2.3.1*).

D. It complies with the limits of the assay.

TESTS

Solution S
Dissolve 5.0 g in 42 mL of *1 M sodium hydroxide* and dilute to 50 mL with *distilled water R*.

Appearance
The substance to be examined is not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Ether-insoluble substances
Dissolve 1.0 g in 25 mL of *ether R*. The solution is not more opalescent than the solvent used for the test.

Sugars and other reducing substances
To 1 mL of solution S add 1 mL of *1 M hydrochloric acid*, heat to boiling, allow to cool and add 1.5 mL of *1 M sodium hydroxide* and 2 mL of *cupri-tartaric solution R*. Heat to boiling. No red or greenish precipitate is formed.

Methanol (*2.4.24*)
Maximum 50 ppm, if intended for use in the manufacture of parenteral preparations.

Citric, oxalic and phosphoric acids
To 5 mL of solution S add *dilute ammonia R1* until slightly alkaline (*2.2.4*). Add 1 mL of *calcium chloride solution R*. Heat on a water-bath for 5 min. Both before and after heating, any opalescence in the solution is not more intense than that in a mixture of 1 mL of *water R* and 5 mL of solution S.

Sulfates (*2.4.13*)
Maximum 200 ppm.

Dilute 7.5 mL of solution S to 15 mL with *distilled water R*.

Calcium (*2.4.3*)
Maximum 200 ppm.

Dilute 5 mL of solution S to 15 mL with *distilled water R*.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

Bacterial endotoxins (*2.6.14*)
Less than 5 IU/g if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins. Before use, neutralise the test solution to pH 7.0-7.5 with *strong sodium hydroxide solution R* and shake vigorously.

ASSAY
Place 1.000 g in a ground-glass-stoppered flask and add 10 mL of *water R* and 20.0 mL of *1 M sodium hydroxide*. Close the flask and allow to stand for 30 min. Using 0.5 mL of *phenolphthalein solution R* as indicator, titrate with *1 M hydrochloric acid* until the pink colour is discharged.

1 mL of *1 M sodium hydroxide* is equivalent to 90.1 mg of $C_3H_6O_3$.

(*S*)-enantiomer
Transfer an amount of the substance to be examined equivalent to 2.00 g of lactic acid into a round-bottomed flask, add 25 mL of *1 M sodium hydroxide* and boil gently for 15 min. Cool down and adjust to pH 7.0 using *1 M hydrochloric acid*. Add 5.0 g of *ammonium molybdate R*, dissolve and dilute to 50.0 mL with *water R*. Filter and measure the angle of optical rotation (*2.2.7*). Calculate the percentage content of (*S*)-enantiomer using the expression:

$$50 + \left(24.18 \times \alpha \times \frac{2.222}{m} \times \frac{90}{c}\right)$$

α = angle of optical rotation (absolute value),
m = mass of the substance to be examined, in grams,
c = percentage content of $C_3H_6O_3$ in the substance to be examined.

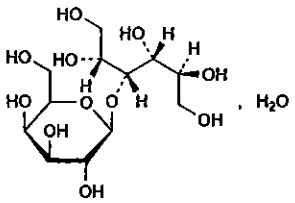
The complex of (*S*)-lactic acid formed under these test conditions is laevorotatory.

LABELLING
The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

Ph Eur

Lactitol Monohydrate

(Ph. Eur. monograph 1337)



$C_{12}H_{24}O_{11},H_2O$ 362.3 *81025-04-9*

Action and use
Osmotic laxative.

Ph Eur

DEFINITION
4-*O*-β-D-Galactopyranosyl-D-glucitol monohydrate.

Content
96.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Very soluble in water, slightly soluble to very slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION
First identification: B.
Second identification: A, C.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison lactitol monohydrate CRS.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 50 mg of the substance to be examined in *methanol R* and dilute to 20 mL with the same solvent.

Reference solution (a) Dissolve 5 mg of *lactitol monohydrate CRS* in *methanol R* and dilute to 2 mL with the same solvent.

Reference solution (b) Dissolve 2.5 mg of *sorbitol CRS* (impurity E) in 1 mL of reference solution (a) and dilute to 10 mL with *methanol R*.

Plate *TLC silica gel G plate R.*

Mobile phase *water R, acetonitrile R* (25:75 *V/V*).

Application 2 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *4-aminobenzoic acid solution R* and dry in a current of cold air until the acetone is removed; heat at 100 °C for 15 min and allow to cool; spray with a 2 g/L solution of *sodium periodate R* and dry in a current of cold air; heat at 100 °C for 15 min.

System suitability The chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Solution S

Dissolve 5.000 g in *carbon dioxide-free water R* and dilute to 50.0 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_7 (*2.2.2, Method II*).

Acidity or alkalinity

To 10 mL of solution S add 10 mL of *carbon dioxide-free water R*. To 10 mL of this solution add 0.05 mL of *phenolphthalein solution R*. Not more than 0.2 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink. To a further 10 mL of the solution add 0.05 mL of *methyl red solution R*. Not more than 0.3 mL of *0.01 M hydrochloric acid* is required to change the colour of the indicator to red.

Specific optical rotation (*2.2.7*)

+ 13.5 to + 15.5 (anhydrous substance), determined on solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 10.0 mL with the same solvent.

Test solution (b) Dilute 2.0 mL of test solution (a) to 50.0 mL with *water R*.

Reference solution (a) Dissolve 5.0 mg of *lactitol monohydrate CRS* and 5 mg of *glycerol R* in *water R* and dilute to 25.0 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of test solution (a) to 100.0 mL with *water R*. Dilute 5.0 mL of this solution to 100.0 mL with *water R*.

Reference solution (c) Dilute 2.5 mL of reference solution (a) to 10.0 mL with *water R*.

Column:
- *size*: *l* = 0.30 m, Ø = 7.8 mm;
- *stationary phase*: *strong cation-exchange resin (calcium form) R*;
- *temperature*: 60 °C.

Mobile phase *water for chromatography R.*

Flow rate 0.6 mL/min.

Detection Differential refractometer maintained at a constant temperature (e.g. 35 °C).

Injection 100 µL; inject test solution (a) and reference solutions (b) and (c).

Run time 2.5 times the retention time of lactitol.

Relative retention With reference to lactitol (retention time = about 13 min): impurity A = about 0.7; impurity B = about 0.8; glycerol = about 1.3; impurity C = about 1.5; impurity D = about 1.8; impurity E = about 1.9.

System suitability Reference solution (c):
- *resolution*: minimum 5.0 between the peaks due to lactitol and glycerol.

Limits:
- *impurity B*: not more than the area of the peak due to lactitol in the chromatogram obtained with reference solution (c) (1.0 per cent);
- *total of other impurities*: not more than the area of the peak due to lactitol in the chromatogram obtained with reference solution (c) (1.0 per cent);
- *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Reducing sugars

Maximum 0.2 per cent.

Dissolve 5.0 g in 3 mL of *water R* with gentle heating. Cool and add 20 mL of *cupri-citric solution R* and a few glass beads. Heat so that boiling begins after 4 min and maintain boiling for 3 min. Cool rapidly and add 100 mL of a 2.4 per cent *V/V* solution of *glacial acetic acid R* and 20.0 mL of *0.025 M iodine*. With continuous shaking, add 25 mL of a mixture of 6 volumes of *hydrochloric acid R* and 94 volumes of *water R*. When the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulfate* using 1 mL of *starch solution R* added towards the end of the titration, as indicator. Not less than 12.8 mL of *0.05 M sodium thiosulfate* is required.

Water (*2.5.12*)

4.5 per cent to 5.5 per cent, determined on 0.300 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

Microbial contamination

TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*).

TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

Absence of *Escherichia coli* (*2.6.13*).

Absence of *Salmonella* (*2.6.13*).

Absence of *Pseudomonas aeruginosa* (*2.6.13*).

ASSAY

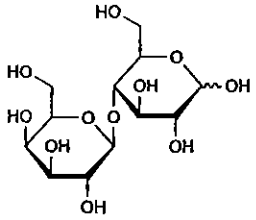
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (a).

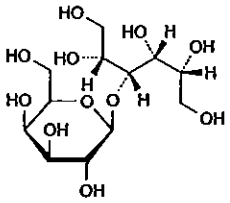
Calculate the percentage content of $C_{12}H_{24}O_{11}$ taking into account the assigned content of *lactitol monohydrate CRS*.

IMPURITIES

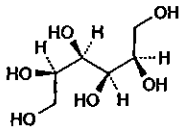
Specified impurities *A, B, C, D, E.*



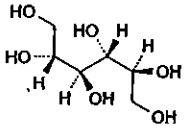
A. 4-*O*-β-D-galactopyranosyl-D-glucopyranose (lactose),



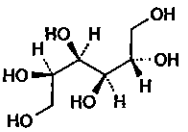
B. 3-*O*-β-D-galactopyranosyl-D-mannitol (lactulitol),



C. D-mannitol,



D. galactitol (dulcitol),

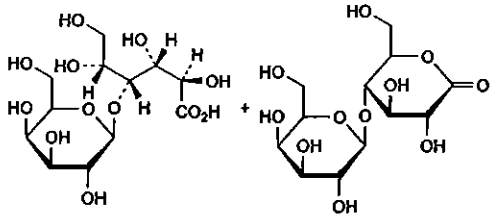


E. D-glucitol (D-sorbitol).

Ph Eur

Lactobionic Acid

(Ph. Eur. monograph 1647)



$C_{12}H_{22}O_{12}$ (acid form)	358.3	*96-82-2*
$C_{12}H_{20}O_{11}$ (δ-lactone)	340.3	*5965-65-1*

Ph Eur

DEFINITION

Mixture in variable proportions of 4-*O*-β-D-galactopyranosyl-D-gluconic acid and 4-*O*-β-D-galactopyranosyl-D-glucono-1,5-lactone.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Freely soluble in water, slightly soluble in glacial acetic acid, in anhydrous ethanol and in methanol.

mp

About 125 °C with decomposition.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *lactobionic acid CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *water R*, dry at 105 °C and record new spectra using the residues.

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *water R* and dilute to 1 mL with the same solvent.

Reference solution Dissolve 10 mg of *lactobionic acid CRS* in *water R* and dilute to 1 mL with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *concentrated ammonia R1, ethyl acetate R, water R, methanol R* (2:2:2:4 *V/V/V/V*).

Application 5 µL.

Development Over 3/4 of the plate.

Detection Spray 3 times with *ammonium molybdate solution R6* and heat in an oven at 110 °C for 15 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and colour to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5 (*2.2.2, Method II*).

Dissolve 3.0 g in 25 mL of *water R*.

Specific optical rotation (*2.2.7*)

+ 23.0 to + 29.0 (anhydrous substance).

Dissolve 1.0 g in 80 mL of *water R* and dilute to 100.0 mL with the same solvent. Allow to stand for 24 h.

Reducing sugars

Maximum 0.2 per cent, calculated as glucose.

Dissolve 5.0 g in 25 mL of *water R* with the aid of gentle heat. Cool and add 20 mL of *cupri-citric solution R* and a few glass beads. Heat so that boiling begins after 4 min and maintain boiling for 3 min. Cool rapidly and add 100 mL of a 2.4 per cent *V/V* solution of *glacial acetic acid R* and 20.0 mL of *0.025 M iodine*. With continuous shaking, add 25 mL of a mixture of 6 volumes of *hydrochloric acid R* and 94 volumes of *water R* and, when the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulfate* using 1 mL of *starch solution R*, added towards the end of the titration, as indicator. Not less than 12.8 mL of *0.05 M sodium thiosulfate* is required.

Water (*2.5.12*)

Maximum 5.0 per cent, determined on 0.50 g.

Use a mixture of 1 volume of *formamide R* and 2 volumes of *methanol R* as solvent.

Total ash (*2.4.16*)
Maximum 0.2 per cent.

ASSAY
Dissolve 0.350 g in 50 mL of *carbon dioxide-free water R*, previously heated to 30 °C. Immediately titrate with *0.1 M sodium hydroxide* and determine the 2 equivalence points potentiometrically (*2.2.20*).

The first equivalence point (V_1) corresponds to the acid form of lactobionic acid and the second equivalence point ($V_2 - V_1$) corresponds to the δ-lactone form.

1 mL of *0.1 M sodium hydroxide* is equivalent to 35.83 mg of $C_{12}H_{22}O_{12}$.

1 mL of *0.1 M sodium hydroxide* is equivalent to 34.03 mg of $C_{12}H_{20}O_{11}$.

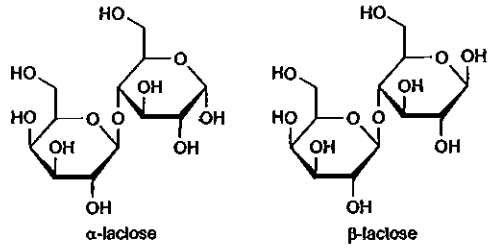
The sum of the 2 results is expressed as a percentage content of lactobionic acid.

Ph Eur

Lactose[1]

Anhydrous Lactose

(Ph. Eur. monograph 1061)



$C_{12}H_{22}O_{11}$ 342.3 *63-42-3*

Action and use
Excipient.

Ph Eur

DEFINITION
O-β-D-Galactopyranosyl-(1→4)-β-D-glucopyranose or mixture of *O*-β-D-galactopyranosyl-(1→4)-α-D-glucopyranose and *O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranose.

♦CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water, practically insoluble in ethanol (96 per cent).♦

IDENTIFICATION
First identification: A, ◊D.

Second identification: B, C, D◊

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison anhydrous lactose CRS.

◊B. Thin-layer chromatography (*2.2.27*).

Solvent mixture water R, methanol R (40:60 *V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 20 mL with the solvent mixture.

Reference solution (a) Dissolve 10 mg of *anhydrous lactose CRS* in the solvent mixture and dilute to 20 mL with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *fructose R*, 10 mg of *glucose R*, 10 mg of lactose monohydrate R and 10 mg of *sucrose R* in the solvent mixture and dilute to 20 mL with the solvent mixture.

Plate TLC silica gel plate R.

Mobile phase water R, methanol R, glacial acetic acid R, ethylene chloride R (10:15:25:50 *V/V/V/V*); measure the volumes accurately, as a slight excess of water produces cloudiness.

Application 2 µL; thoroughly dry the points of application.

Development A Over 3/4 of the plate.

Drying A In a current of warm air.

Development B Immediately, over 3/4 of the plate, after renewing the mobile phase.

Drying B In a current of warm air.

Detection Spray with a solution of 0.5 g of *thymol R* in a mixture of 5 mL of *sulfuric acid R* and 95 mL of *ethanol (96 per cent) R*; heat at 130 °C for 10 min.

System suitability Reference solution (b):
— the chromatogram shows 4 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 0.25 g in 5 mL of *water R*. Add 5 mL of *ammonia R* and heat in a water-bath at 80 °C for 10 min. A red colour develops.

D. Water (see Tests).◊

TESTS
Solution S
Dissolve 1.0 g in boiling *water R*, allow to cool and dilute to 10.0 mL with *water R*.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_7 (*2.2.2, Method II*).

Acidity or alkalinity
Dissolve 6.0 g by heating in 25 mL of *carbon dioxide-free water R*, cool and add 0.3 mL of *phenolphthalein solution R1*. The solution is colourless. Not more than 0.4 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink or red.

Specific optical rotation (*2.2.7*)
+ 54.4 to + 55.9 (anhydrous substance).

Dissolve 10.0 g in 80 mL of *water R* with heating at 50 °C. Allow to cool and add 0.2 mL of *dilute ammonia R1*. Allow to stand for 30 min and dilute to 100.0 mL with *water R*.

Absorbance: proteins and light-absorbing impurities (*2.2.25*)
Test solution (a) Solution S.

Test solution (b) Dilute 1.0 mL of test solution (a) to 10.0 mL with *water R*.

Spectral range 400 nm for test solution (a) and 210-300 nm for test solution (b).

Results:
— at 400 nm: maximum 0.04 for test solution (a);

[1] *This monograph has undergone pharmacopoeial harmonisation. See chapter 5.8. Pharmacopoeial harmonisation.*

— from 210 nm to 220 nm: maximum 0.25 for test solution (b);
— from 270 nm to 300 nm: maximum 0.07 for test solution (b).

Water (*2.5.12*)
Maximum 1.0 per cent, determined on 1.00 g, using a mixture of 1 volume of *formamide R* and 2 volumes of *methanol R* as solvent.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

Microbial contamination
TAMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

Absence of *Escherichia coli* (*2.6.13*).

◊FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for lactose used as filler/diluent in solid dosage forms (compressed and powder).

Particle-size distribution (*2.9.31* or *2.9.38*)

Bulk and tapped density (*2.9.34*)
Determine the bulk density and the tapped density. Calculate the Hausner ratio.

α-Lactose and β-lactose
Gas chromatography (*2.2.28*).

Silylation reagent *dimethyl sulfoxide R, N-trimethylsilylimidazole R, pyridine R* (19.5:22:58.5 *V/V/V*).

Test solution Introduce 10 mg of the substance to be examined into a vial with a screw cap and add 4 mL of the silylation reagent. Sonicate for 20 min at room temperature, allow to cool and transfer 400 µL to an injection vial. Add 1 mL of *pyridine R*, close the vial and mix well.

Reference solution Prepare a mixture of *α-lactose monohydrate R* and *β-lactose R* to obtain an anomeric ratio of about 1:1 based on the labelled anomeric contents of the α-lactose monohydrate and the β-lactose. Introduce 10 mg of the mixture into a vial with a screw cap and add 4 mL of the silylation reagent. Sonicate for 20 min at room temperature, allow to cool, and transfer 400 µL to an injection vial. Add 1 mL of *pyridine R*, close the vial and mix well.

Precolumn:
— *material*: intermediate-polarity deactivated fused silica;
— *size*: *l* = 2 m, Ø = 0.53 mm.

Column:
— *material*: fused silica;
— *size*: *l* = 15 m, Ø = 0.25 mm;
— *stationary phase*: *phenyl(5)methyl(95)polysiloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R.*

Flow rate 2.8 mL/min.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 1	80
	1 - 3	80 → 150
	3 - 15.5	150 → 300
	15.5 - 17.5	300
Injection port		275 or use cold on-column injection
Detector		325

Detection Flame ionisation.

Injection 0.5 µL, splitless or by cold on-column injection.

Relative retention With reference to β-lactose (retention time = about 12 min): α-lactose = about 0.9.

System suitability Reference solution:
— *resolution*: minimum 3.0 between the peaks due to α-lactose and β-lactose.

Calculate the percentage content of α-lactose using the following expression:

$$\frac{100 S_a}{S_a + S_b}$$

Calculate the percentage content of β-lactose using the following expression:

$$\frac{100 S_b}{S_a + S_b}$$

S_a = area of the peak due to α-lactose;
S_b = area of the peak due to β-lactose.

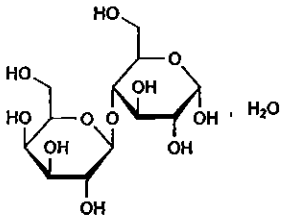
Loss on drying (*2.2.32*)
Determine on 1.000 g by drying in an oven at 80 °C for 2 h. ◊

Ph Eur

Lactose Monohydrate[1]

(Ph. Eur. monograph 0187)

NOTE: The name Lactose was formerly used in the United Kingdom.



$C_{12}H_{22}O_{11},H_2O$ 360.3

Action and use
Excipient.

Ph Eur

DEFINITION
O-β-D-Galactopyranosyl-(1→4)-α-D-glucopyranose monohydrate.

[1] *This monograph has undergone pharmacopoeial harmonisation. See chapter 5.8 Pharmacopoeial harmonisation.*

♦**CHARACTERS**

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, practically insoluble in ethanol (96 per cent).♦

IDENTIFICATION

First identification: A, ◊*D.*

Second identification: B, C, D◊

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *lactose monohydrate CRS.*

◊B. Thin-layer chromatography (*2.2.27*).

Solvent mixture *water R, methanol R* (40:60 *V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 20 mL with the solvent mixture.

Reference solution (a) Dissolve 10 mg of *lactose monohydrate CRS* in the solvent mixture and dilute to 20 mL with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *fructose R*, 10 mg of *glucose R*, 10 mg of *lactose monohydrate R* and 10 mg of *sucrose R* in the solvent mixture and dilute to 20 mL with the solvent mixture.

Plate *TLC silica gel plate R.*

Mobile phase *water R, methanol R, glacial acetic acid R, ethylene chloride R* (10:15:25:50 *V/V/V/V*); measure the volumes accurately, as a slight excess of water produces cloudiness.

Application 2 µL; thoroughly dry the points of application.

Development A Over 3/4 of the plate.

Drying A In a current of warm air.

Development B Immediately, over 3/4 of the plate, after renewing the mobile phase.

Drying B In a current of warm air.

Detection Spray with a solution of 0.5 g of *thymol R* in a mixture of 5 mL of *sulfuric acid R* and 95 mL of *ethanol (96 per cent) R*; heat at 130 °C for 10 min.

System suitability Reference solution (b):

— the chromatogram shows 4 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 0.25 g in 5 mL of *water R*. Add 5 mL of *ammonia R* and heat in a water-bath at 80 °C for 10 min. A red colour develops.

D. Water (see Tests).◊

TESTS

Solution S

Dissolve 1.0 g in boiling *water R*, allow to cool and dilute to 10.0 mL with *water R*.

Appearance of solution

Solution S is clear (*2.2.1*) ◊and not more intensely coloured than reference solution BY_7 (*2.2.2, Method II*)◊.

Acidity or alkalinity

Dissolve 6.0 g by heating in 25 mL of *carbon dioxide-free water R*, cool and add 0.3 mL of *phenolphthalein solution R1*. The solution is colourless. Not more than 0.4 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink or red.

Specific optical rotation (*2.2.7*)

+ 54.4 to + 55.9 (anhydrous substance).

Dissolve 10.0 g in 80 mL of *water R* with heating at 50 °C. Allow to cool and add 0.2 mL of *dilute ammonia R1*. Allow to stand for 30 min and dilute to 100.0 mL with *water R*.

Absorbance: proteins and light-absorbing impurities (*2.2.25*)

Test solution (a) Solution S.

Test solution (b) Dilute 1.0 mL of test solution (a) to 10.0 mL with *water R*.

Spectral range 400 nm for test solution (a) and 210-300 nm for test solution (b).

Results:

— at 400 nm: maximum 0.04 for test solution (a);

— from 210 nm to 220 nm: maximum 0.25 for test solution (b);

— from 270 nm to 300 nm: maximum 0.07 for test solution (b).

Water (*2.5.12*)

4.5 per cent to 5.5 per cent, determined on 0.50 g, using a mixture of 1 volume of *formamide R* and 2 volumes of *methanol R* as solvent.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

Microbial contamination

TAMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

Absence of *Escherichia coli* (*2.6.13*).

◊**FUNCTIONALITY-RELATED CHARACTERISTICS**

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for lactose monohydrate used as a filler/diluent in solid dosage forms (compressed and powder).

Particle size distribution (*2.9.31* or *2.9.38*)

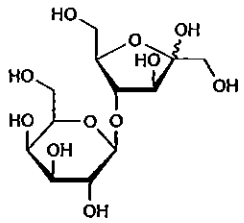
Bulk and tapped density (*2.9.34*)

Determine the bulk density and the tapped density. Calculate the Hausner ratio.◊

Ph Eur

Lactulose

(Ph. Eur. monograph 1230)



$C_{12}H_{22}O_{11}$ 342.3 *4618-18-2*

Action and use
Osmotic laxative.

Preparation
Lactulose Oral Powder

Ph Eur

DEFINITION

4-*O*-β-D-Galactopyranosyl-D-*arabino*-hex-2-ulofuranose.

Content
95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water, sparingly soluble in methanol, practically insoluble in toluene.

mp
About 168 °C.

IDENTIFICATION

First identification: B, C, D, E.

Second identification: A, C, D, E.

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 10.0 mL with the same solvent.

Reference solution Dissolve 50.0 mg of *lactulose CRS* in *water R* and dilute to 10.0 mL with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *glacial acetic acid R*, 50 g/L solution of *boric acid R*, *methanol R*, *ethyl acetate R* (10:15:20:55 *V/V/V/V*).

Application 2 µL.

Development Over 3/4 of the plate.

Drying At 100-105 °C for 5 min; allow to cool.

Detection Spray with a 1.0 g/L solution of *1,3-dihydroxynaphthalene R* in a mixture of 10 volumes of *sulfuric acid R* and 90 volumes of *methanol R*; heat at 110 °C for 5 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (b).

C. Dissolve 50 mg in 10 mL of *water R*. Add 3 mL of *cupri-tartaric solution R* and heat. A red precipitate is formed.

D. Dissolve 0.125 g in 5 mL of *water R*. Add 5 mL of *ammonia R*. Heat in a water-bath at 80 °C for 10 min. A red colour develops.

E. Specific optical rotation (see Tests).

TESTS

Solution S
Dissolve 3.0 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

pH (*2.2.3*)
3.0 to 7.0.

To 10 mL of solution S add 0.1 mL of a saturated solution of *potassium chloride R*.

Specific optical rotation (*2.2.7*)
−50.0 to −46.0 (anhydrous substance).

Dissolve 1.25 g in *water R*, add 0.2 mL of *concentrated ammonia R* and dilute to 25.0 mL with *water R*.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 1.00 g of the substance to be examined in 10 mL of *water R*. Add 12.5 mL of *acetonitrile R* with gentle heating and dilute to 25.0 mL with *water R*.

Reference solution (a) To 3.0 mL of the test solution add 48.5 mL of *acetonitrile R* with gentle heating and dilute to 100.0 mL with *water R*.

Reference solution (b) Dissolve 1.00 g of *lactulose CRS* in 10 mL of *water R*. Add 12.5 mL of *acetonitrile R* with gentle heating and dilute to 25.0 mL with *water R*.

Reference solution (c) Dissolve 10 mg of *lactulose R*, 10 mg of *epilactose R* (impurity A) and 10 mg of *lactose monohydrate R* (impurity C) in 2 mL of *water R*. Add 2.5 mL of *acetonitrile R* with gentle heating and dilute to 5 mL with *water R*.

Reference solution (d) To 5.0 mL of the test solution add 47.5 mL of *acetonitrile R* with gentle heating and dilute to 100.0 mL with *water R*. Dilute 5.0 mL of this solution to 100.0 mL with a mixture of equal volumes of *acetonitrile R* and *water R*.

Column 1:
— *size*: *l* = 0.05 m, Ø = 4.6 mm;
— *stationary phase*: *aminopropylsilyl silica gel for chromatography R* (3 µm);
— *temperature*: 38 ± 1 °C.

Column 2:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *aminopropylsilyl silica gel for chromatography R* (3 µm);
— *temperature*: 38 ± 1 °C.

Columns 1 and 2 are coupled in series.

Mobile phase Dissolve 0.253 g of *sodium dihydrogen phosphate R* in 200 mL of *water for chromatography R* and dilute to 1000 mL with *acetonitrile R*.

Flow rate 1.0 mL/min.

Detection Differential refractometer maintained at a constant temperature (e.g. 35 °C).

Injection 20 µL of the test solution and reference solutions (a), (c) and (d).

Run time Twice the retention time of lactulose.

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A and C.

Relative retention With reference to lactulose (retention time = about 18 min): impurity A = about 0.9; impurity C = about 1.2.

System suitability Reference solution (c):
— *peak-to-valley ratio*: minimum 5.0, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to lactulose.

Limits:
— *impurity C*: not more than the area of the peak due to lactulose in the chromatogram obtained with reference solution (a) (3.0 per cent);
— *unspecified impurities*: for each impurity, not more than twice the area of the peak due to lactulose in the chromatogram obtained with reference solution (d) (0.5 per cent);
— *total*: not more than the area of the peak due to lactulose in the chromatogram obtained with reference solution (a) (3.0 per cent);
— *disregard limit*: the area of the peak due to lactulose in the chromatogram obtained with reference solution (d) (0.25 per cent).

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Methanol

Head-space gas chromatography (*2.2.28*).

Internal standard solution Mix 0.5 mL of *propanol R* and 100.0 mL of *water R*. Dilute 1.0 mL of the solution to 100.0 mL with *water R*. Dilute 5.0 mL of this solution to 50.0 mL with *water R*.

Test solution To 79 mg of the substance to be examined in a 20 mL vial add 1.0 mL of the internal standard solution and 5 µL of a 0.1 per cent *V/V* solution of *methanol R*.

Reference solution To 1.0 mL of the internal standard solution in a 20 mL vial add 5 µL of a 0.1 per cent *V/V* solution of *methanol R*.

Column:
— *size*: l = 2 m, Ø = 2 mm;
— *stationary phase*: *ethylvinylbenzene-divinylbenzene copolymer R* (180 µm).

Carrier gas *helium for chromatography R*.

Flow rate 30 mL/min.

Static head-space conditions that may be used:
— *equilibration temperature*: 60 °C;
— *equilibration time*: 1 h;
— *pressurisation time*: 1 min.

Temperature:
— *column*: 140 °C;
— *injection port*: 200 °C;
— *detector*: 220 °C.

Detection Flame ionisation.

Injection 1 mL of the gaseous phase.

Calculate the content of methanol, taking its density (*2.2.5*) at 20 °C to be 0.79 g/mL.

Limit:
— *methanol*: calculate the ratio (R) of the area of the peak due to methanol to the area of the peak due to the internal standard in the chromatogram obtained with the reference solution; calculate the ratio of the area of the peak due to methanol to the area of the peak due to the internal standard in the chromatogram obtained with the test solution: this ratio is not greater than $2R$ (50 ppm).

Boron

Maximum 9 ppm.

Avoid where possible the use of glassware.

Reference solution Dissolve 50.0 mg of *boric acid R* in *water R* and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of the solution to 100.0 mL with *water R*. *Keep in a well-closed polyethylene container.*

In 4 polyethylene 25 mL flasks, place separately:
— 0.50 g of the substance to be examined dissolved in 2.0 mL of *water R* (solution A);
— 0.50 g of the substance to be examined dissolved in 1.0 mL of the reference solution and 1.0 mL of *water R* (solution B);
— 1.0 mL of the reference solution and 1.0 mL of *water R* (solution C);
— 2.0 mL of *water R* (solution D).

To each flask add 4.0 mL of *acetate-edetate buffer solution pH 5.5 R*. Mix and add 4.0 mL of freshly prepared *azomethine H solution R*. Mix and allow to stand for 1 h. Measure the absorbance (*2.2.25*) of solutions A, B and C at 420 nm, using solution D as the compensation liquid. The test is not valid unless the absorbance of solution C is at least 0.25. The absorbance of solution B is not less than twice that of solution A.

Water (*2.5.12*)

Maximum 2.5 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

Microbial contamination

TAMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

Absence of *Escherichia coli* (*2.6.13*).

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (b).

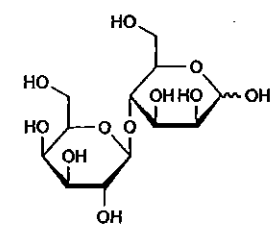
System suitability Reference solution (b):
— *symmetry factor*: 0.6 to 2.0 for the principal peak.

Calculate the percentage content of $C_{12}H_{22}O_{11}$ taking into account the assigned content of *lactulose CRS*.

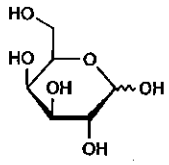
IMPURITIES

Specified impurities *C*.

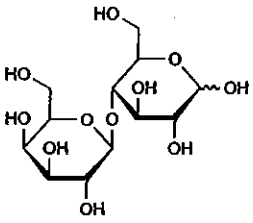
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, D, E.*



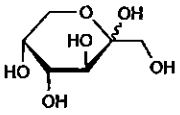
A. 4-*O*-β-D-galactopyranosyl-D-mannopyranose (epilactose),



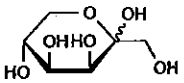
B. D-galactopyranose (galactose),



C. 4-*O*-β-D-galactopyranosyl-D-glucopyranose (lactose),



D. D-*arabino*-hex-2-ulopyranose (fructose),



E. D-*lyxo*-hex-2-ulopyranose (tagatose).

Ph Eur

Lactulose Solution

(Liquid Lactulose, Ph. Eur. monograph 0924)

Action and use
Osmotic laxative.

Ph Eur

DEFINITION

Aqueous solution of 4-*O*-β-D-galactopyranosyl-D-*arabino*-hex-2-ulofuranose normally prepared by alkaline isomerisation of lactose. It may contain other sugars including lactose, epilactose, galactose, tagatose and fructose.

Content
Minimum 620 g/L of lactulose ($C_{12}H_{22}O_{11}$; M_r 342.3) and 95.0 per cent to 105.0 per cent of the content of lactulose stated on the label.

It may contain a suitable antimicrobial preservative.

CHARACTERS

Appearance
Clear, viscous liquid, colourless or pale brownish-yellow.

Solubility
Miscible with water. It may be a supersaturated solution or may contain crystals that disappear on heating.

A 10 per cent *V/V* solution is laevorotatory.

IDENTIFICATION

First identification: B, C, D.

Second identification: A, C, D.

A. Thin-layer chromatography (*2.2.27*).

Test solution Dilute 0.50 g of the substance to be examined in *water R* and dilute to 50.0 mL with the same solvent.

Reference solution Dissolve 60.0 mg of *lactulose CRS* in *water R* and dilute to 10.0 mL with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *glacial acetic acid R*, 50 g/L solution of *boric acid R*, *methanol R*, *ethyl acetate R* (10:15:20:55 *V/V/V/V*).

Application 2 µL.

Development Over 3/4 of the plate.

Drying At 100-105 °C for 5 min and allow to cool.

Detection Spray with a 1.0 g/L solution of *1,3-dihydroxynaphthalene R* in a mixture of 10 volumes of *sulfuric acid R* and 90 volumes of *methanol R*; heat at 110 °C for 5 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (b).

C. To 0.1 g add 10 mL of *water R* and 3 mL of *cupri-tartaric solution R* and heat. A red precipitate is formed.

D. To 0.25 g add 5 mL of *water R* and 5 mL of *ammonia R*. Heat in a water-bath at 80 °C for 10 min. A red colour develops.

TESTS

Solution S
Mix 10 g with *carbon dioxide-free water R* and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

pH (*2.2.3*)
3.0 to 7.0.

To 10 mL of solution S add 0.1 mL of a saturated solution of *potassium chloride R*.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Mix 4.00 g of the substance to be examined and 20 mL of *water R*. Add 25.0 mL of *acetonitrile R* with gentle heating and dilute to 50.0 mL with *water R*.

Reference solution (a) To 5.0 mL of the test solution add 47.5 mL of *acetonitrile R* with gentle heating and dilute to 100.0 mL with *water R*.

Reference solution (b) Dissolve 2.00 g of *lactulose CRS* in 20 mL of *water R*. Add 25.0 mL of *acetonitrile R* with gentle heating and dilute to 50.0 mL with *water R*.

Reference solution (c) Dissolve 65 mg of *fructose CRS* (impurity D) in a mixture of equal volumes of *acetonitrile R* and *water R* and dilute to 100.0 mL with the same mixture of solvents.

Reference solution (d) Dissolve 1 g of *lactulose for peak identification CRS* (containing impurities A, B, C, E, F, G and H) in reference solution (c) and dilute to 25 mL with reference solution (c).

Reference solution (e) Dilute 5.0 mL of reference solution (a) to 100.0 mL with a mixture of equal volumes of *acetonitrile R* and *water R*.

Column 1:
— *size*: *l* = 0.05 m, Ø = 4.6 mm;
— *stationary phase*: *aminopropylsilyl silica gel for chromatography R* (3 µm);
— *temperature*: 38 ± 1 °C.

Column 2:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *aminopropylsilyl silica gel for chromatography R* (3 µm);
— *temperature*: 38 ± 1 °C.

Columns 1 and 2 are coupled in series.

Mobile phase Dissolve 0.253 g of *sodium dihydrogen phosphate R* in 200 mL of *water for chromatography R* and dilute to 1000 mL with *acetonitrile R*.

Flow rate 1.0 mL/min.

Detection Differential refractometer maintained at a constant temperature (e.g. 35 °C).

Injection 20 µL of the test solution and of reference solutions (a), (d) and (e).

Run time Twice the retention time of lactulose.

Identification of impurities Use the chromatogram supplied with *lactulose for peak identification CRS* and the chromatogram obtained with reference solution (d) to identify the peaks due to impurities A, B, C, D, E, F, G and H.

Relative retention With reference to lactulose (retention time = about 18 min): impurity F = about 0.2; impurity E = about 0.38; impurity D = about 0.42; impurity B = about 0.6; impurity G = about 0.8; impurity A = about 0.9; impurity C = about 1.2; impurity H = about 1.5.

System suitability Reference solution (d):
— *peak-to-valley ratio*: minimum 5.0, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to lactulose.

Limits:
— *impurity B*: not more than 3 times the area of the peak due to lactulose in the chromatogram obtained with reference solution (a) (15.0 per cent);
— *impurities A, C*: for each impurity, not more than twice the area of the peak due to lactulose in the chromatogram obtained with reference solution (a) (10.0 per cent);
— *impurities E, F*: for each impurity, not more than 0.8 times the area of the peak due to lactulose in the chromatogram obtained with reference solution (a) (4.0 per cent);
— *impurities G, H*: for each impurity, not more than 0.3 times the area of the peak due to lactulose in the chromatogram obtained with reference solution (a) (1.5 per cent);
— *impurity D*: not more than 0.2 times the area of the peak due to lactulose in the chromatogram obtained with reference solution (a) (1.0 per cent);
— *unspecified impurities*: for each impurity, not more than 0.1 times the area of the peak due to lactulose in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *sum of impurities eluting after impurity H*: not more than 0.26 times the area of the peak due to lactulose in the chromatogram obtained with reference solution (a) (1.3 per cent);
— *total (excluding impurities B and C)*: not more than 2.4 times the area of the peak due to lactulose in the chromatogram obtained with reference solution (a) (12.0 per cent);
— *disregard limit*: not more than the area of the peak due to lactulose in the chromatogram obtained with reference solution (e) (0.25 per cent).

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Methanol

Head-space gas chromatography (*2.2.28*).

Internal standard solution Mix 0.5 mL of *propanol R* and 100.0 mL of *water R*. Dilute 1.0 mL of the solution to 100.0 mL with *water R*. Dilute 5.0 mL of this solution to 50.0 mL with *water R*.

Test solution To 0.13 g of the substance to be examined in a 20 mL vial add 1.0 mL of the internal standard solution and 5 µL of a 0.1 per cent *V/V* solution of *methanol R*.

Reference solution To 1.0 mL of the internal standard solution in a 20 mL vial add 5 µL of a 0.1 per cent *V/V* solution of *methanol R*.

Column:
— *size*: *l* = 2 m, Ø = 2 mm;
— *stationary phase*: *ethylvinylbenzene-divinylbenzene copolymer R* (180 µm).

Carrier gas *helium for chromatography R*.

Flow rate 30 mL/min.

Static head-space conditions that may be used:
— *equilibration temperature*: 60 °C;
— *equilibration time*: 1 h;
— *pressurisation time*: 1 min.

Temperature:
— *column*: 140 °C;
— *injection port*: 200 °C;
— *detector*: 220 °C.

Detection Flame ionisation.

Injection 1 mL of the gaseous phase.

Calculate the content of methanol, taking its density (*2.2.5*) at 20 °C to be 0.79 g/mL.

Limit:
— *methanol*: calculate the ratio (*R*) of the area of the peak due to methanol to the area of the peak due to the internal standard in the chromatogram obtained with the reference solution; calculate the ratio of the area of the peak due to methanol to the area of the peak due to the internal standard in the chromatogram obtained with the test solution: this ratio is not greater than 2*R* (30 ppm).

Sulfites

Maximum 30 ppm.

Mix 5.0 g with 40 mL of *water R*, add 2.0 mL of *0.1 M sodium hydroxide* and dilute to 100 mL with *water R*. To 10.0 mL of this solution, add 1.0 mL of *hydrochloric acid R1*, 2.0 mL of *decolorised fuchsin solution R1* and 2.0 mL of a 0.5 per cent *V/V* solution of *formaldehyde R*. Allow to stand for 30 min and measure the absorbance (*2.2.25*) at 583 nm using as the compensation liquid a solution prepared at the same time and in the same manner with 10.0 mL of *water R* instead of the solution of the substance to be examined. The absorbance is not greater than that of a reference solution prepared at the same time and in the same manner using 10.0 mL of *sulfite standard solution (1.5 ppm SO_2) R* instead of the solution of the substance to be examined.

Boron
Maximum 5 ppm.
Avoid where possible the use of glassware.
Reference solution Dissolve 56.0 mg of *boric acid R* in *water R* and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of this solution to 100.0 mL with *water R*. *Keep in a well-closed polyethylene container.*
In 4 polyethylene 25 mL flasks, place separately:
— 1.00 g of the substance to be examined and 1 mL of *water R* (solution A);
— 1.00 g of the substance to be examined and 1 mL of the reference solution (solution B);
— 1 mL of the reference solution and 1 mL of *water R* (solution C);
— 2 mL of *water R* (solution D).
To each flask, add 4.0 mL of *acetate-edetate buffer solution pH 5.5 R*. Mix and add 4.0 mL of freshly prepared *azomethine H solution R*. Mix and allow to stand for 1 h. Measure the absorbance (*2.2.25*) of solutions A, B and C at 420 nm, using solution D as the compensation liquid. The test is not valid unless the absorbance of solution C is at least 0.25. The absorbance of solution B is not less than twice that of solution A.

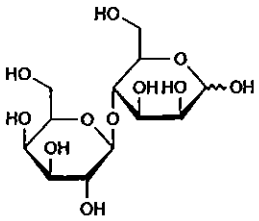
Sulfated ash (*2.4.14*)
Maximum 0.2 per cent, determined on 1.5 g and calculated with reference to the declared content of lactulose.

Microbial contamination
TAMC: acceptance criterion 10^2 CFU/g (*2.6.12*).
TYMC: acceptance criterion 10^1 CFU/g (*2.6.12*).
Absence of *Escherichia coli* (*2.6.13*).

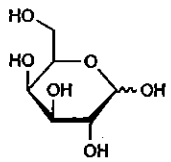
ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.
Injection Test solution and reference solution (b).
System suitability Reference solution (b):
— *symmetry factor*: 0.6 to 2.0 for the principal peak.
Calculate the percentage content of $C_{12}H_{22}O_{11}$ taking into account the assigned content of *lactulose CRS*.

LABELLING
The label states the declared content of lactulose.

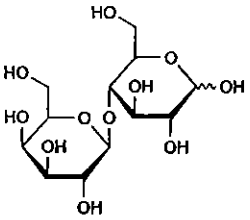
IMPURITIES
Specified impurities *A, B, C, D, E, F, G, H.*



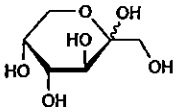
A. 4-*O*-β-D-galactopyranosyl-D-mannopyranose (epilactose),



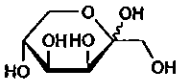
B. D-galactopyranose (galactose),



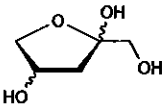
C. 4-*O*-β-D-galactopyranosyl-D-glucopyranose (lactose),



D. D-*arabino*-hex-2-ulopyranose (fructose),



E. D-*lyxo*-hex-2-ulopyranose (tagatose),

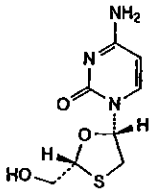


F. (2*R*,4*R*)-2-(hydroxymethyl)oxolane-2,4-diol,
G. unknown structure,
H. unknown structure.

Ph Eur

Lamivudine

(Ph. Eur. monograph 2217)



$C_8H_{11}N_3O_3S$ 229.3 *134678-17-4*

Action and use
Nucleoside reverse transcriptase inhibitor; antiviral (HIV).

Preparations
Lamivudine Tablets
Zidovudine and Lamivudine Tablets
Abacavir, Zidovudine and Lamivudine Tablets
Abacavir and Lamivudine Tablets

Ph Eur

DEFINITION
4-Amino-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl] pyrimidin-2(1*H*)-one.

Content
97.5 per cent to 102.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white powder.

Solubility
Soluble in water, sparingly soluble in methanol, slightly soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification: B, C.

Second identification: A, B.

A. Specific optical rotation (*2.2.7*): −99 to −97 (dried substance).

Dissolve 0.250 g in *water R* and dilute to 50.0 mL with the same solvent.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *lamivudine CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

C. Enantiomeric purity (see Tests).

TESTS

Absorbance (*2.2.25*)
Maximum 0.3 at 440 nm, using a path length of 4 cm.

Dissolve 1.00 g in *water R*, using sonication if necessary, and dilute to 20.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *salicylic acid R* in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 100.0 mL with the mobile phase.

Reference solution (c) Dissolve 50.0 mg of *lamivudine CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (d) Dissolve 5 mg of *cytosine R* and 5 mg of *uracil R* in the mobile phase and dilute to 100 mL with the mobile phase. Dilute 2 mL of the solution to 10 mL with the mobile phase.

Reference solution (e) Dissolve 5 mg of *lamivudine for system suitability 1 CRS* (containing impurities A and B) in 2 mL of the mobile phase. Add 1 mL of reference solution (d) and dilute to 10 mL with the mobile phase.

Column:
- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 35 °C.

Mobile phase Mix 5 volumes of *methanol R* and 95 volumes of a 1.9 g/L solution of *ammonium acetate R*, previously adjusted to pH 3.8 with *glacial acetic acid R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 277 nm.

Injection 10 µL.

Run time 3 times the retention time of lamivudine.

Identification of impurities Use the chromatograms obtained with reference solutions (b) and (e) to identify the peaks due to impurities A, B, C, E and F.

Relative retention With reference to lamivudine (retention time = about 9 min): impurity E = about 0.28; impurity F = about 0.32; impurity A = about 0.36; impurity B = about 0.91; impurity J = about 1.45; impurity C = about 2.32.

System suitability Reference solution (e):
- *resolution*: minimum 1.5 between the peaks due to impurities F and A; minimum 1.5 between the peaks due to impurity B and lamivudine.

Limits:
- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity E = 0.6; impurity F = 2.2; impurity J = 2.2;
- *impurity A*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
- *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *total*: not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.6 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Enantiomeric purity
Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution Dissolve 25.0 mg of the substance to be examined in *water R* and dilute to 100.0 mL with the same solvent.

Reference solution Dissolve the contents of a vial of *lamivudine for system suitability 2 CRS* (containing impurity D) in 1.0 mL of *water R*.

Column:
- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: beta-cyclodextrin derivative of silica gel for chiral separation R* (5 µm);
- *temperature*: maintain at constant temperature between 15 °C and 30 °C; the temperature may be adjusted to optimise the resolution between lamivudine and impurity D; a lower temperature favours improved resolution.

Mobile phase Mix 5 volumes of *methanol R* and 95 volumes of a 7.7 g/L solution of *ammonium acetate R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 270 nm.

Injection 10 µL.

Run time Twice the retention time of lamivudine.

Relative retention With reference to lamivudine (retention time = about 8 min): impurity D = about 1.2; impurity B and enantiomer = about 1.3 and 1.5.

System suitability Reference solution:
- *peak-to-valley-ratio*: minimum 15, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the

curve separating this peak from the peak due to lamivudine.

Calculate the sum of the percentage contents of all impurity peaks with a relative retention from 1.2 to 1.5. Subtract the percentage content of impurity B as obtained in the test for related substances.

Limit:

— *impurity D*: maximum 0.3 per cent.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (c).

Calculate the percentage content of $C_8H_{11}N_3O_3S$ using the chromatograms obtained with the test solution and reference solution (c) and the declared content of $C_8H_{11}N_3O_3S$ in *lamivudine CRS*.

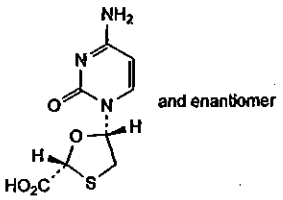
STORAGE

Protected from light.

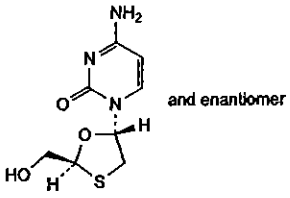
IMPURITIES

Specified impurities *A, B, C, D.*

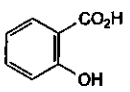
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *E, F, G, H, I, J.*



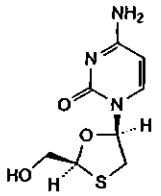
A. (2*RS*,5*SR*)-5-(4-amino-2-oxopyrimidin-1(2*H*)-yl)-1,3-oxathiolane-2-carboxylic acid,



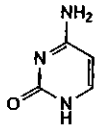
B. 4-amino-1-[(2*RS*,5*RS*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2(1*H*)-one ((±)-*trans*-lamivudine),



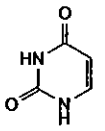
C. 2-hydroxybenzenecarboxylic acid (salicylic acid),



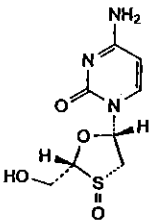
D. 4-amino-1-[(2*S*,5*R*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2(1*H*)-one,



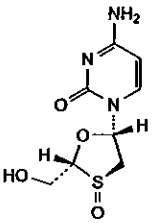
E. 4-aminopyrimidin-2(1*H*)-one (cytosine),



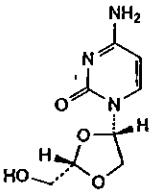
F. pyrimidine-2,4(1*H*,3*H*)-dione (uracil),



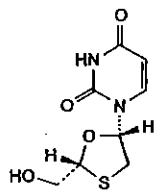
G. 4-amino-1-[(2*R*,3*S*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2(1*H*)-one *S*-oxide,



H. 4-amino-1-[(2*R*,3*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2(1*H*)-one *S*-oxide,



I. 4-amino-1-[(2*S*,4*S*)-2-(hydroxymethyl)-1,3-dioxolan-4-yl] pyrimidin-2(1*H*)-one,

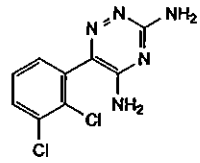


J. 1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl] pyrimidine-2,4(1*H*,3*H*)-dione.

Ph Eur

Lamotrigine

(Ph. Eur. monograph 1756)



$C_9H_7Cl_2N_5$ 256.1 *84057-84-1*

Action and use
Antiepileptic.

Preparations
Lamotrigine Dispersible Tablets
Lamotrigine Tablets

Ph Eur

DEFINITION
6-(2,3-Dichlorophenyl)-1,2,4-triazine-3,5-diamine.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Very slightly soluble in water, slightly soluble in anhydrous ethanol.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *lamotrigine CRS*.

TESTS

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 20 mg of the substance to be examined in 5 mL of *methanol R* and dilute to 100.0 mL with a 10.3 g/L solution of *hydrochloric acid R*.

Reference solution (a) Dissolve 5 mg of *lamotrigine for system suitability CRS* (containing impurity G) in 2.5 mL of *methanol R* and dilute to 25.0 mL with a 10.3 g/L solution of *hydrochloric acid R*.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with a 10.3 g/L solution of *hydrochloric acid R*. Dilute 2.0 mL of this solution to 10.0 mL with a 10.3 g/L solution of *hydrochloric acid R*.

Reference solution (c) Dissolve 5.0 mg of *lamotrigine impurity E CRS* in a mixture of 0.25 mL of *hydrochloric acid R* and 45 mL of *methanol R* and dilute to 50.0 mL with *methanol R*. Dilute 5.0 mL of the solution to 100.0 mL with a 10.3 g/L solution of *hydrochloric acid R*. To 4.0 mL of this solution add 5 mL of *methanol R* and dilute to 100.0 mL with a 10.3 g/L solution of *hydrochloric acid R*.

Reference solution (d) Dissolve 10 mg of *lamotrigine for peak identification CRS* (containing impurities A, E and F) in 2.5 mL of *methanol R* and dilute to 50.0 mL with a 10.3 g/L solution of *hydrochloric acid R*.

Blank solution Mix 5 volumes of *methanol R* and 95 volumes of a 10.3 g/L solution of *hydrochloric acid R*.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 35 °C.

Mobile phase:
— *mobile phase A*: mix 1 volume of *triethylamine R* and 150 volumes of a 2.7 g/L solution of *potassium dihydrogen phosphate R*; adjust to pH 2.0 with *phosphoric acid R*;
— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 4	85	15
4 - 14	85 → 20	15 → 80

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 270 nm.

Injection 10 µL of the test solution, reference solutions (a), (b) and (d) and the blank solution.

Identification of impurities Use the chromatogram supplied with *lamotrigine for peak identification CRS* and the chromatogram obtained with reference solution (d) to identify the peaks due to impurities A, E and F; use the chromatogram supplied with *lamotrigine for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peak due to impurity G.

Relative retention With reference to lamotrigine (retention time = about 7 min): impurity G = about 1.1; impurity A = about 1.3; impurity E = about 1.7; impurity F = about 1.8.

System suitability Reference solution (a):
— *peak-to-valley ratio*: minimum 1.2, where H_p = height above the baseline due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to lamotrigine.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity F by 1.3;
— *impurity F*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
— *impurities A, G*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak due to impurity E.

Impurity E

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Mobile phase *acetonitrile for chromatography R*, mobile phase A (35:65 *V/V*).

Detection Spectrophotometer at 210 nm.

Injection Test solution and reference solutions (d) and (c).

Run time 10 min.

Retention time Impurity E = about 5.5 min; impurity F = about 8.5 min.

System suitability Reference solution (d):

— the chromatogram obtained is similar to the chromatogram supplied with *lamotrigine for peak identification CRS*.

Limit:

— *impurity E*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 2.000 g by drying in an oven at 105 °C at a pressure not exceeding 0.7 kPa for 3 h.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 2.0 g.

ASSAY

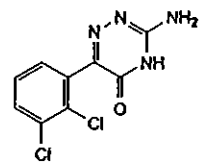
Dissolve 0.200 g in 60 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 mL of *0.1 M perchloric acid* is equivalent to 25.61 mg of $C_9H_7Cl_2N_5$.

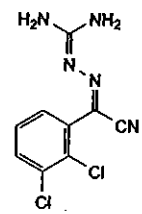
IMPURITIES

Specified impurities *A, E, F, G.*

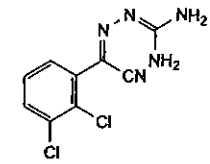
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D.*



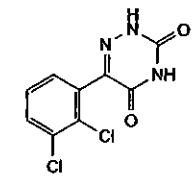
A. 3-amino-6-(2,3-dichlorophenyl)-1,2,4-triazin-5(4*H*)-one,



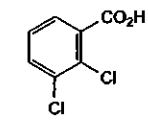
B. (2*E*)-[2-(diaminomethylidene)diazanylidene](2,3-dichlorophenyl)acetonitrile,



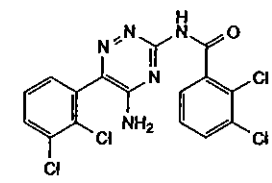
C. (2*Z*)-[2-(diaminomethylidene)diazanylidene](2,3-dichlorophenyl)acetonitrile,



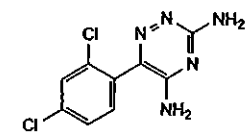
D. 6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5(2*H*,4*H*)-dione,



E. 2,3-dichlorobenzoic acid,



F. *N*-[5-amino-6-(2,3-dichlorophenyl)-1,2,4-triazin-3-yl]-2,3-dichlorobenzamide,

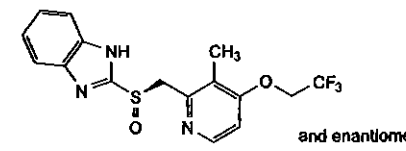


G. 6-(2,4-dichlorophenyl)-1,2,4-triazine-3,5-diamine.

Ph Eur

Lansoprazole

(Ph. Eur. monograph 2219)



$C_{16}H_{14}F_3N_3O_2S$ 369.4 *103577-45-3*

Action and use

Proton pump inhibitor; treatment of peptic ulcer disease.

Preparations

Lansoprazole Gastro-resistant Capsules

Lansoprazole Gastro-resistant Tablets

Ph Eur

DEFINITION

2-[(*RS*)-[[3-Methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl]methyl]sulfinyl]-1*H*-benzimidazole.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or brownish powder.

Solubility

Practically insoluble in water, soluble in anhydrous ethanol, very slightly soluble in acetonitrile.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *lansoprazole CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *anhydrous ethanol R*, evaporate to dryness and record new spectra using the residues.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution B_2 or BY_2 (*2.2.2, Method II*).

Dissolve 1.0 g in *dimethylformamide R* and dilute to 20 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use and protect them from light.*

Solvent mixture Mix 1 volume of *triethylamine R* and 60 volumes of *water R* and adjust to pH 10.5 with *phosphoric acid R*; mix this solution with 40 volumes of *acetonitrile R1.*

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 10 mL with the solvent mixture.

Reference solution (a) Dissolve the contents of a vial of *lansoprazole for peak identification CRS* (containing impurities A and B) in 1.0 mL of the solvent mixture.

Reference solution (b) Dilute 2.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 5 mg of *2-hydroxybenzimidazole R* (impurity D) and 5 mg of *2-mercaptobenzimidazole R* (impurity E) in the solvent mixture and dilute to 100 mL with the solvent mixture. Dilute 1 mL of this solution to 10 mL with the solvent mixture.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped amidohexadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 1 volume of *triethylamine R* and 60 volumes of *water R* and adjust to pH 6.2 with *phosphoric acid R*; mix this solution with 40 volumes of *acetonitrile R1.*

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 285 nm.

Injection 10 µL.

Run time 3 times the retention time of lansoprazole.

Identification of impurities Use the chromatogram supplied with *lansoprazole for peak identification CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A and B; use the chromatogram obtained with reference solution (c) to identify the peaks due to impurities D and E.

Relative retention With reference to lansoprazole (retention time = about 7 min): impurity D = about 0.4; impurity A = about 0.5; impurity E = about 0.6; impurity B = about 1.2.

System suitability Reference solution (a):

— *resolution*: minimum 3.0 between the peaks due to lansoprazole and impurity B.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity E by 0.4;

— *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent);

— *impurities A, D, E*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.6 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.32*)

Maximum 0.1 per cent, determined on 0.150-0.200 g using the evaporation technique:

— *temperature*: 50-70 °C;

— *heating time*: 15 min;

— *flow rate*: 150 mL/min.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 40 mL of *ethanol (96 per cent) R* and dilute to 50 mL with *water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M sodium hydroxide* is equivalent to 36.94 mg of $C_{16}H_{14}F_3N_3O_2S$.

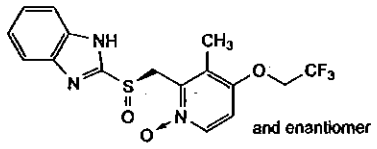
STORAGE

In an airtight container, protected from light.

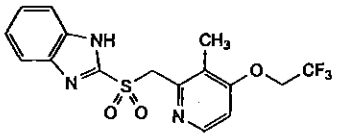
IMPURITIES

Specified impurities *A, B, D, E.*

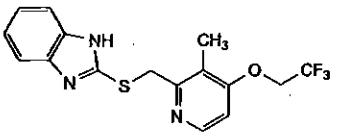
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C, F.*



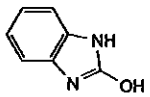
A. 2-[(*RS*)-[[3-methyl-1-oxido-4-(2,2,2-trifluoroethoxy)pyridin-2-yl]methyl]sulfinyl]-1*H*-benzimidazole,



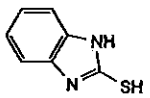
B. 2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl]methyl]sulfonyl]-1*H*-benzimidazole,



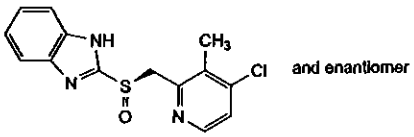
C. 2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl]methyl]sulfanyl]-1*H*-benzimidazole,



D. 1*H*-benzimidazol-2-ol,



E. 1*H*-benzimidazole-2-thiol,

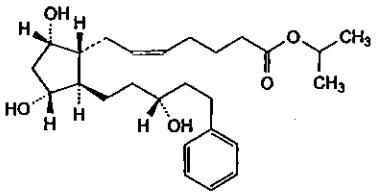


F. 2-[(*RS*)-[(4-chloro-3-methylpyridin-2-yl)methyl]sulfinyl]-1*H*-benzimidazole.

Ph Eur

Latanoprost

(Ph. Eur. monograph 2230)



$C_{26}H_{40}O_5$ 432.6 *130209-82-4*

Action and use
Prostaglandin analogue; treatment of intraocular pressure

Ph Eur

DEFINITION

Propan-2-yl (5*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(3*R*)-3-hydroxy-5-phenylpentyl]cyclopentyl]hept-5-enoate.

Content
94.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
Clear, colourless or yellow, viscous, oily liquid.

Solubility
Practically insoluble in water, very soluble in acetonitrile, freely soluble in anhydrous ethanol.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of latanoprost.*

B. Specific optical rotation (see Tests).

TESTS

Specific optical rotation (*2.2.7*)
+ 32.0 to + 37.0 (anhydrous substance).

Dissolve 0.100 g in *acetonitrile R* and dilute to 10.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Test solution Dissolve 20.0 mg of the substance to be examined in 2 mL of *anhydrous ethanol R* and dilute to 10.0 mL with *heptane R*.

Reference solution (a) Dissolve the contents of a vial of *latanoprost for system suitability CRS* (containing impurities E and F) in 200 µL of *anhydrous ethanol R* then add 800 µL of *heptane R*.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *heptane R*. Dilute 1.0 mL of this solution to 10.0 mL with *heptane R*.

Reference solution (c) Dissolve 20.0 mg of *latanoprost CRS* in 2 mL of *anhydrous ethanol R* and dilute to 10.0 mL with *heptane R*.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *silica gel for chromatography R* (5 µm);
— *temperature*: 30 °C.

Mobile phase *anhydrous ethanol R, heptane R* (6:94 *V/V*).

Flow rate 1.3 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µL of the test solution and reference solutions (a) and (b).

Run time Twice the retention time of latanoprost.

Identification of impurities Use the chromatogram supplied with *latanoprost for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities E and F.

Relative retention With reference to latanoprost (retention time = about 16 min): impurity E = about 0.9; impurity F = about 1.1.

System suitability Reference solution (a):
— *resolution*: minimum 2.0 between the peaks due to latanoprost and impurity F.

Calculation of percentage contents:
— for each impurity, use the concentration of latanoprost in reference solution (b).

Limits:
- *impurity F*: maximum 3.5 per cent;
- *impurity E*: maximum 0.4 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *sum of impurities other than F*: maximum 1.0 per cent;
- *reporting threshold*: 0.05 per cent.

Impurity H

Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R, water R* (30:70 *V/V*).

Test solution Dissolve 25.0 mg of the substance to be examined in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

Reference solution (a) Dissolve the contents of a vial of *latanoprost impurity H CRS* in 1.0 mL of the solvent mixture.

Reference solution (b) To 50 µL of the test solution add 450 µL of reference solution (a).

Column:
- *size*: *l* = 0.15 m, Ø = 4 mm;
- *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 60 °C.

Mobile phase:
- *mobile phase A*: *phosphoric acid R, acetonitrile R1, water for chromatography R* (0.1:30:70 *V/V/V*);
- *mobile phase B*: *phosphoric acid R, water for chromatography R, acetonitrile R1* (0.1:20:80 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 9	100	0
9 - 10	100 → 0	0 → 100
10 - 15	0	100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 200 nm.

Injection 50 µL.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity H.

Relative retention With reference to latanoprost (retention time = about 12 min): impurity H = about 0.7.

System suitability Reference solution (b):
- *resolution*: minimum 5.0 between the peaks due to impurity H and latanoprost.

Calculation of percentage content:
- use the concentration of impurity H in reference solution (a).

Limit:
- *impurity H*: maximum 0.15 per cent.

Water (*2.5.32*)

Maximum 0.50 per cent, determined on 0.100 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (c).

Calculate the percentage content of $C_{26}H_{40}O_5$ taking into account the assigned content of *latanoprost CRS*.

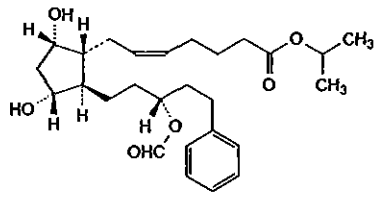
STORAGE

At a temperature of 2 °C to 8 °C.

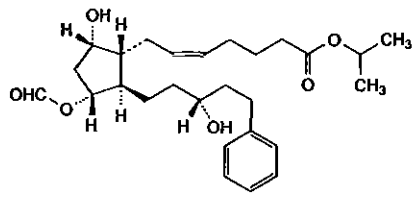
IMPURITIES

Specified impurities *E, F, H.*

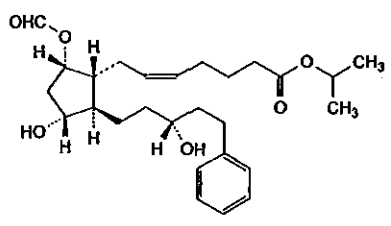
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, G, J.*



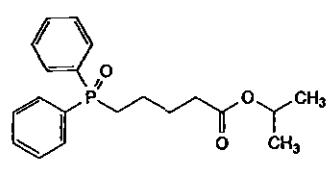
A. propan-2-yl (5*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-2-[(3*R*)-3-(formyloxy)-5-phenylpentyl]-3,5-dihydroxycyclopentyl]hept-5-enoate,



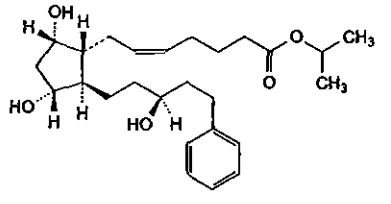
B. propan-2-yl (5*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3-(formyloxy)-5-hydroxy-2-[(3*R*)-3-hydroxy-5-phenylpentyl]cyclopentyl]hept-5-enoate,



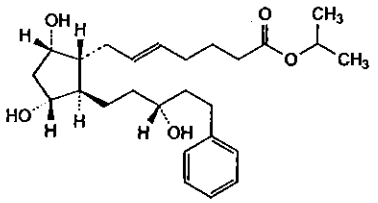
C. propan-2-yl (5*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-5-(formyloxy)-3-hydroxy-2-[(3*R*)-3-hydroxy-5-phenylpentyl]cyclopentyl]hept-5-enoate,



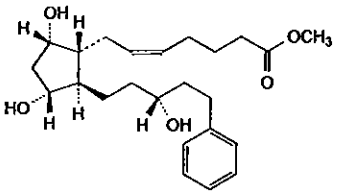
D. propan-2-yl 5-(diphenylphosphoryl)pentanoate,



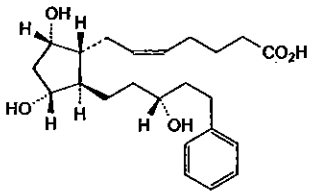
E. propan-2-yl (5*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(3*S*)-3-hydroxy-5-phenylpentyl]cyclopentyl]hept-5-enoate,



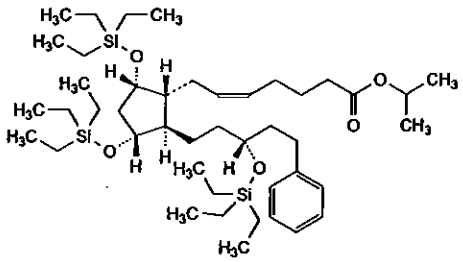
F. propan-2-yl (5*E*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(3*R*)-3-hydroxy-5-phenylpentyl]cyclopentyl]hept-5-enoate,



G. methyl (5*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(3*R*)-3-hydroxy-5-phenylpentyl]cyclopentyl]hept-5-enoate,



H. (5*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(3*R*)-3-hydroxy-5-phenylpentyl]cyclopentyl]hept-5-enoic acid,



J. propan-2-yl (5*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-2-[(3*R*)-5-phenyl-3-[(triethylsilyl)oxy]pentyl]-3,5-bis[(triethylsilyl)oxy]cyclopentyl]hept-5-enoate.

Ph Eur

Lauromacrogol 400

(Ph. Eur. monograph 2046)

Action and use
Non-ionic surfactant; sclerosant.

Ph Eur

DEFINITION

Mixture of lauryl alcohol (dodecanol) monoethers of mixed macrogols. It may contain some free macrogols and it contains various amounts of free lauryl alcohol. The number of moles of ethylene oxide reacted per mole of lauryl alcohol is 9. The name of the substance is followed by a number (400) corresponding approximately to the average molecular mass of the macrogol portion.

This monograph applies to lauromacrogol 400 used as active substance.

CHARACTERS

Appearance
White or almost white, unctuous and hygroscopic mass, melting at 24 °C into a colourless or yellowish, viscous liquid.

Solubility
Freely soluble in water, very soluble in acetone and in ethanol (96 per cent).

IDENTIFICATION

A. Hydroxyl value (see Tests).

B. Saponification value (see Tests).

C. Warm the substance to be examined in an incubator at 50 °C for 1 h until fully molten and clear. Transfer 50 mL to a warmed cloud-point tube (flat-bottomed glass tube 30-33.5 mm in internal diameter and 115-125 mm high). Insert the tube into a cooling bath that allows the outer surface of the tube to be in contact with chilled air, contained within a cylindrical metal container (internal diameter 9.5-12.5 mm greater than the external diameter of the sample tube, 115 mm high) that is surrounded by iced water. The base of the glass tube rests on a 6 mm thick cork disc, which prevents direct thermal contact with the cooled metal cylinder. Stir the substance to be examined continuously with a thermometer so that the temperature is constant throughout the substance. Periodically lift the tube out of the cooling bath to check for signs of cloudiness at the bottom of the tube. Examine the tube against a bright light source. When cloudiness is first observed, check more frequently until the substance becomes completely cloudy and the thermometer, suspended in the centre of the substance, is only just visible when viewed horizontally. Record the temperature. It is 20 °C to 25 °C.

TESTS

Appearance
The molten substance to be examined is clear (*2.2.1*) and not more intensely coloured than reference solution GY_6 (*2.2.2, Method I*).

Alkalinity
Dissolve 2.0 g in a hot mixture of 10 mL of *carbon dioxide-free water R* and 10 mL of *ethanol (96 per cent) R*.
Add 0.1 mL of *bromothymol blue solution R1*. Not more than 0.5 mL of *0.1 M hydrochloric acid* is required to change the colour of the indicator to yellow.

Acid value (*2.5.1*)
Maximum 1.0, determined on 5.0 g.

Hydroxyl value (*2.5.3, Method A*)
90 to 105, determined on 2.0 g.

Iodine value (*2.5.4, Method A*)
Maximum 2.0.

Peroxide value
Maximum 5.0.

Introduce 10.0 g into a 100 mL beaker, dissolve with *glacial acetic acid R* and dilute to 20 mL with the same solvent. Add 1 mL of *saturated potassium iodide solution R*, mix and allow to stand for 1 min. Add 50 mL of *carbon dioxide-free water R*. Titrate with *0.01 M sodium thiosulfate*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

Determine the peroxide value using the following expression:

$$\frac{(n_1 - n_2) \times M \times 1000}{m}$$

n_1 = volume of *0.01 M sodium thiosulfate* required for the substance to be examined, in millilitres;
n_2 = volume of *0.01 M sodium thiosulfate* required for the blank titration, in millilitres;
M = molarity of the sodium thiosulfate solution, in moles per litre;
m = mass of the substance to be examined, in grams.

Saponification value (*2.5.6*)
Maximum 3.0.

Free lauryl alcohol (dodecanol)
Gas chromatography (*2.2.28*).

Test solution Dissolve 0.200 g of the substance to be examined in *acetone R* and dilute to 10.0 mL with the same solvent.

Reference solution Dissolve 2.00 g of *lauryl alcohol R* in *acetone R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of the solution to 50.0 mL with *acetone R*.

Column:
— *material*: fused silica;
— *size*: l = 30 m, Ø = 0.25 mm;
— *stationary phase*: *phenyl(5)methyl(95)polysiloxane R* (film thickness 0.1 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1 mL/min.

Split ratio 50:1.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 1	120
	1 - 23	120 → 350
	23 - 33	350
Injection port		300
Detector		350

Detection Flame ionisation.

Injection 1.0 µL.

Retention time Lauryl alcohol = about 5 min.

Limit:
— *free lauryl alcohol*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (2.0 per cent).

Free macrogols
Size-exclusion chromatography (*2.2.30*).

Test solution Dissolve 5.0 g of the substance to be examined in the mobile phase and dilute to 250.0 mL with the mobile phase.

Reference solution (a) Dissolve about 0.4 g of *macrogol 1000 R* in the mobile phase and dilute to 250.0 mL with the mobile phase.

Reference solution (b) Dilute 50.0 mL of reference solution (a) to 100.0 mL with the mobile phase.

Precolumns (2):
— *size*: l = 0.125 m, Ø = 4 mm;
— *stationary phase*: spherical *octadecylsilyl silica gel for chromatography R* (5 µm) with a pore size of 10 nm.

Column:
— *size*: l = 0.30 m, Ø = 7.8 mm;
— *stationary phase*: *hydroxylated polymethacrylate gel R* (6 µm) with a pore size of 12 nm.

Connect both precolumns to the column using a 3-way valve and switch the mobile phase flow according to the following programme:
— 0-114 s: precolumn 1 and column;
— 115 s to the end: precolumn 2 and column;
— 115 s to 8 min: flow back of precolumn 1.

Mobile phase *water R, methanol R* (2:8 *V/V*).

Flow rate 1.1 mL/min.

Detection Refractometer.

Injection 20 µL.

Calculate the percentage content of free macrogols using the following expression:

$$\frac{A_1 \times m_2 \times 200}{m_1 \times (A_2 + 2A_3)}$$

m_1 = mass of the substance to be examined in the test solution, in grams;
m_2 = mass of *macrogol 1000 R* in reference solution (a), in grams;
A_1 = area of the peak due to free macrogols in the chromatogram obtained with the test solution;
A_2 = area of the peak due to macrogol 1000 in the chromatogram obtained with reference solution (a);
A_3 = area of the peak due to macrogol 1000 in the chromatogram obtained with reference solution (b).

Limit:
— *free macrogols*: maximum 3.0 per cent.

Average chain length of the fatty alcohol and average number of moles of ethylene oxide
Nuclear magnetic resonance spectrometry (*2.2.33*).

Test solution If the substance is in the solid state at room temperature, heat gently before sampling. Dissolve 0.4 mL of the substance to be examined in 0.3 mL of a mixture of 1 volume of *deuterated methanol R* and 2 volumes of *deuterated chloroform R*, containing 0.1 mol/L of *chromium(III) acetylacetonate R* as a relaxation aid.

Apparatus High resolution FT-NMR spectrometer operating at minimum 300 MHz.

Acquisition of ^{13}C NMR spectra The following parameters may be used:
— *sweep width*: 250 ppm (−15 ppm to 235 ppm);
— *irradiation frequency offset*: 110 ppm;
— *time domain*: 64 K;
— *pulse delay*: 3 s;
— *pulse program*: zgig 30 (inverse gated, 30° excitation pulse);
— *dummy scans*: 4;
— *number of scans*: 2048.

Processing and plotting The following parameters may be used:
— *size*: 64 K (zero-filling);
— *window multiplication*: exponential;
— *Lorentzian broadening factor*: 1 Hz.

Use the CD_3OD signal for shift referencing. The shift of the central peak of the multiplet is set to 49.0 ppm.

Plot the spectral region δ 0.0-80.0 ppm. Compare the spectrum with the spectrum in Figure 2046.-1. The shift values lie near the values given in Table 2046.-1.

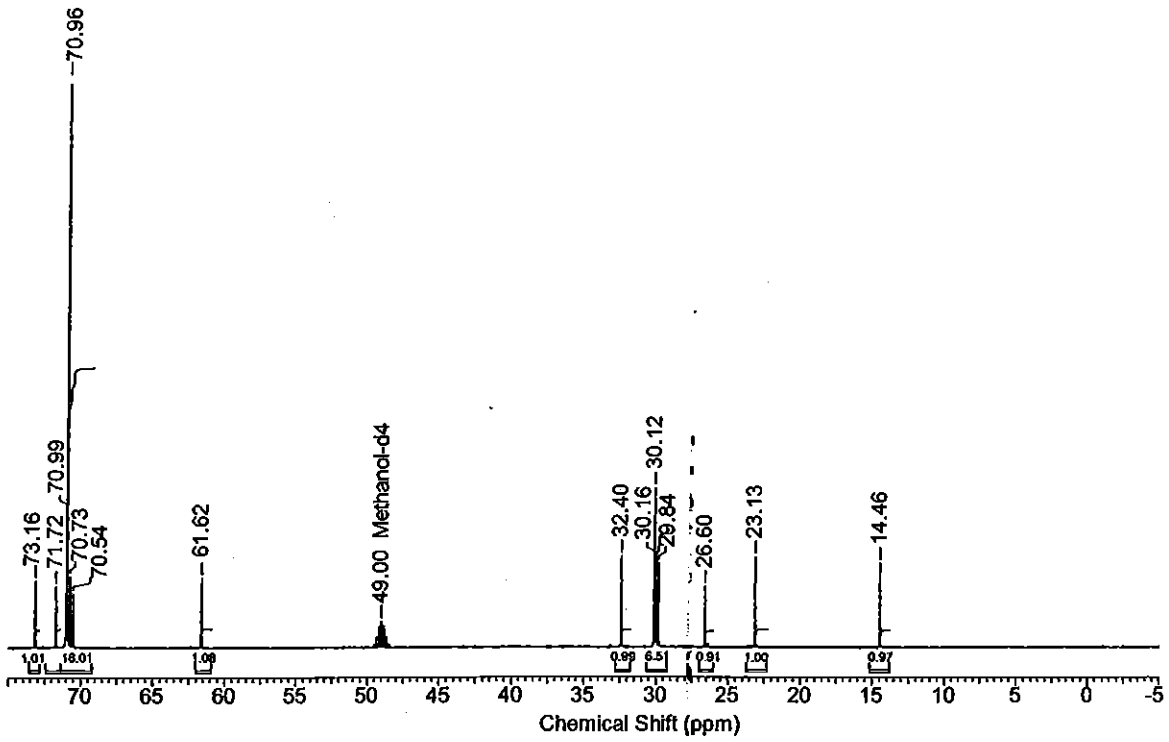


Figure 2046.-1. – *^{13}C NMR spectrum of lauromacrogol 400*

Table 2046.-1. – *Shift values*

Signal	Shift (ppm)	Normalised integrals
CH_3	14.4	0.989
CH_2 (alkyl chain)	23.2	1.000
CH_2 (alkyl chain)	25.5	1.001
CH_2's (alkyl chain)	30	7.410
CH_2 (alkyl chain)	32.5	0.963
CH_2 (-CH_2-OH) (end CH_2-group of macrogol)	61.6	1.001
CH_2's (macrogol)	70.7	16.25
CH_2 (R-CH-O-macrogol) (CH_2 in alpha position)	72.6	0.998
CH_2 (macrogol)	73.1	0.929

System suitability:
— *signal-to-noise ratio*: minimum 150, for the smallest relevant peak (CH_2 at 73.1 ppm);
— *peak width at half-height*: maximum 0.05 ppm, for the central $CDCl_3$ signal (at δ 78.6 ppm).

Calculation of the average chain length of the fatty alcohol and the average number of moles of ethylene oxide Define the signal at 23.2 ppm as 1.000 and normalise the integrals of the other signals listed in Table 2046.-1.

The average chain length of the fatty alcohol is calculated using the following expression:

$$\Sigma_{14-33}\, I_{n,i} + I_{n,72.6}$$

$\Sigma_{14-33}\, I_{n,i}$ = sum of the normalised integrals of the signals from 14 ppm to 33 ppm;
$I_{n,72.6}$ = normalised integral of the signal at 72.6 ppm.

The average number of moles of ethylene oxide is calculated using the following expression:

$$0.5 \times \left(I_{n,62} + I_{n,71} + I_{n,73}\right)$$

$I_{n,62}$, $I_{n,71}$, $I_{n,73}$ = normalised integral of the signals at 62 ppm, 71 ppm and 73 ppm respectively.

The sum of the normalised integrals of the signals at 62 ppm, 71 ppm and 73 ppm corresponds to the average number of methylene groups in the macrogol part of lauromacrogol 400.

Limits:
— *average chain length of the fatty alcohol*: 10.0 to 14.0;
— *average number of moles of ethylene oxide*: 7.0 to 11.0.

Ethylene oxide and dioxan (*2.4.25, Method A*)
Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Water (*2.5.12*)
Maximum 2.0 per cent, determined on 0.500 g.

Total ash (*2.4.16*)
Maximum 0.2 per cent, determined on 2.0 g.

Ph Eur

Lauroyl Macrogolglycerides

(Ph. Eur. monograph 1231)

Action and use
Excipient.

Ph Eur

DEFINITION

Mixtures of monoesters, diesters and triesters of glycerol and monoesters and diesters of macrogols with a mean relative molecular mass between 300 and 1500.

They are obtained by partial alcoholysis of saturated oils mainly containing triglycerides of lauric (dodecanoic) acid, using macrogol, or by esterification of glycerol and macrogol with saturated fatty acids, or by mixing glycerol esters and condensates of ethylene oxide with the fatty acids of these hydrogenated oils.

CHARACTERS

Appearance
Pale yellow waxy solid.

Solubility
Dispersible in hot water, freely soluble in methylene chloride.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 1.0 g of the substance to be examined in *methylene chloride R* and dilute to 20 mL with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *hexane R, ether R* (30:70 *V/V*).

Application 10 µL.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with a 0.1 g/L solution of *rhodamine B R* in *ethanol (96 per cent) R* and examine in ultraviolet light at 365 nm.

Results The chromatogram shows a spot due to triglycerides with an R_F value of about 0.9 (R_{st} 1) and spots due to 1,3-diglycerides (R_{st} 0.7), to 1,2-diglycerides (R_{st} 0.6), to monoglycerides (R_{st} 0.1) and to esters of macrogol (R_{st} 0).

B. Hydroxyl value (see Tests).

C. Saponification value (see Tests).

D. Composition of fatty acids (see Tests).

TESTS

Drop point (*2.2.17*)
Introduce into the cup the substance to be examined, which has been melted by heating for 1 h in an oven at 100 ± 2 °C, and allow to stand for 5 h at about 5 °C.

Ethylene oxide units per molecule (nominal value)	Type of macrogol	Drop point
6	300	33 - 38
8	400	36 - 41
12	600	38 - 43
32	1500	42.5 - 47.5

Acid value (*2.5.1*)
Maximum 2.0, determined on 2.0 g.

Hydroxyl value (*2.5.3, Method A*)
Use 1.0 g.

Ethylene oxide units per molecule (nominal value)	Type of macrogol	Hydroxyl value
6	300	65 - 85
8	400	60 - 80
12	600	50 - 70
32	1500	36 - 56

Peroxide value (*2.5.5, Method A*)
Maximum 6.0, determined on 2.0 g.

Saponification value (*2.5.6*)
Use 2.0 g.

Ethylene oxide units per molecule (nominal value)	Type of macrogol	Saponification value
6	300	190 - 204
8	400	170 - 190
12	600	150 - 170
32	1500	79 - 93

Alkaline impurities
Introduce 5.0 g into a test tube and carefully add a mixture, neutralised if necessary with *0.01 M hydrochloric acid* or with *0.01 M sodium hydroxide*, of 0.05 mL of a 0.4 g/L solution of *bromophenol blue R* in *ethanol (96 per cent) R*, 0.3 mL of *water R* and 10 mL of *ethanol (96 per cent) R*. Shake and allow to stand. Not more than 1.0 mL of *0.01 M hydrochloric acid* is required to change the colour of the upper layer to yellow.

Free glycerol
Maximum 3.0 per cent.

Dissolve 1.20 g in 25.0 mL of *methylene chloride R*. Heat if necessary. After cooling, add 100 mL of *water R*. Shake and add 25.0 mL of *periodic acetic acid solution R*. Shake and allow to stand for 30 min. Add 40 mL of a 75 g/L solution of *potassium iodide R*. Allow to stand for 1 min. Add 1 mL of *starch solution R*. Titrate the iodine with *0.1 M sodium thiosulfate*. Carry out a blank titration.

1 mL of *0.1 M sodium thiosulfate* is equivalent to 2.3 mg of glycerol.

Composition of fatty acids (*2.4.22, Method A*)
Composition of the fatty-acid fraction of the substance:
— *caprylic acid*: maximum 15.0 per cent;
— *capric acid*: maximum 12.0 per cent;
— *lauric acid*: 30.0 per cent to 50.0 per cent;
— *myristic acid*: 5.0 per cent to 25.0 per cent;
— *palmitic acid*: 4.0 per cent to 25.0 per cent;
— *stearic acid*: 5.0 per cent to 35.0 per cent.

Ethylene oxide and dioxan (*2.4.25*)
Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Water (*2.5.12*)
Maximum 1.0 per cent, determined on 1.00 g. Use a mixture of 30 volumes of *anhydrous methanol R* and 70 volumes of *methylene chloride R* as solvent.

Total ash (*2.4.16*)
Maximum 0.1 per cent.

LABELLING

The label states the type of macrogol used (mean relative molecular mass) or the number of units of ethylene oxide per molecule (nominal value).

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for lauroyl macrogolglycerides used as self-emulsifying agents, solubilisers, modified-release agents and wetting agents for powders and tablets.

Hydroxyl value
(see Tests).

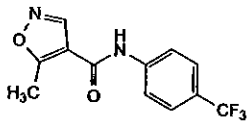
Saponification value
(see Tests).

Composition of fatty acids
(see Tests).

Ph Eur

Leflunomide

(Ph. Eur. monograph 2330)



$C_{12}H_9F_3N_2O_2$ 270.2

Action and use
Immunomodulator.

Preparation
Leflunomide Tablets

Ph Eur

DEFINITION
5-Methyl-*N*-[4-(trifluoromethyl)phenyl]isoxazole-4-carboxamide.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Practically insoluble in water, freely soluble in methanol, sparingly soluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Preparation Heat the substance to be examined and the reference substance at 130 °C for 10 min.

Comparison *leflunomide CRS*.

TESTS

Related substances
Liquid chromatography (*2.2.29*). *Store all solutions protected from light.*

Test solution (a) Dissolve 25.0 mg of the substance to be examined in 5 mL of *acetonitrile for chromatography R* and dilute to 50.0 mL with the mobile phase.

Test solution (b) Dissolve 0.125 g of the substance to be examined in 5 mL of *acetonitrile for chromatography R* and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dilute 5.0 mL of test solution (a) to 50.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve 12.5 mg of *leflunomide impurity A CRS* in 5 mL of *acetonitrile for chromatography R* and dilute to 100.0 mL with the mobile phase. Dilute 10.0 mL of the solution to 100.0 mL with the mobile phase. Dilute 2.0 mL of this solution to 100.0 mL with the mobile phase.

Reference solution (c) Dissolve 25.0 mg of *leflunomide CRS* in 5 mL of *acetonitrile for chromatography R* and dilute to 50.0 mL with the mobile phase.

Reference solution (d) Dissolve the contents of 1 vial of *leflunomide for peak identification CRS* (containing impurities B and C) in 2.0 mL of the mobile phase and sonicate for 10 min.

Column:
— *size*: l = 0.125 m, Ø = 4.0 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 5 volumes of *triethylamine R* with 650 volumes of *water for chromatography R*, adjust to pH 3.4 ± 0.1 with *phosphoric acid R* and add 350 volumes of *acetonitrile for chromatography R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µL of test solutions (a) and (b) and reference solutions (a), (b) and (d).

Run time Twice the retention time of leflunomide.

Identification of impurities Use the chromatogram supplied with *leflunomide for peak identification CRS* and the chromatogram obtained with reference solution (d) to identify the peaks due to impurities B and C.

Relative retention With reference to leflunomide (retention time = about 25 min): impurity B = about 0.2; impurity A = about 0.4; impurity C = about 0.9.

System suitability Reference solution (d):
— *peak-to-valley ratio*: minimum 3, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to leflunomide.

Limits Test solution (a):
— *impurity B*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
— *sum of impurities C and E*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *sum of impurities other than B*: not more twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Limit Test solution (b):
— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.01 per cent).

Loss on drying (*2.2.32*)
Maximum 0.3 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 4 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (a) and reference solution (c).

Calculate the percentage content of $C_{12}H_9F_3N_2O_2$ from the declared content of *leflunomide CRS*.

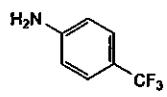
STORAGE

Protected from light.

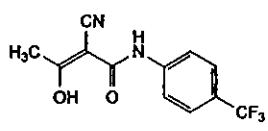
IMPURITIES

Specified impurities *A, B.*

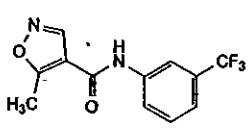
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C, D, E, F, G, H.*



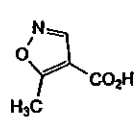
A. 4-(trifluoromethyl)aniline,



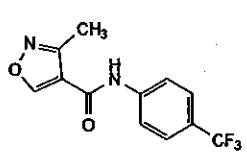
B. (2*Z*)-2-cyano-3-hydroxy-*N*-[4-(trifluoromethyl)phenyl]but-2-enamide (teriflunomide),



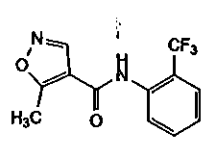
C. 5-methyl-*N*-[3-(trifluoromethyl)phenyl]isoxazole-4-carboxamide,



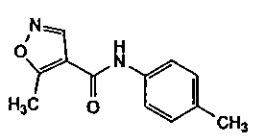
D. 5-methylisoxazole-4-carboxylic acid,



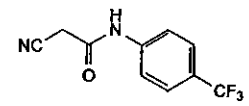
E. 3-methyl-*N*-[4-(trifluoromethyl)phenyl]isoxazole-4-carboxamide,



F. 5-methyl-*N*-[2-(trifluoromethyl)phenyl]isoxazole-4-carboxamide,



G. 5-methyl-*N*-(4-methylphenyl)isoxazole-4-carboxamide,

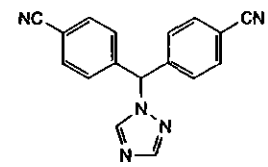


H. 2-cyano-*N*-[4-(trifluoromethyl)phenyl]acetamide.

Ph Eur

Letrozole

(Ph. Eur. monograph 2334)



$C_{17}H_{11}N_5$ 285.3 *112809-51-5*

Action and use

Aromatase inhibitor; treatment of breast carcinoma.

Preparation

Letrozole Tablets

Ph Eur

DEFINITION

4,4′-[(1*H*-1,2,4-Triazol-1-yl)methylene]dibenzonitrile.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or yellowish, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in methanol.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *letrozole CRS.*

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 25.0 mg of the substance to be examined in 15 mL of *acetonitrile R* and dilute to 50.0 mL with *water R*.

Test solution (b) To 2.0 mL of test solution (a) add 30 mL of *acetonitrile R* and dilute to 100.0 mL with *water R*.

Reference solution (a) Dilute 2 mL of test solution (a) to 10 mL with a mixture of 30 volumes of *acetonitrile R* and 70 volumes of *water R*. Dissolve the contents of a vial of *letrozole impurity A CRS* in 1 mL of this solution.

Reference solution (b) To 2.0 mL of test solution (a) add 30.0 mL of *acetonitrile R* and dilute to 100.0 mL with *water R*. To 1.0 mL of this solution add 6.0 mL of *acetonitrile R* and dilute to 20.0 mL with *water R*.

Reference solution (c) Dissolve 25.0 mg of *letrozole CRS* in 15 mL of *acetonitrile R* and dilute to 50.0 mL with *water R*. To 2.0 mL of this solution add 30 mL of *acetonitrile R* and dilute to 100.0 mL with *water R*.

Column:

— *size*: *l* = 0.125 m, Ø = 4.6 mm;

— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A: water for chromatography R;*
— *mobile phase B: acetonitrile for chromatography R;*

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 4	70	30
4 - 29	70 → 30	30 → 70

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µL of test solution (a) and reference solutions (a) and (b).

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity A.

Relative retention With reference to letrozole (retention time = about 13 min): impurity A = about 0.6.

System suitability Reference solution (a):
— *resolution*: minimum 5.0 between the peaks due to impurity A and letrozole.

Calculation of percentage contents:
— for each impurity, use the concentration of letrozole in reference solution (b).

Limits:

— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.3 per cent;
— *reporting threshold*: 0.05 per cent.

Water (*2.5.12*)
Maximum 0.3 per cent, determined on 1.00 g. Use a validated pyridine-free iodosulfurous reagent.

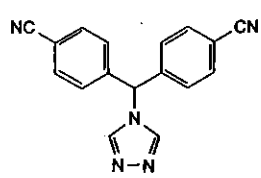
ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution (b) and reference solution (c).

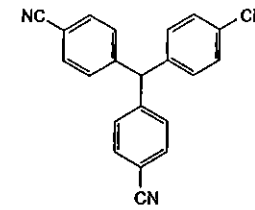
System suitability Reference solution (c):
— *symmetry factor*: maximum 1.7 for the peak due to letrozole.

Calculate the percentage content of $C_{17}H_{11}N_5$ from the assigned content of *letrozole CRS*.

IMPURITIES
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B.*



A. 4,4′-[(4*H*-1,2,4-triazol-4-yl)methylene]dibenzonitrile,

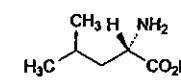


B. 4,4′,4″-methanetriyltribenzonitrile.

Ph Eur

Leucine

(Ph. Eur. monograph 0771)



$C_6H_{13}NO_2$ 131.2 *61-90-5*

Action and use
Amino acid.

Ph Eur

DEFINITION
(2*S*)-2-Amino-4-methylpentanoic acid.
Product of fermentation or of protein hydrolysis.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder or shiny flakes.

Solubility
Sparingly soluble in water, practically insoluble in ethanol (96 per cent). It dissolves in dilute mineral acids and in dilute solutions of alkali hydroxides.

IDENTIFICATION
First identification: A, B.
Second identification: A, C.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*)
Comparison *leucine CRS.*

C. Thin-layer chromatography (*2.2.27*).
Test solution Dissolve 10 mg of the substance to be examined in a 10.3 g/L solution of *hydrochloric acid R* and dilute to 50 mL with the same solution.
Reference solution Dissolve 10 mg of *leucine CRS* in a 10.3 g/L solution of *hydrochloric acid R* and dilute to 50 mL with the same solution.
Plate *TLC silica gel plate R.*
Mobile phase *glacial acetic acid R, water R, butanol R* (20:20:60 *V/V/V*).
Application 5 µL.
Development Over 2/3 of the plate.
Drying In air.
Detection Spray with *ninhydrin solution R* and heat at 105 °C for 15 min.
Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to

the principal spot in the chromatogram obtained with the reference solution.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Dissolve 0.5 g in a 103 g/L solution of *hydrochloric acid R* and dilute to 10 mL with the same solution.

Specific optical rotation (*2.2.7*)

+ 14.5 to + 16.5 (dried substance).

Dissolve 1.00 g in *hydrochloric acid R1* and dilute to 25.0 mL with the same acid.

Ninhydrin-positive substances

Amino acid analysis (*2.2.56*). For analysis, use Method 1.

The concentrations of the test solutions and the reference solutions may be adapted according to the sensitivity of the equipment used. The concentrations of all solutions are adjusted so that the system suitability requirements described in general chapter *2.2.46* are fulfilled, keeping the ratios of concentrations between all solutions as described.

Solution A *dilute hydrochloric acid R1* or a sample preparation buffer suitable for the apparatus used.

Test solution (a) Dissolve 30.0 mg of the substance to be examined in solution A and dilute to 50.0 mL with solution A.

Test solution (b) Dilute 1.0 mL of test solution (a) to 25.0 mL with solution A.

Reference solution (a) Dilute 1.0 mL of test solution (a) to 100.0 mL with solution A. Dilute 2.0 mL of this solution to 10.0 mL with solution A.

Reference solution (b) Dissolve 30.0 mg of *isoleucine R* (impurity A) in solution A and dilute to 100.0 mL with solution A. Dilute 1.0 mL of the solution to 250.0 mL with solution A. Dilute 1.0 mL of this solution to 10.0 mL with solution A.

Reference solution (c) Dissolve 30.0 mg of *proline R* in solution A and dilute to 100.0 mL with solution A. Dilute 1.0 mL of the solution to 250.0 mL with solution A.

Reference solution (d) Dilute 6.0 mL of *ammonium standard solution (100 ppm NH_4) R* to 50.0 mL with solution A. Dilute 1.0 mL of this solution to 100.0 mL with solution A.

Reference solution (e) Dissolve 30 mg of *isoleucine R* (impurity A) and 30 mg of *leucine R* in solution A and dilute to 50.0 mL with solution A. Dilute 1.0 mL of the solution to 200.0 mL with solution A.

Blank solution Solution A.

Inject suitable, equal amounts of the test solutions, blank solution and reference solutions (a), (b), (c) and (e) into the amino acid analyser. Run a program suitable for the determination of physiological amino acids.

System suitability Reference solution (e):

— *resolution*: minimum 1.5 between the peaks due to impurity A and leucine.

Calculation of percentage contents:

— for impurity A in test solution (b), use the concentration of impurity A in reference solution (b);

— for any ninhydrin-positive substance detected at 570 nm in test solution (a), use the concentration of leucine in reference solution (a);

— for any ninhydrin-positive substance detected at 440 nm in test solution (a), use the concentration of proline in reference solution (c); if a peak is above the reporting threshold at both wavelengths, use the result obtained at 570 nm for quantification.

Limits:

— *impurity A at 570 nm*: maximum 0.8 per cent;

— *any ninhydrin-positive substance*: for each impurity, maximum 0.2 per cent;

— *total*: maximum 1.0 per cent;

— *reporting threshold*: 0.05 per cent.

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Chlorides (*2.4.4*)

Maximum 200 ppm.

Dissolve 0.25 g in *water R* and dilute to 15 mL with the same solvent.

Sulfates (*2.4.13*)

Maximum 300 ppm.

Dissolve 0.5 g in 3 mL of *dilute hydrochloric acid R* and dilute to 15 mL with *distilled water R*.

Ammonium

Amino acid analysis (*2.2.56*) as described in the test for ninhydrin-positive substances with the following modifications.

Injection Test solution (a), reference solution (d) and blank solution.

Limit:

— *ammonium at 570 nm*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.02 per cent), taking into account the peak due to ammonium in the chromatogram obtained with the blank solution.

Iron (*2.4.9*)

Maximum 10 ppm.

In a separating funnel, dissolve 1.0 g in 10 mL of *dilute hydrochloric acid R*. Shake with 3 quantities, each of 10 mL, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 mL of *water R* and shake for 3 min. Use the aqueous layer.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 3 mL of *anhydrous formic acid R*. Add 30 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 13.12 mg of $C_6H_{13}NO_2$.

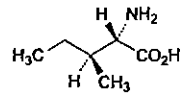
STORAGE

Protected from light.

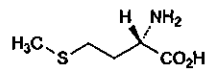
IMPURITIES

Specified impurities *A*.

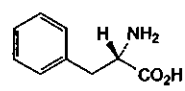
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D, E*.



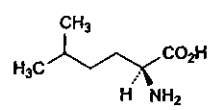
A. (2*S*,3*S*)-2-amino-3-methylpentanoic acid (isoleucine),



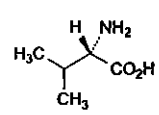
B. (2*S*)-2-amino-4-(methylsulfanyl)butanoic acid (methionine),



C. (2*S*)-2-amino-3-phenylpropanoic acid (phenylalanine),



D. (2*S*)-2-amino-5-methylhexanoic acid (5-methylnorleucine),

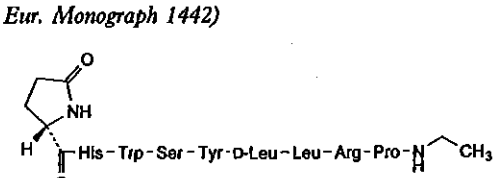


E. (2*S*)-2-amino-3-methylbutanoic acid (valine).

Ph Eur

Leuprorelin

(Ph. Eur. Monograph 1442)



$C_{59}H_{84}N_{16}O_{12}$ 1209 *53714-56-0*

Action and use
Gonadotropin releasing hormone (gonadorelin) analogue; treatment of prostate cancer.

Preparation
Leuprorelin Injection

Ph Eur

DEFINITION

5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-*N*-ethyl-L-prolinamide.

Synthetic nonapeptide analogue of the hypothalamic peptide, gonadorelin. It is obtained by chemical synthesis and is available as an acetate.

Content
97.0 per cent to 103.0 per cent (anhydrous and acetic acid-free substance).

CHARACTERS

Appearance
Hygroscopic, white or almost white powder.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs of *potassium bromide R*.

Comparison *Ph. Eur. reference spectrum of leuprorelin.*

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (b).

C. Amino acid analysis (*2.2.56*). For hydrolysis use Method 1 and for analysis use Method 1.

Express the content of each amino acid in moles. Calculate the relative proportions of the amino acids taking one seventh of the sum of the number of moles of histidine, glutamic acid, leucine, proline, tyrosine and arginine as equal to 1. The values fall within the following limits: serine present; glutamic acid = 0.85 to 1.1; proline = 0.85 to 1.1; leucine = 1.8 to 2.2; tyrosine = 0.85 to 1.1; histidine = 0.85 to 1.1 and arginine = 0.85 to 1.1. Not more than traces of other amino acids are present, with the exception of tryptophan.

TESTS

Specific optical rotation (*2.2.7*)
−38.0 to −42.0 (anhydrous and acetic acid-free substance).

Dissolve the substance to be examined in a 1 per cent *V/V* solution of *glacial acetic acid R* to obtain a concentration of 10.0 mg/mL.

Related substances
Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution (a) Dissolve the substance to be examined in the mobile phase to obtain a concentration of 1.0 mg/mL.

Test solution (b) Dilute 0.5 mL of test solution (a) to 10.0 mL with the mobile phase.

Reference solution (a) Dissolve *leuprorelin CRS* in the mobile phase to obtain a concentration of 1.0 mg/mL.

Reference solution (b) Dilute 0.5 mL of reference solution (a) to 10.0 mL with the mobile phase.

Resolution solution Dilute 5.0 mL of reference solution (a) to 50.0 mL with *water R*. To 5 mL of the solution add 100 µL of *1 M sodium hydroxide* and shake vigorously. Heat in an oven at 100 °C for 60 min, cool immediately and add 50 µL of *dilute phosphoric acid R*. Shake vigorously.

Column:
— *size*: *l* = 0.10 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase Dissolve about 15.2 g of *triethylamine R* in 800 mL of *water R*, adjust to pH 3.0 with *phosphoric acid R* and dilute to 1000 mL with *water R*. Add 850 mL of this solution to 150 mL of a mixture of 2 volumes of *propanol R* and 3 volumes of *acetonitrile R*.

Flow rate 1.0-1.5 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µL of test solution (a) and the resolution solution.

Run time 90 min.

Relative retention With reference to leuprorelin (retention time = 41-49 min): impurity E = about 0.7; impurity F = about 0.7; impurity H = about 0.78; impurity A = about 0.8; impurity B = about 0.9; impurity I = about 0.94; impurity J = about 1.09;

impurity C = about 1.2; impurity G = about 1.3; impurity K = about 1.31; impurity D = about 1.5.

System suitability Resolution solution:

— *resolution*: minimum 1.5 between the peaks due to impurity B and leuprorelin.

Limits:

— *impurity D*: maximum 1.0 per cent;

— *impurities A, B, C*: for each impurity, maximum 0.5 per cent;

— *unspecified impurities*: for each impurity, maximum 0.5 per cent;

— *total*: maximum 2.5 per cent;

— *disregard limit*: 0.1 per cent.

Acetic acid (*2.5.34*)

4.7 per cent to 9.0 per cent.

Test solution Dissolve 10.0 mg of the substance to be examined in a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A and dilute to 10.0 mL with the same mixture of mobile phases.

Water (*2.5.32*)

Maximum 5.0 per cent.

Sulfated ash (*2.4.14*)

Maximum 0.3 per cent.

Bacterial endotoxins (*2.6.14, Method D*)

Less than 16.7 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Run time 60 min.

Injection 20 µL of test solution (b) and reference solution (b).

Calculate the content of leuprorelin ($C_{59}H_{84}N_{16}O_{12}$) using the areas of the peaks and the declared content of $C_{59}H_{84}N_{16}O_{12}$ in *leuprorelin CRS*.

STORAGE

In an airtight container, protected from light, at a temperature not exceeding 30 °C.

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

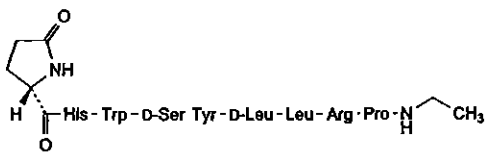
LABELLING

The label states the mass of peptide in the container.

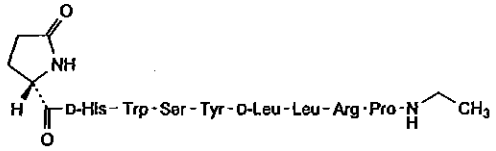
IMPURITIES

Specified impurities *A, B, C, D.*

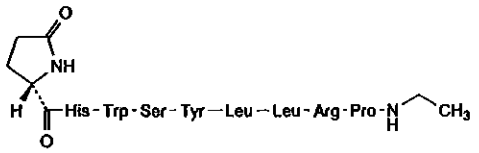
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *E, F, G, H, I, J, K.*



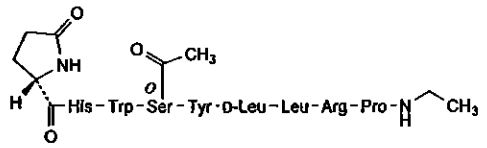
A. [4-D-serine]leuprorelin,



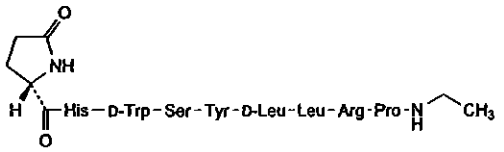
B. [2-D-histidine]leuprorelin,



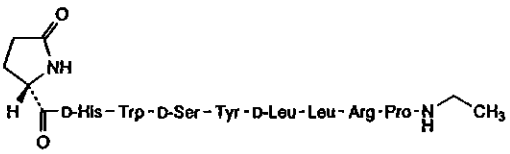
C. [6-L-leucine]leuprorelin,



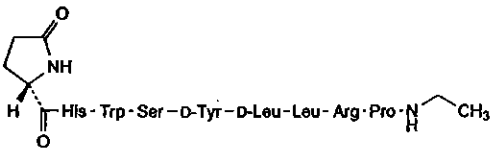
D. [4-(*O*-acetyl-L-serine)]leuprorelin,



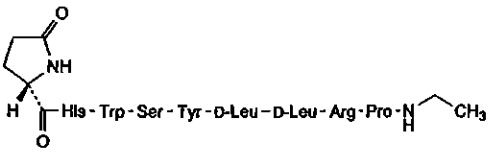
E. [3-D-tryptophane]leuprorelin,



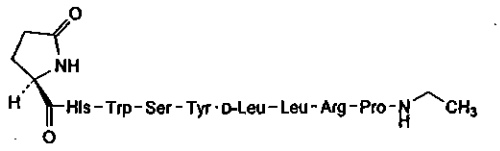
F. [2-D-histidine,4-D-serine]leuprorelin,



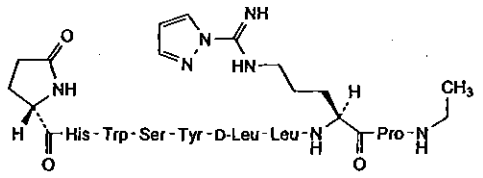
G. [5-D-tyrosine]leuprorelin,



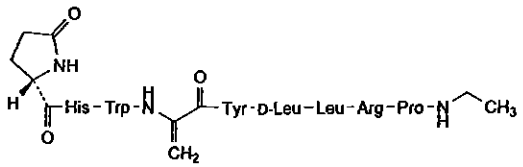
H. [7-D-leucine]leuprorelin,



I. [1-(5-oxo-D-proline)]leuprorelin,



J. [8-[5-*N*-[imino(1*H*-pyrazol-1-yl)methyl]-L-ornithine]] leuprorelin,

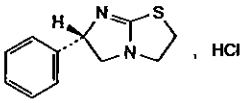


K. [4-dehydroalanine]leuprorelin.

Ph Eur

Levamisole Hydrochloride

(Ph. Eur. monograph 0726)



$C_{11}H_{13}ClN_2S$ · 240.8 *16595-80-5*

Action and use
Immunostimulant; antihelminthic.

Ph Eur

DEFINITION
(6*S*)-6-Phenyl-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole hydrochloride.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water, soluble in ethanol (96 per cent), slightly soluble in methylene chloride.

IDENTIFICATION
A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *levamisole hydrochloride CRS.*

C. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S
Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 50.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

pH (*2.2.3*)
3.0 to 4.5 for solution S.

Specific optical rotation (*2.2.7*)
−128 to −121 (dried substance), determined on solution S.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use, protect from light and keep below 25 °C.*

Test solution Dissolve 0.100 g of the substance to be examined in *methanol R*, add 1.0 mL of *concentrated ammonia R* and dilute to 10.0 mL with *methanol R.*

Reference solution (a) Dissolve 10 mg of *levamisole hydrochloride for system suitability CRS* (containing impurities A, B, C, D and E) in *methanol R*, add 0.1 mL of *concentrated ammonia R* and dilute to 1.0 mL with *methanol R.*

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *methanol R.* Dilute 5.0 mL of this solution to 25.0 mL with *methanol R.*

Column:
— *size: l* = 0.10 m, Ø = 4.6 mm;
— *stationary phase: base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
— *mobile phase A*: dissolve 0.5 g of *ammonium dihydrogen phosphate R* in 90 mL of *water R*, adjust to pH 6.5 with a 40 g/L solution of *sodium hydroxide R* and dilute to 100 mL with *water R*;
— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 8	90 → 30	10 → 70
8 - 10	30	70

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 215 nm.

Equilibration At least 4 min with the mobile phase at the initial composition.

Injection 10 µL.

Identification of impurities Use the chromatogram supplied with *levamisole hydrochloride for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D and E.

Relative retention With reference to levamisole (retention time = about 3 min): impurity A = about 0.9; impurity B = about 1.4; impurity C = about 1.5; impurity D = about 1.6; impurity E = about 2.0.

System suitability:
— the chromatogram obtained with reference solution (a) is similar to the chromatogram supplied with *levamisole hydrochloride for system suitability CRS*;
— *symmetry factor*: maximum 3.5 for the principal peak in the chromatogram obtained with reference solution (b).

Limits:
— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 2.0; impurity B = 1.7; impurity C = 2.9; impurity D = 1.3; impurity E = 2.7;
— *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

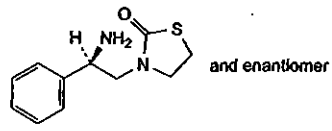
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.200 g in 30 mL of *ethanol (96 per cent) R* and add 5.0 mL of *0.01 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 24.08 mg of $C_{11}H_{13}ClN_2S$.

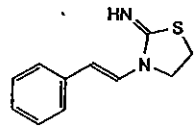
STORAGE
Protected from light.

IMPURITIES
Specified impurities *A, B, C, D, E.*

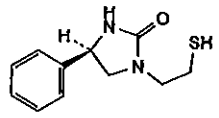


and enantiomer

A. 3-[(2*RS*)-2-amino-2-phenylethyl]thiazolidin-2-one,

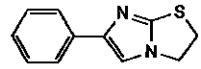


B. 3-[(*E*)-2-phenylethenyl]thiazolidin-2-imine,

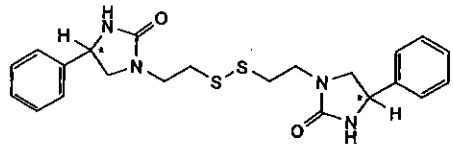


and enantiomer

C. (4*RS*)-4-phenyl-1-(2-sulfanylethyl)imidazolidin-2-one,



D. 6-phenyl-2,3-dihydroimidazo[2,1-*b*]thiazole,

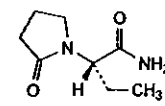


E. 1,1′-[(disulfane-1,2-diyl)bis(ethylene)]bis[(4*RS*)-4-phenylimidazolidin-2-one].

Ph Eur

Levetiracetam

(Ph. Eur. monograph 2535)



$C_8H_{14}N_2O_2$ 170.2 *102767-28-2*

Action and use
Antiepileptic.

Ph Eur

DEFINITION
(2*S*)-2-(2-Oxopyrrolidin-1-yl)butanamide.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white powder.

Solubility
Very soluble in water, soluble in acetonitrile, practically insoluble in heptane.

IDENTIFICATION
Carry out either tests A, B or tests B, C.

A. Specific optical rotation (*2.2.7*): −82 to −76.

Dissolve 0.500 g in *water R* and dilute to 25.0 mL with the same solvent.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *levetiracetam CRS.*

C. Enantiomeric purity (see Tests).

TESTS
Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Dissolve 2.0 g in *water R* and dilute to 10.0 mL with the same solvent.

Enantiomeric purity
Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution Dissolve 0.200 g of the substance to be examined in *2-propanol R* and dilute to 10.0 mL with the same solvent. Dilute 1.0 mL of the solution to 20.0 mL with the mobile phase.

Reference solution (a) Dissolve 5 mg of the substance to be examined and 5 mg of *levetiracetam impurity D CRS* in the mobile phase and dilute to 5.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *cellulose derivative of silica gel for chiral separation R* (10 µm).

Mobile phase *2-propanol R, heptane R* (18:82 *V/V*).

Flow rate 0.8 mL/min.

Detection Spectrophotometer at 205 nm.

Injection 20 µL.

Run time 1.4 times the retention time of levetiracetam.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity D.

Relative retention With reference to levetiracetam (retention time = about 12 min): impurity D = about 0.8.

System suitability Reference solution (a):
— *resolution*: minimum 1.5 between the peaks due to impurity D and levetiracetam;
— *symmetry factor*: maximum 2.4 for the peak due to levetiracetam.

Limit:
— *impurity D*: maximum 0.8 per cent.
— *reporting threshold*: 0.10 per cent (reference solution (b)).

Impurity G

Liquid chromatography (*2.2.29*).

Buffer solution Dissolve 1.22 g of *sodium decanesulfonate R* in 850 mL of *water for chromatography R*, add 1.3 mL of *phosphoric acid R*, adjust to pH 3.0 with a 200 g/L solution of *potassium hydroxide R* and dilute to 1000.0 mL with *water for chromatography R*.

Test solution Dissolve 20 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dissolve 2.0 mg of *levetiracetam impurity G CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 20.0 mL with the mobile phase.

Reference solution (c) To 1.0 mL of reference solution (a) add 1.0 mL of the test solution and dilute to 20.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 27 °C.

Mobile phase *acetonitrile R1*, buffer solution (15:85 *V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 200 nm.

Injection 50 µL of the test solution and reference solutions (b) and (c).

Run time 5 times the retention time of levetiracetam.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity G.

Relative retention With reference to levetiracetam (retention time = about 4 min): impurity G = about 3.8.

System suitability Reference solution (c):
— *resolution*: minimum 5.0 between the peaks due to levetiracetam and impurity G.

Calculation of percentage content:
— for impurity G, use the concentration of impurity G in reference solution (b).

Limit:
— *impurity G*: maximum 0.05 per cent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 50.0 mg of the substance to be examined in mobile phase A and dilute to 10.0 mL with mobile phase A.

Test solution (b) Dilute 1.0 mL of test solution (a) to 50.0 mL with mobile phase A.

Reference solution (a) Dissolve 5 mg of *levetiracetam impurity A CRS* and 5 mg of *levetiracetam impurity E CRS* in mobile phase A, add 1.0 mL of test solution (a) and dilute to 100.0 mL with mobile phase A.

Reference solution (b) Dilute 1.0 mL of test solution (a) to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 20.0 mL with mobile phase A.

Reference solution (c) Dissolve 5.0 mg of *levetiracetam impurity C CRS* in mobile phase A and dilute to 100.0 mL with mobile phase A. Dilute 1.0 mL of the solution to 40.0 mL with mobile phase A.

Reference solution (d) Dissolve 50.0 mg of *levetiracetam CRS* in mobile phase A and dilute to 10.0 mL with mobile phase A. Dilute 1.0 mL of the solution to 50.0 mL with mobile phase A.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: *acetonitrile R1, phosphate buffer solution pH 5.5 R* (5:95 *V/V*);
— *mobile phase B*: *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	100	0
3 - 20	100 → 71	0 → 29
20 - 25	71	29

Flow rate 0.9 mL/min.

Detection Spectrophotometer at 205 nm.

Injection 10 µL of test solution (a) and reference solutions (a), (b) and (c).

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A and E; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity C.

Relative retention With reference to levetiracetam (retention time = about 11 min): impurity C = about 0.5; impurity A = about 0.7; impurity E = about 0.9.

System suitability Reference solution (a):
— *resolution*: minimum 3.5 between the peaks due to impurity E and levetiracetam.

Calculation of percentage contents:
— for impurity C, use the concentration of impurity C in reference solution (c);
— for impurities other than C, use the concentration of levetiracetam in reference solution (b).

Limits:
— *impurity A*: maximum 0.3 per cent;
— *impurity C*: maximum 250 ppm;
— *unspecified impurities*: for each impurity, maximum 0.05 per cent;
— *total*: maximum 0.4 per cent;
— *reporting threshold*: 0.03 per cent, except for impurity C.

Water (*2.5.32*)

Maximum 0.5 per cent, determined on 0.300 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

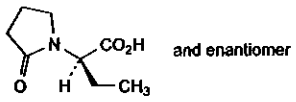
Injection Test solution (b) and reference solution (d).

Calculate the percentage content of $C_8H_{14}N_2O_2$ taking into account the assigned content of *levetiracetam CRS*.

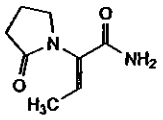
IMPURITIES

Specified impurities *A, C, D, G.*

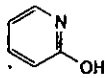
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, E.*



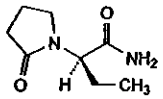
A. (2*RS*)-2-(2-oxopyrrolidin-1-yl)butanoic acid,



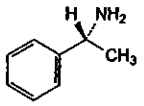
B. (2*Z*)-2-(2-oxopyrrolidin-1-yl)but-2-enamide,



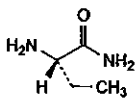
C. pyridin-2-ol,



D. (2*R*)-2-(2-oxopyrrolidin-1-yl)butanamide ((*R*)-etiracetam),



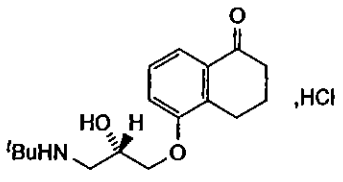
E. (1*R*)-1-phenylethan-1-amine,



G. (2*S*)-2-aminobutanamide.

Ph Eur

Levobunolol Hydrochloride



$C_{17}H_{25}NO_3,HCl$ 327.9 *27912-14-7*

Action and use

Beta-adrenoceptor antagonist.

Preparation

Levobunolol Eye Drops

DEFINITION

Levobunolol Hydrochloride is (*S*)-5-(3-*tert*-butylamino-2-hydroxypropoxy)-1,2,3,4-tetrahydronaphthalen-1-one hydrochloride. It contains not less than 98.5% and not more than 101.0% of $C_{17}H_{25}NO_3,HCl$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or pinkish white, crystalline powder.

Freely soluble in *water*, sparingly soluble in *ethanol (96%)*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of levobunolol hydrochloride *(RS 200)*.

B. Yields the reactions characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH of a 5% w/v solution, 4.5 to 6.5, Appendix V L.

Specific optical rotation

In a 3% w/v solution in *methanol*, –19.0 to –20.0, calculated with reference to the dried substance, Appendix V F.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using solutions in the mobile phase containing (1) 0.10% w/v of the substance being examined, (2) 0.00050% w/v of the substance being examined, (3) 0.0050% w/v of each of *levobunolol hydrochloride BPCRS* and *atenolol*.

The chromatographic procedure described under Assay may be used.

For solution (1) allow the chromatography to proceed for 3 times the retention time of the principal peak. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 8.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of any *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Loss on drying

When dried over *phosphorus pentoxide* at 110° at a pressure not exceeding 2 kPa for 4 hours, loses not more than 0.5% of its weight. Use 1 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using solutions in the mobile phase containing (1) 0.01% w/v of the substance being examined, (2) 0.01% w/v of *levobunolol hydrochloride BPCRS* and (3) 0.0050% w/v of each of *levobunolol hydrochloride BPCRS* and *atenolol*.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 3.9 mm) packed with *end-capped octylsilyl silica gel for chromatography* (10 µm) (Lichrosorb RP 8 is suitable), (b) a solution prepared by mixing 53 volumes of 0.005M *sodium heptanesulfonate* in *methanol* with 47 volumes of 0.005M *sodium heptanesulfonate* in *water* containing 1 mL of 0.5M *sulfuric acid* as the mobile phase with a flow rate of 1 mL per minute and (c) a detection wavelength of 223 nm.

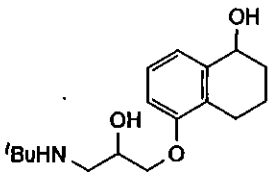
The test is not valid unless in the chromatogram obtained with solution (3) the *resolution factor* between the two principal peaks is at least 8.

Calculate the content of $C_{17}H_{25}NO_3$,HCl from the declared content of $C_{17}H_{25}NO_3$,HCl in *levobunolol hydrochloride BPCRS*.

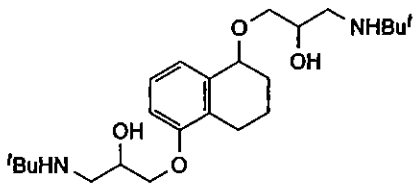
STORAGE

Levobunolol Hydrochloride should be protected from light.

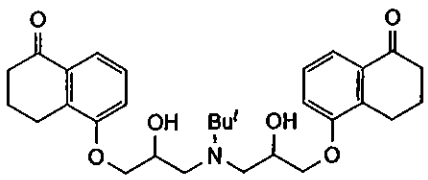
IMPURITIES



A. 5-(3-*tert*-butylamino-2-hydroxypropoxy)-1,2,3,4-tetrahydro-1-naphthol



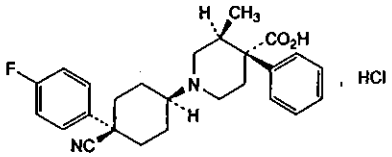
B. 1,1′-(1,2,3,4-tetrahydro-1,5-naphthalenedioxy)bis(3-*tert*-butylamino)-2-propanol



C. *meso*-5,5′-[(3,3′-*tert*-butylamino)bis(2-hydroxypropoxy)] bis-3,4-dihydronaphthalen-1(2 *H*)-one

Levocabastine Hydrochloride

(Ph. Eur. monograph 1484)



$C_{26}H_{30}ClFN_2O_2$ 457.0 *79547-78-7*

Action and use
Histamine H_1 receptor antagonist; antihistamine.

Ph Eur

DEFINITION

(3*S*,4*R*)-1-[*cis*-4-Cyano-4-(4-fluorophenyl)cyclohexyl]-3-methyl-4-phenylpiperidine-4-carboxylic acid hydrochloride.

Content
98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Practically insoluble in water, sparingly soluble in methanol, slightly soluble in ethanol (96 per cent) and in a 2 g/L solution of sodium hydroxide.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *levocabastine hydrochloride CRS*.

C. Dissolve 50 mg in a mixture of 0.4 mL of *ammonia R* and 2 mL of *water R*. Mix, allow to stand for 5 min and filter. Acidify the filtrate with *dilute nitric acid R*. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S
Dissolve 0.250 g in *methanol R* and dilute to 25.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*)
−106 to −102 (dried substance), determined on solution S.

Related substances
Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Test solution Dissolve 50 mg of the substance to be examined in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dissolve the contents of a vial of *levocabastine for system suitability 1 CRS* (containing impurities A, B, E, J and K) in 1 mL of *methanol R*.

Reference solution (b) Dilute 1.0 mL of the test solution to 20.0 mL with *methanol R*. Dilute 1.0 mL of this solution to 10.0 mL with *methanol R*.

Column:
— *size*: *l* = 0.10 m, Ø = 2.1 mm;
— *stationary phase*: *end-capped ethylene-bridged phenylsilyl silica gel for chromatography (hybrid material) R* (1.7 µm);
— *temperature*: 60 °C.

Mobile phase:
— *mobile phase A*: 17 g/L solution of *tetrabutylammonium hydrogen sulfate R*;
— *mobile phase B*: *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 0.5	95	5
0.5 - 3.5	95 → 90	5 → 10
3.5 - 6.0	90 → 85	10 → 15
6.0 - 11.0	85 → 70	15 → 30
11.0 - 14.5	70 → 20	30 → 80
14.5 - 15.5	20	80

Flow rate 0.45 mL/min.

Detection Spectrophotometer at 214 nm.

Injection 2.0 µL.

Identification of impurities Use the chromatogram supplied with *levocabastine for system suitability 1 CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, E, J and K.

Relative retention With reference to levocabastine (retention time = about 6.5 min): impurity A = about 0.85; impurity J = about 0.86; impurity B = about 0.90; impurity E = about 0.94; impurity K = about 1.07.

System suitability Reference solution (a):
— *peak-to-valley ratio*: minimum 2.9, where H_p = height above the baseline of the peak due to impurity K and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to levocabastine; minimum 5.0, where H_p = height above the baseline of the peak due to impurity J and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity A.

Calculation of percentage contents:
— for each impurity, use the concentration of levocabastine hydrochloride in reference solution (b).

Limits:
— *impurity E*: maximum 0.4 per cent;
— *impurity A*: maximum 0.2 per cent;
— *impurity B*: maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.6 per cent;
— *reporting threshold*: 0.05 per cent.

Impurity C

Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Test solution Dissolve 50 mg of the substance to be examined in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dissolve the contents of a vial of *levocabastine for system suitability 2 CRS* (containing impurity C) in 1 mL of *methanol R*.

Reference solution (b) Dilute 1.0 mL of the test solution to 20.0 mL with *methanol R*. Dilute 1.0 mL of this solution to 10.0 mL with *methanol R*.

Column:
— *size*: *l* = 0.15 m, Ø = 2.1 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography compatible with 100 per cent aqueous mobile phases R* (1.8 µm);
— *temperature*: 35 °C.

Mobile phase:
— *mobile phase A*: 17 g/L solution of *tetrabutylammonium hydrogen sulfate R*;
— *mobile phase B*: *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 0.5	90	10
0.5 - 15.5	90 → 80	10 → 20
15.5 - 20.5	80 → 50	20 → 50

Flow rate 0.30 mL/min.

Detection Spectrophotometer at 214 nm.

Injection 2.0 µL.

Identification of impurities Use the chromatogram supplied with *levocabastine for system suitability 2 CRS* and the chromatogram obtained with reference solution (a) to identify the peak due to impurity C.

Relative retention With reference to levocabastine (retention time = about 16 min): impurity C = about 0.98.

System suitability Reference solution (a):
— *peak-to-valley ratio*: minimum 10.0, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to levocabastine.

Calculation of percentage content:
— for impurity C, use the concentration of levocabastine hydrochloride in reference solution (b).

Limit:
— *impurity C*: maximum 0.3 per cent.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Dissolve 0.175 g in 50 mL of *ethanol (96 per cent) R*, previously neutralised to *phenol red solution R*, and add 5.0 mL of *water R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume at the 2nd point of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 22.85 mg of $C_{26}H_{30}ClFN_2O_2$.

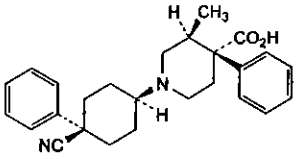
STORAGE

Protected from light.

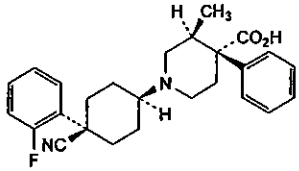
IMPURITIES

Specified impurities *A, B, C, E.*

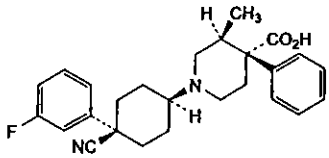
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *D, F, G, H, I, J, K, L.*



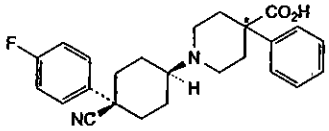
A. (3*S*,4*R*)-1-(*cis*-4-cyano-4-phenylcyclohexyl)-3-methyl-4-phenylpiperidine-4-carboxylic acid,



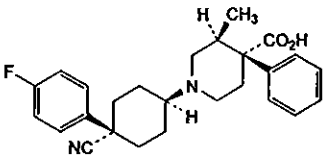
B. (3*S*,4*R*)-1-[*cis*-4-cyano-4-(2-fluorophenyl)cyclohexyl]-3-methyl-4-phenylpiperidine-4-carboxylic acid,



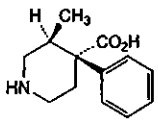
C. (3*S*,4*R*)-1-[*cis*-4-cyano-4-(3-fluorophenyl)cyclohexyl]-3-methyl-4-phenylpiperidine-4-carboxylic acid,



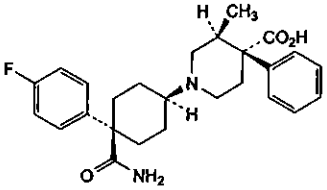
D. 1-[*cis*-4-cyano-4-(4-fluorophenyl)cyclohexyl]-4-phenylpiperidine-4-carboxylic acid,



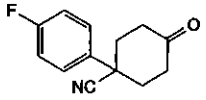
E. (3*S*,4*R*)-1-[*trans*-4-cyano-4-(4-fluorophenyl)cyclohexyl]-3-methyl-4-phenylpiperidine-4-carboxylic acid,



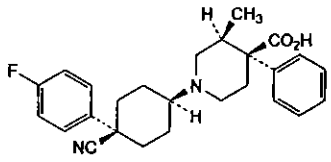
F. (3*S*,4*R*)-3-methyl-4-phenylpiperidine-4-carboxylic acid,



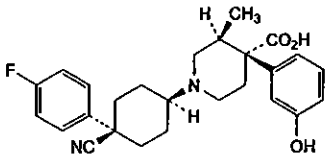
G. (3*S*,4*R*)-1-[*cis*-4-carbamoyl-4-(4-fluorophenyl)cyclohexyl]-3-methyl-4-phenylpiperidine-4-carboxylic acid,



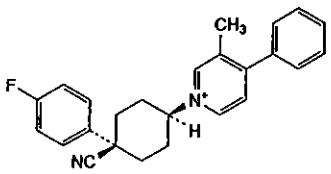
H. 1-(4-fluorophenyl)-4-oxocyclohexanecarbonitrile,



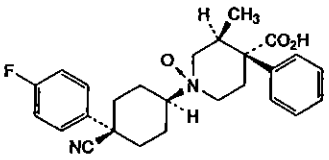
I. (3*S*,4*S*)-1-[*cis*-4-cyano-4-(4-fluorophenyl)cyclohexyl]-3-methyl-4-phenylpiperidine-4-carboxylic acid,



J. (3*S*,4*R*)-1-[*cis*-4-cyano-4-(4-fluorophenyl)cyclohexyl]-4-(3-hydroxyphenyl)-3-methylpiperidine-4-carboxylic acid,



K. 1-[*cis*-4-cyano-4-(4-fluorophenyl)cyclohexyl]-3-methyl-4-phenylpyridinium,

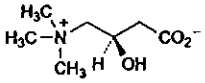


L. (3*S*,4*R*)-1-[*cis*-4-cyano-4-(4-fluorophenyl)cyclohexyl]-3-methyl-4-phenylpiperidine-4-carboxylic acid 1-oxide.

Ph Eur

Levocarnitine

(Ph. Eur. monograph 1339)



$C_7H_{15}NO_3$ 161.2 *541-15-1*

Action and use
Carnitine substitute.

Ph Eur

DEFINITION
(3*R*)-3-Hydroxy-4-(trimethylazaniumyl)butanoate.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder or colourless crystals, hygroscopic.

Solubility
Freely soluble in water, soluble in warm ethanol (96 per cent), practically insoluble in acetone.

IDENTIFICATION

First identification: A, B.

Second identification: A, C.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs, prepared using substance previously dried *in vacuo* at 50 °C for 5 h.

Comparison *levocarnitine CRS.*

C. To 1 mL of solution S (see Tests) add 9 mL of *water R*, 10 mL of *dilute sulfuric acid R* and 30 mL of *ammonium reineckate solution R*. A pink precipitate is formed. Allow to stand for 30 min. Filter and wash with *water R*, with *ethanol (96 per cent) R* and then with *acetone R* and dry at 80 °C. The precipitate melts (*2.2.14*) at 147 °C to 150 °C.

TESTS

Solution S

Dissolve 5.00 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50.0 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)

6.5 to 8.5.

Dilute 10 mL of solution S to 20 mL with *carbon dioxide-free water R*.

Specific optical rotation (*2.2.7*)

−32.0 to −29.0 (anhydrous substance), determined on solution S at 25 °C.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 20.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 12.5 mg of *levocarnitine impurity A CRS* in *water R* and dilute to 50.0 mL with the same solvent. Dilute 2.0 mL of the solution to 20.0 mL with the mobile phase.

Reference solution (c) Dissolve 50 mg of the substance to be examined in 10 mL of reference solution (b).

Column:

— *size*: *l* = 0.30 m, Ø = 3.9 mm;

— *stationary phase*: *aminopropylsilyl silica gel for chromatography R* (10 µm);

— *temperature*: 30 °C.

Mobile phase Mix 35 volumes of a 6.81 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 4.7 with *dilute sodium hydroxide solution R*, and 65 volumes of *acetonitrile R1*.

Flow rate 1 mL/min.

Detection Spectrophotometer at 205 nm.

Injection 25 µL.

Run time Twice the retention time of levocarnitine.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity A.

Relative retention With reference to levocarnitine (retention time = about 10 min): impurity A = about 1.1.

System suitability Reference solution (c):

— *resolution*: minimum 1.5 between the peaks due to levocarnitine and impurity A.

Limits:

— *impurity A*: not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total (excluding impurity A)*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Chlorides (*2.4.4*)

Maximum 200 ppm.

Dilute 2.5 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)

Maximum 300 ppm.

Dilute 5 mL of solution S to 15 mL with *distilled water R*.

Water (*2.5.12*)

Maximum 1.0 per cent, determined on 2.00 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.125 g in a mixture of 3 volumes of *anhydrous formic acid R* and 50 volumes of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 16.12 mg of $C_7H_{15}NO_3$.

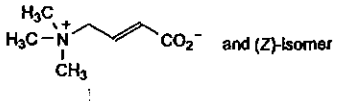
STORAGE

In an airtight container.

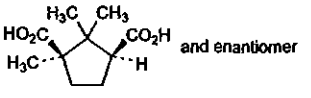
IMPURITIES

Specified impurities *A.*

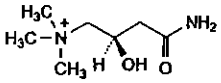
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D.*



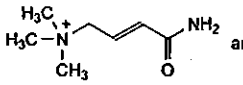
A. (2*EZ*)-4-(trimethylazaniumyl)but-2-enoate,



B. (1*RS*,3*SR*)-1,2,2-trimethylcyclopentane-1,3-dicarboxylic acid (camphoric acid),



C. (2*R*)-4-amino-2-hydroxy-*N*,*N*,*N*-trimethyl-4-oxobutan-1-aminium (carnitinamide),

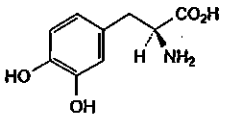


D. (2*EZ*)-4-amino-*N*,*N*,*N*-trimethyl-4-oxobut-2-en-1-aminium.

Ph Eur

Levodopa

(Ph. Eur. monograph 0038)



$C_9H_{11}NO_4$ 197.2 *59-92-7*

Action and use
Dopamine precursor; treatment of Parkinson's disease.

Preparations
Co-beneldopa Capsules
Co-beneldopa Dispersible Tablets
Co-beneldopa Prolonged-release Capsules
Co-careldopa Tablets

When L-dopa is prescribed or demanded, Levodopa shall be dispensed or supplied.

Ph Eur

DEFINITION
(2*S*)-2-Amino-3-(3,4-dihydroxyphenyl)propanoic acid.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Slightly soluble in water, practically insoluble in ethanol (96 per cent). It is freely soluble in *1 M hydrochloric acid* and sparingly soluble in *0.1 M hydrochloric acid*.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *levodopa CRS*.

TESTS

Appearance of solution
The solution is not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).
Dissolve 1.0 g in a 103 g/L solution of *hydrochloric acid R* and dilute to 25 mL with the same solution.

pH (*2.2.3*)
4.5 to 7.0.
Shake 0.10 g with 10 mL of *carbon dioxide-free water R* for 15 min.

Related substances
Liquid chromatography (*2.2.29*). *Use freshly prepared solutions.*
Solution A 10.3 g/L solution of *hydrochloric acid R*.
Test solution Dissolve 0.100 g of the substance to be examined in solution A and dilute to 25 mL with solution A.
Reference solution (a) Dilute 1.0 mL of the test solution to 50.0 mL with solution A. Dilute 5.0 mL of this solution to 100.0 mL with solution A.
Reference solution (b) Dissolve 8 mg of *tyrosine R* (impurity B) and 4 mg of *3-methoxy-L-tyrosine R* (L-isomer of impurity C) in 2 mL of the test solution and dilute to 50 mL with solution A. Dilute 5 mL of this solution to 100 mL with solution A.
Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: spherical *di-isobutyloctadecylsilyl silica gel for chromatography R* (5 µm) with a pore size of 8 nm.
Mobile phase:
— *mobile phase A*: 0.1 M phosphate buffer solution pH 3.0 R;
— *mobile phase B*: *methanol R*, 0.1 M phosphate buffer solution pH 3.0 R (18:85 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 18	90	10
18 - 22	90 → 0	10 → 100
22 - 35	0	100

Flow rate 1 mL/min.
Detection Spectrophotometer at 280 nm.
Injection 20 µL.
Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B and C.
Relative retention With reference to levodopa (retention time = about 6 min): impurity A = about 0.7; impurity B = about 2; impurity C = about 3.5.
System suitability Reference solution (b):
— *resolution*: minimum 10 between the peaks due to levodopa and impurity B.
Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity B by 2.2;
— *impurity B*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *impurity C*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent);
— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
— *disregard limit*: 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.03 per cent).

Enantiomeric purity
Liquid chromatography (*2.2.29*). *Use freshly prepared solutions.*

Test solution Dissolve 25 mg of the substance to be examined in the mobile phase and dilute to 25 mL with the mobile phase.

Reference solution (a) Dilute 5.0 mL of the test solution to 20.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution (b) Dissolve 10 mg of *D-dopa R* (impurity D) in 10 mL of the test solution. Dilute 1 mL of this solution to 100 mL with the mobile phase.

Column:
— *size*: *l* = 0.15 m, Ø = 3.9 mm;
— *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve separately 200 mg of *copper acetate R* and 387 mg of *N,N-dimethyl-L-phenylalanine R* in 250 mL of *water R*; mix the 2 solutions and adjust immediately to pH 4.0 with *acetic acid R*; add 50 mL of *methanol R* and dilute to 1000 mL with *water R*; mix and filter.

Flow rate 1 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µL.

Run time Twice the retention time of levodopa.

Relative retention With reference to levodopa (retention time = about 7 min): impurity D = about 0.4.

System suitability Reference solution (b):
— *resolution*: minimum 5 between the peaks due to impurity D and levodopa.

Limit:
— *impurity D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent).

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 0.500 g by drying in an oven at 105 °C.

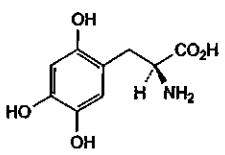
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.150 g, heating if necessary, in 5 mL of *anhydrous formic acid R*. Add 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

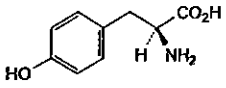
1 mL of *0.1 M perchloric acid* is equivalent to 19.72 mg of $C_9H_{11}NO_4$.

STORAGE
Protected from light.

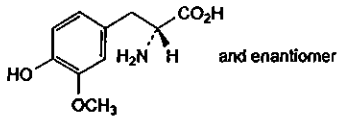
IMPURITIES
Specified impurities *A, B, C, D.*



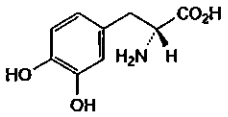
A. (2*S*)-2-amino-3-(2,4,5-trihydroxyphenyl)propanoic acid,



B. (2*S*)-2-amino-3-(4-hydroxyphenyl)propanoic acid (tyrosine),



C. (2*RS*)-2-amino-3-(4-hydroxy-3-methoxyphenyl)propanoic acid (3-methoxy-DL-tyrosine),

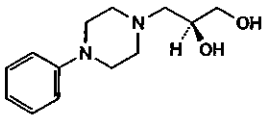


D. (2*R*)-2-amino-3-(3,4-dihydroxyphenyl)propanoic acid (D-dopa).

Ph Eur

Levodropropizine

(Ph. Eur. monograph 1535)



$C_{13}H_{20}N_2O_2$ 236.3 *99291-25-5*

Action and use
Cough suppressant.

Ph Eur

DEFINITION
(2*S*)-3-(4-Phenylpiperazin-1-yl)propane-1,2-diol.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white powder.

Solubility
Slightly soluble in water, freely soluble in dilute acetic acid and in methanol, slightly soluble in ethanol (96 per cent).

IDENTIFICATION
Carry out either tests A, B or tests B, C.

A. Specific optical rotation (*2.2.7*): −33.5 to −30.0 (dried substance).

Dissolve 1.50 g in a 21 g/L solution of *hydrochloric acid R* and dilute to 50.0 mL with the same acid.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *levodropropizine CRS.*

C. Enantiomeric purity (see Tests).

TESTS
pH (*2.2.3*)
9.2 to 10.2.

Suspend 2.5 g in *carbon dioxide-free water R*, heat to dissolve, cool to room temperature and dilute to 100 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 25.0 mg of *levodropropizine impurity B CRS* in *methanol R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of this solution to 100.0 mL with the mobile phase.

Reference solution (b) Mix 1 mL of the test solution with 1 mL of reference solution (a).

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped extra-dense bonded octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 12 volumes of *methanol R* and 88 volumes of a 6.81 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.0 with *phosphoric acid R*.

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL.

Run time Twice the retention time of levodropropizine.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity B.

Relative retention With reference to levodropropizine (retention time = about 7 min): impurity B = about 1.2.

System suitability Reference solution (b):
- *resolution*: minimum 2.0 between the peaks due to levodropropizine and impurity B.

Limits:
- *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *unspecified impurities*: for each impurity, not more than 0.2 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 1.2 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (a) (0.6 per cent);
- *disregard limit*: 0.1 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (a) (0.05 per cent).

Impurity C

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use. Use vials sealed with a crimp-top to prevent evaporation of the solvent.*

Test solution Dissolve 50 mg of *sodium diethyldithiocarbamate R* and 1.0 g of the substance to be examined in *methanol R* and dilute to 5.0 mL with the same solvent. Heat at 60 °C for 20 min and then cool to room temperature. Add 5 mL of *water R* and 0.5 mL of *phosphoric acid R*. Extract with 5 mL of *methylene chloride R*.

Reference solution (a) Dissolve 0.100 g of *levodropropizine impurity C CRS* in *methanol R* and dilute to 50.0 mL with the same solvent. Dilute 1.0 mL of the solution to 20.0 mL with *methanol R*. Dilute 1.0 mL of this solution to 100.0 mL with *methanol R*.

Reference solution (b) Dissolve 50 mg of *sodium diethyldithiocarbamate R* in reference solution (a) and dilute to 5.0 mL with reference solution (a). Heat at 60 °C for 20 min and then cool to room temperature. Add 5 mL of *water R* and 0.5 mL of *phosphoric acid R*. Extract with 5 mL of *methylene chloride R*.

Reference solution (c) Dissolve 50 mg of *sodium diethyldithiocarbamate R* and 1.0 g of the substance to be examined in reference solution (a) and dilute to 5.0 mL with reference solution (a). Heat at 60 °C for 20 min and then cool to room temperature. Add 5 mL of *water R* and 0.5 mL of *phosphoric acid R*. Extract with 5 mL of *methylene chloride R*.

Blank solution Dissolve 50 mg of *sodium diethyldithiocarbamate R* in *methanol R* and dilute to 5.0 mL with the same solvent. Heat at 60 °C for 20 min and then cool to room temperature. Add 5 mL of *water R* and 0.5 mL of *phosphoric acid R*. Extract with 5 mL of *methylene chloride R*.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *aminopropylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *methanol R, tetrahydrofuran R, heptane R* (15:15:70 *V/V/V*).

Flow rate 1 mL/min.

Detection Spectrophotometer at 278 nm.

Injection 20 µL of the blank solution, the test solution and reference solutions (b) and (c).

Run time 3 times the retention time of impurity C.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity C.

Retention time Impurity C = about 8 min.

System suitability Reference solution (b):
- *signal-to-noise ratio*: minimum 50 for the peak due to impurity C.

Calculate the content of impurity C in parts per million using the following expression:

$$\frac{A}{B-A} \times 5$$

A = area of the peak due to impurity C in the chromatogram obtained with the test solution;

B = area of the peak due to impurity C in the chromatogram obtained with reference solution (c).

Limit:
- *impurity C*: maximum 5 ppm.

Enantiomeric purity

Liquid chromatography (*2.2.29*).

Solvent mixture *anhydrous ethanol R, hexane R* (40:60 *V/V*).

Test solution Dissolve 10.0 mg of the substance to be examined in 10.0 mL of the solvent mixture. Dilute 1.0 mL of this solution to 50.0 mL with the solvent mixture.

Reference solution (a) Dissolve 10 mg of *levodropropizine CRS* in 10.0 mL of the solvent mixture. Dilute 1.0 mL of this solution to 50.0 mL with the solvent mixture.

Reference solution (b) Dissolve 10.0 mg of *levodropropizine impurity A CRS* in 10 mL of the solvent mixture. Dilute 1 mL of this solution to 50 mL with the solvent mixture.

Reference solution (c) Dilute 1.0 mL of reference solution (b) to 50.0 mL with the solvent mixture.

Reference solution (d) Dilute 0.5 mL of reference solution (b) to 25 mL with reference solution (a).

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *cellulose derivative of silica gel for chiral separation R* (10 µm).

Mobile phase *diethylamine R, anhydrous ethanol R, hexane R* (0.2:5:95 *V/V/V*).

Flow rate 0.8 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL of the test solution and reference solutions (a), (c) and (d).

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peak due to impurity A.

Relative retention With reference to levodropropizine (retention time = about 28 min): impurity A = about 0.9.

System suitability:
— *retention times*: the retention times of the principal peaks in the chromatograms obtained with the test solution and reference solution (a) are similar;
— *resolution*: minimum 1.3 between the peaks due to impurity A and levodropropizine in the chromatogram obtained with reference solution (d).

Limit:
— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (2 per cent).

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 0.500 g by drying *in vacuo* at 60 °C at a pressure of 0.15-0.25 kPa for 4 h.

Sulfated ash (*2.4.14*)
Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 50 mL of *anhydrous acetic acid R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M perchloric acid*. Read the volume added at the 2nd point of inflexion.

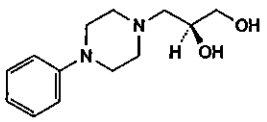
1 mL of *0.1 M perchloric acid* is equivalent to 11.82 mg of $C_{13}H_{20}N_2O_2$.

STORAGE

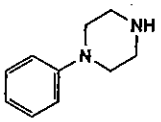
Protected from light.

IMPURITIES

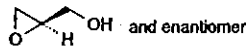
Specified impurities *A, B, C.*



A. (2*R*)-3-(4-phenylpiperazin-1-yl)propane-1,2-diol (dextrodropropizine),



B. 1-phenylpiperazine,



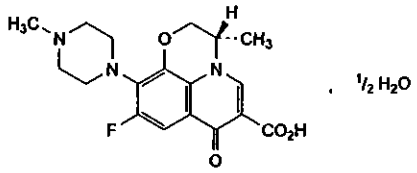
and enantiomer

C. [(2*RS*)-oxiran-2-yl]methanol (glycidol).

Ph Eur

Levofloxacin Hemihydrate

(Ph. Eur. monograph 2598)



$C_{18}H_{20}FN_3O_4,½H_2O$ 370.4 *138199-71-0*

Action and use
Fluoroquinolone antibacterial.

Ph Eur

DEFINITION

(3*S*)-9-Fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid hemihydrate.

Content
98.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance
Light yellowish-white or light yellow, crystalline powder.

Solubility
Sparingly soluble in water, freely soluble in acetic acid, sparingly soluble in methanol, slightly soluble in anhydrous ethanol.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *levofloxacin hemihydrate CRS.*

B. Examine the chromatograms obtained in the test for related substances.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (b).

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution GY_3 (*2.2.2, Method II*).

Dissolve 0.100 g in *water R* and dilute to 10 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Buffer solution Solution containing 1.25 g/L of *copper sulfate pentahydrate R*, 1.3 g/L of *isoleucine R* and 8.5 g/L of *ammonium acetate R* in *water for chromatography R*.

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve the contents of a vial of *levofloxacin for system suitability CRS* (containing impurities A, B and G) in 1 mL of the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 45 °C.

Mobile phase *methanol R*, buffer solution (30:70 *V/V*).

Flow rate 0.8 mL/min.

Detection Spectrophotometer at 360 nm.

Injection 25 µL.

Run time 3 times the retention time of levofloxacin.

Identification of impurities Use the chromatogram supplied with *levofloxacin for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B and G.

Relative retention With reference to levofloxacin (retention time = about 20 min): impurity B = about 0.50; impurity G = about 0.56; impurity A = about 1.22.

System suitability Reference solution (b):
— *resolution*: minimum 1.5 between the peaks due to impurities B and G.

Calculation of percentage contents:
— *correction factor*: multiply the peak area of impurity B by 1.3;
— for each impurity, use the concentration of levofloxacin hemihydrate in reference solution (a).

Limits:
— *impurity A*: maximum 0.5 per cent;
— *impurity B*: maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.6 per cent;
— *reporting threshold*: 0.05 per cent.

Impurity F

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 5.0 mg of *levofloxacin impurity F CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 25.0 mL with the mobile phase.

Reference solution (c) Dilute 4 mL of reference solution (a) to 10 mL with the mobile phase. Dilute 1 mL of this solution to 10 mL with the test solution.

Mobile phase *methanol R*, buffer solution (50:50 *V/V*).

Detection Spectrophotometer at 320 nm.

Injection Test solution and reference solutions (b) and (c).

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity F.

Relative retention With reference to levofloxacin (retention time = about 6 min): impurity F = about 1.8.

System suitability Reference solution (c):
— *resolution*: minimum 5.0 between the peaks due to levofloxacin and impurity F.

Calculation of percentage content:
— for impurity F, use the concentration of impurity F in reference solution (b).

Limit:
— *impurity F*: maximum 0.2 per cent.

Water (*2.5.12*)

2.0 per cent to 3.0 per cent, determined on 0.500 g using a mixture of equal volumes of *formamide R* and *methanol R* as solvent.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Dissolve 0.300 g in 100 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 36.14 mg of $C_{18}H_{20}FN_3O_4$.

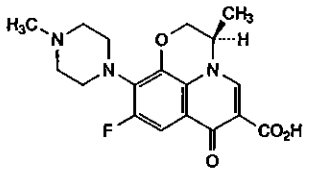
STORAGE

Protected from light.

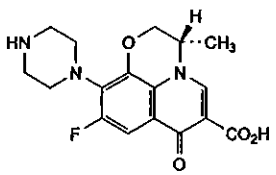
IMPURITIES

Specified impurities *A, B, F.*

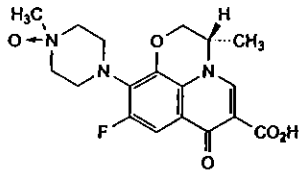
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C, D, E, G, H, I.*



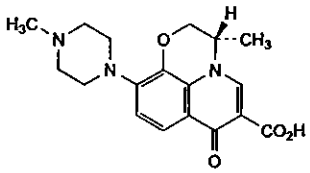
A. (3*R*)-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid (enantiomer of levofloxacin),



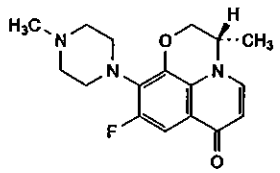
B. (3*S*)-9-fluoro-3-methyl-7-oxo-10-(piperazin-1-yl)-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid,



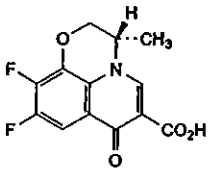
C. 4-[(3*S*)-6-carboxy-9-fluoro-3-methyl-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazin-10-yl]-1-methylpiperazine 1-oxide,



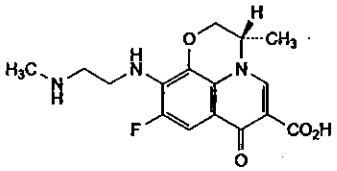
D. (3*S*)-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid,



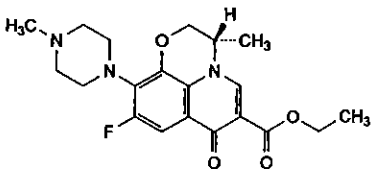
E. (3*S*)-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazin-7-one,



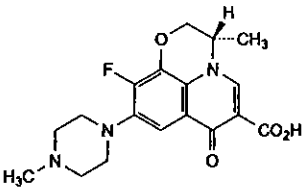
F. (3*S*)-9,10-difluoro-3-methyl-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid,



G. (3*S*)-9-fluoro-3-methyl-10-[[2-(methylamino)ethyl]amino]-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid,



H. ethyl (3*S*)-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylate,

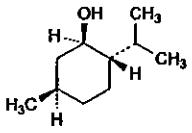


I. (3*S*)-10-fluoro-3-methyl-9-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid.

Ph Eur

Levomenthol

(Ph. Eur. monograph 0619)



$C_{10}H_{20}O$ 156.3 *2216-51-5*

Action and use
Decongestant.

Preparations
Levomenthol Cream
Menthol and Benzoin Inhalation

Ph Eur

DEFINITION
(1*R*,2*S*,5*R*)-5-Methyl-2-(1-methylethyl)cyclohexanol.

CHARACTERS

Appearance
Prismatic or acicular, colourless, shiny crystals.

Solubility
Practically insoluble in water, very soluble in ethanol (96 per cent) and in light petroleum, freely soluble in fatty oils and in liquid paraffin, very slightly soluble in glycerol.

mp
About 43 °C.

IDENTIFICATION
First identification: A, C.
Second identification: B, D.
A. Specific optical rotation (see Tests).
B. Thin-layer chromatography (*2.2.27*).
Test solution Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 5 mL with the same solvent.
Reference solution Dissolve 25 mg of *menthol CRS* in *methanol R* and dilute to 5 mL with the same solvent.
Plate *TLC silica gel G plate R.*
Mobile phase *ethyl acetate R, toluene R* (5:95 *V/V*).
Application 2 µL.
Development Over a path of 15 cm.
Drying In air, until the solvents have evaporated.
Detection Spray with *anisaldehyde solution R* and heat at 100-105 °C for 5-10 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. Examine the chromatograms obtained in the test for related substances.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in position and approximate dimensions to the principal peak in the chromatogram obtained with reference solution (c).

D. Dissolve 0.20 g in 0.5 mL of *anhydrous pyridine R*. Add 3 mL of a 150 g/L solution of *dinitrobenzoyl chloride R* in *anhydrous pyridine R*. Heat on a water-bath for 10 min. Add 7.0 mL of *water R* in small quantities with stirring and allow to stand in iced water for 30 min. A precipitate is formed. Allow to stand and decant the supernatant. Wash the precipitate with 2 quantities, each of 5 mL, of iced *water R*, recrystallise from 10 mL of *acetone R*, wash with iced *acetone R* and dry at 75 °C at a pressure not exceeding 2.7 kPa for 30 min. The crystals melt (*2.2.14*) at 154 °C to 157 °C.

TESTS

Solution S

Dissolve 2.50 g in 10 mL of *ethanol (96 per cent) R* and dilute to 25.0 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

Dissolve 1.0 g in *ethanol (96 per cent) R* and dilute to 10 mL with the same solvent. Add 0.1 mL of *phenolphthalein solution R*. The solution is colourless. Not more than 0.5 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink.

Specific optical rotation (*2.2.7*)

−48 to −51, determined on solution S.

Related substances

Gas chromatography (*2.2.28*).

Test solution (a) Dissolve 0.20 g of the substance to be examined in *methylene chloride R* and dilute to 50.0 mL with the same solvent.

Test solution (b) Dilute 1.0 mL of test solution (a) to 10.0 mL with *methylene chloride R*.

Reference solution (a) Dissolve 40.0 mg of the substance to be examined and 40.0 mg of *isomenthol R* in *methylene chloride R* and dilute to 100.0 mL with the same solvent.

Reference solution (b) Dilute 0.10 mL of test solution (a) to 100.0 mL with *methylene chloride R*.

Reference solution (c) Dissolve 40.0 mg of *menthol CRS* in *methylene chloride R* and dilute to 100.0 mL with the same solvent.

Column:
- *material*: glass;
- *size*: *l* = 2.0 m, Ø = 2 mm;
- *stationary phase*: diatomaceous earth for gas chromatography R impregnated with 15 per cent *m/m* of *macrogol 1500 R*.

Carrier gas *nitrogen for chromatography R*.

Flow rate 30 mL/min.

Temperature:
- *column*: 120 °C;
- *injection port*: 150 °C;
- *detector*: 200 °C.

Detection Flame ionisation.

Injection 1 µL.

Run time Twice the retention time of menthol.

System suitability:
- *resolution*: minimum 1.4 between the peaks due to menthol and isomenthol in the chromatogram obtained with reference solution (a);
- *signal-to-noise ratio*: minimum 5 for the principal peak in the chromatogram obtained with reference solution (b).

Limits Test solution (a):
- *total*: not more than 1 per cent of the area of the principal peak;
- *disregard limit*: 0.05 per cent of the area of the principal peak.

Residue on evaporation

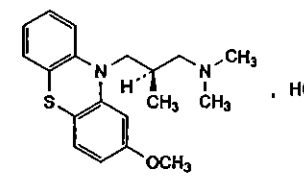
Maximum 0.05 per cent.

Evaporate 2.00 g on a water-bath and heat in an oven at 100-105 °C for 1 h. The residue weighs not more than 1.0 mg.

Ph Eur

Levomepromazine Hydrochloride

(Ph. Eur. monograph 0505)



$C_{19}H_{25}ClN_2OS$ 364.9 *1236-99-3*

Action and use

Dopamine receptor antagonist; neuroleptic.

Preparation

Levomepromazine Injection

Ph Eur

DEFINITION

(2*R*)-3-(2-Methoxy-10*H*-phenothiazin-10-yl)-*N*,*N*,2-trimethylpropan-1-amine hydrochloride.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or very slightly yellow, crystalline powder, slightly hygroscopic.

Solubility

Freely soluble in water and in ethanol (96 per cent).

It deteriorates when exposed to air and light.

It shows polymorphism (*5.9*). It exists in 2 forms, one melting at about 142 °C and the other at about 162 °C.

IDENTIFICATION

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*). *Prepare the solution protected from bright light and carry out the measurements immediately.*

Test solution Dissolve 50.0 mg in *water R* and dilute to 500.0 mL with the same solvent. Dilute 10.0 mL of the solution to 100.0 mL with *water R*.

Spectral range 230-340 nm.

Absorption maxima 250 nm and 302 nm.

Specific absorbance at the absorption maximum at 250 nm 640 to 700.

B. Identification of phenothiazines by thin-layer chromatography (*2.3.3*): use *levomepromazine hydrochloride CRS* to prepare the reference solution.

C. Introduce 0.2 g into a 100 mL separating funnel. Add 5 mL of *water R* and 0.5 mL of *strong sodium hydroxide solution R*. Shake vigorously with two quantities, each of 10 mL, of *ether R*. Combine the ether layers, dry over *anhydrous sodium sulfate R* and evaporate to dryness. Keep the residue at 100 °C to 105 °C for 15 min and allow to crystallise in iced water. Initiate crystallisation if necessary by scratching the wall of the flask with a glass rod. Dry the crystals at 60 °C for 2 h. The crystals melt (*2.2.14*) at 122 °C to 128 °C.

D. Dissolve 20 mg in 2 mL of *methanol R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 25.0 mL with the same solvent.

Acidity or alkalinity

To 10 mL of solution S, add 0.1 mL of *bromocresol green solution R*. Not more than 0.5 mL of a 0.40 g/L solution of *sodium hydroxide R* or 1.0 mL of a 1.03 g/L solution of *hydrochloric acid R* is required to change the colour of the indicator.

Specific optical rotation (*2.2.7*)

+ 9.5 to + 11.5 (dried substance), determined on solution S.

Related substances

Thin-layer chromatography (*2.2.27*). *Prepare the solutions immediately before use and carry out the test protected from bright light.*

Solvent mixture *diethylamine R, methanol R* (5:95 *V/V*).

Test solution Dissolve 0.2 g of the substance to be examined in the solvent mixture and dilute to 10 mL with the solvent mixture.

Reference solution Dilute 0.5 mL of the test solution to 100 mL with the solvent mixture.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *acetone R, diethylamine R, cyclohexane R* (10:10:80 *V/V/V*).

Application 10 µL.

Development Over 2/3 of the plate.

Drying In air, until the solvents have evaporated.

Detection Examine in ultraviolet light at 254 nm.

Retardation factor Levomepromazine ≠ about 0.5.

Limit:

— *any impurity*: any spot, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 5 mL of *water R* and add 50 mL of *2-propanol R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M sodium hydroxide* is equivalent to 36.49 mg of $C_{19}H_{25}ClN_2OS$.

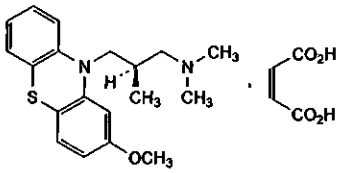
STORAGE

In an airtight container, protected from light.

Ph Eur

Levomepromazine Maleate

(Ph. Eur. monograph 0925)



$C_{23}H_{28}N_2O_5S$ 444.6 *7104-38-3*

Action and use

Dopamine receptor antagonist; neuroleptic.

Preparation

Levomepromazine Tablets

Ph Eur

DEFINITION

Levomepromazine maleate contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (2*R*)-3-(2-methoxy-10*H*-phenothiazin-10-yl)-*N*,*N*,2-trimethylpropan-1-amine (*Z*)-butenedioate, calculated with reference to the dried substance.

CHARACTERS

A white or slightly yellowish, crystalline powder, slightly soluble in water, sparingly soluble in methylene chloride, slightly soluble in alcohol. It deteriorates when exposed to air and light.

It melts at about 186 °C, with decomposition.

IDENTIFICATION

First identification: A, B.

Second identification: A, C, D.

A. Specific optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *levomepromazine maleate CRS*. Examine the substances prepared as discs.

C. Identification test for phenothiazines by thin-layer chromatography (*2.3.3*): use *levomepromazine maleate CRS* to prepare the reference solution.

D. Examine by thin-layer chromatography (*2.2.27*), using *silica gel GF_{254} R* as the coating substance.

Test solution Dissolve 0.20 g of the substance to be examined in a mixture of 10 volumes of *water R* and 90 volumes of *acetone R* and dilute to 10 mL with the same mixture of solvents.

Reference solution Dissolve 50 mg of *maleic acid CRS* in a mixture of 10 volumes of *water R* and 90 volumes of

acetone R and dilute to 10 mL with the same mixture of solvents.

Apply separately to the plate as bands 10 mm by 2 mm 5 µL of each solution. Develop over a path of 12 cm using a mixture of 3 volumes of *water R*, 7 volumes of *anhydrous formic acid R* and 90 volumes of *di-isopropyl ether R*. Dry the plate at 120 °C for 10 min and examine in ultraviolet light at 254 nm. The chromatogram obtained with the test solution shows a zone at the point of application and another zone similar in position and size to the principal zone in the chromatogram obtained with the reference solution.

TESTS

pH (*2.2.3*)

Carry out the test protected from bright light Introduce 0.50 g into a conical flask and add 25.0 mL of *carbon dioxide-free water R*. Shake and allow the solids to settle. The pH of the supernatant solution is 3.5 to 5.5.

Specific optical rotation (*2.2.7*)

Dissolve 1.25 g in *dimethylformamide R* and dilute to 25.0 mL with the same solvent. The specific optical rotation is −7.0 to −8.5, calculated with reference to the dried substance.

Related substances

Carry out the test protected from bright light and prepare the solutions immediately before use Examine by thin-layer chromatography (*2.2.27*), using *silica gel GF_{254} R* as the coating substance.

Test solution Dissolve 0.20 g of the substance to be examined in a mixture of 10 volumes of *water R* and 90 volumes of *acetone R* and dilute to 10 mL with the same mixture of solvents.

Reference solution Dilute 0.5 mL of the test solution to 100 mL with a mixture of 10 volumes of *water R* and 90 volumes of *acetone R*.

Apply separately to the plate 10 µL of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *acetone R*, 10 volumes of *diethylamine R* and 80 volumes of *cyclohexane R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulfated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

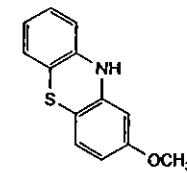
Dissolve 0.350 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 44.46 mg of $C_{23}H_{28}N_2O_5S$.

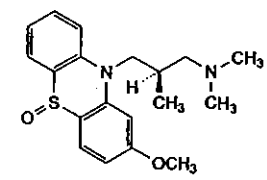
STORAGE

Store protected from light.

IMPURITIES



A. 2-methoxyphenothiazine,

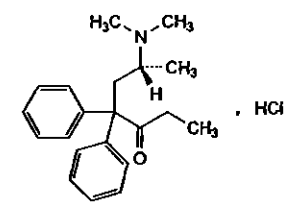


B. 10-[(2*R*)-3-(dimethylamino)-2-methylpropyl]-2-methoxy-10*H*-phenothiazine 5-oxide.

Ph Eur

Levomethadone Hydrochloride

(Ph. Eur. monograph 1787)



$C_{21}H_{28}ClNO$ 345.9 *5967-73-7*

Action and use

Opioid analgesic.

Ph Eur

DEFINITION

(6*R*)-6-(Dimethylamino)-4,4-diphenylheptan-3-one hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: A, C, D.

Second identification: A, B, D.

A. Specific optical rotation (see Tests).

B. Melting point (*2.2.14*): 239 °C to 242 °C.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of methadone hydrochloride.

D. Dilute 1 mL of solution S (see Tests) to 5 mL with *water R* and add 1 mL of *dilute ammonia R1*. Mix, allow to stand for 5 min and filter. The filtrate gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 50.0 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

Dilute 10 mL of solution S to 25 mL with *carbon dioxide-free water R*. To 10 mL of the solution add 0.2 mL of *methyl red solution R* and 0.2 mL of *0.01 M sodium hydroxide*.
The solution is yellow. Add 0.4 mL of *0.01 M hydrochloric acid*. The solution is red.

Specific optical rotation (*2.2.7*)

−125 to −135 (dried substance), determined on solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 50.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 12.0 mg of *imipramine hydrochloride CRS* in the mobile phase and dilute to 10 mL with the mobile phase. To 1 mL of the solution add 5 mL of the test solution and dilute to 10 mL with the mobile phase.

Column:
— *size*: *l* = 0.125 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 25 °C.

Mobile phase Mix 35 volumes of *acetonitrile R* and 65 volumes of an 11.5 g/L solution of *phosphoric acid R* adjusted to pH 3.6 with *tetraethylammonium hydroxide solution R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 210 nm.

Equilibration About 30 min.

Injection 10 µL.

Run time 7 times the retention time of levomethadone.

Retention time Levomethadone = about 5 min.

System suitability Reference solution (b):
— *resolution*: minimum 2.5 between the peaks due to imipramine and levomethadone.

Limits:
— *any impurity*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Dextromethadone

Liquid chromatography (*2.2.29*).

Test solution Dissolve 40.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution Dilute 1.0 mL of the test solution to 10.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 20.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *2-hydroxypropylbetadex for chromatography R* (5 µm);
— *temperature*: 10 °C.

Mobile phase Mix 1 volume of *triethylamine R* adjusted to pH 4.0 with *phosphoric acid R*, 15 volumes of *acetonitrile R* and 85 volumes of a 13.6 g/L solution of *potassium dihydrogen phosphate R*.

Flow rate 0.7 mL/min.

Detection Spectrophotometer at 210 nm.

Equilibration About 30 min.

Injection 10 µL.

Relative retention With reference to levomethadone: dextromethadone = about 1.4.

System suitability Test solution:
— *number of theoretical plates*: minimum 2000, calculated for the peak due to levomethadone;
— *tailing factor*: maximum 3 for the peak due to levomethadone.

Limit:
— *dextromethadone*: not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in a mixture of 40 mL of *water R* and 5 mL of *acetic acid R*. Titrate with *0.1 M silver nitrate*. Determine the end-point potentiometrically (*2.2.20*), using a silver electrode.

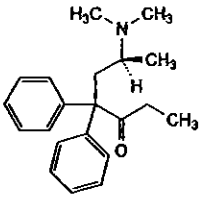
1 mL of *0.1 M silver nitrate* is equivalent to 34.59 mg of $C_{21}H_{28}ClNO$.

STORAGE

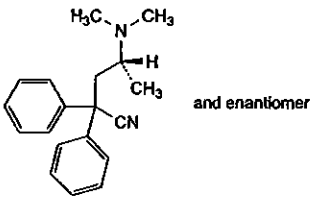
Protected from light.

IMPURITIES

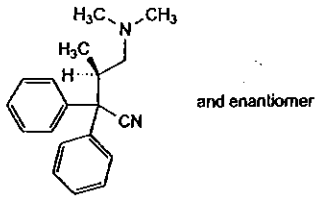
Specified impurities *A, B, C, D, E, F.*



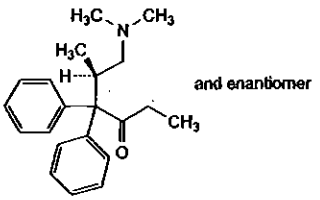
A. (6*S*)-6-(dimethylamino)-4,4-diphenylheptan-3-one,



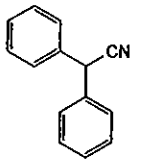
B. (4*RS*)-4-(dimethylamino)-2,2-diphenylpentanenitrile,



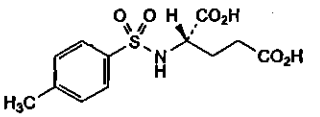
C. (3*RS*)-4-(dimethylamino)-3-methyl-2,2-diphenylbutanenitrile,



D. (5*RS*)-6-(dimethylamino)-5-methyl-4,4-diphenylhexan-3-one,



E. diphenylacetonitrile,

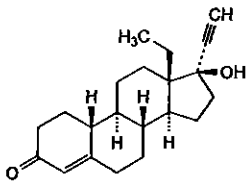


F. (2*S*)-2-[[(4-methylphenyl)sulfonyl]amino]pentanedioic acid (*N*-*p*-tosyl-L-glutamic acid).

Ph Eur

Levonorgestrel

(Ph. Eur. monograph 0926)



$C_{21}H_{28}O_2$ 312.5 *797-63-7*

Action and use
Progestogen.

Preparations
Levonorgestrel Tablets
Levonorgestrel and Ethinylestradiol Tablets

Ph Eur

DEFINITION
13-Ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, sparingly soluble in methylene chloride, slightly soluble in ethanol (96 per cent).

IDENTIFICATION
A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *levonorgestrel CRS.*

TESTS

Specific optical rotation (*2.2.7*)
−35 to −30.

Dissolve 0.200 g in *methylene chloride R* and dilute to 20.0 mL with the same solvent.

Related substances
A. Impurities A, B, H, K, M, O, S, U. Liquid chromatography (*2.2.29*).

Solvent mixture *water R, acetonitrile R* (30:70 *V/V*).

Test solution Dissolve 10.0 mg of the substance to be examined in 7 mL of *acetonitrile R* using sonication and dilute to 10.0 mL with *water R*.

Reference solution (a) Dissolve 5 mg of *levonorgestrel for system suitability 1 CRS* (containing impurities A, H, K, M, O and S) in 3.5 mL of *acetonitrile R* using sonication and dilute to 5 mL with *water R*.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 5.0 mg of *levonorgestrel impurity B CRS* in 35 mL of *acetonitrile R* and dilute to 50.0 mL with *water R*. Dilute 1.0 mL of the solution to 100.0 mL with the solvent mixture.

Reference solution (d) Dissolve 5.0 mg of *norethisterone CRS* (impurity U) in 35 mL of *acetonitrile R* and dilute to 50.0 mL with *water R*. Dilute 1.0 mL of the solution to 100.0 mL with the solvent mixture.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octylsilyl silica gel for chromatography with embedded polar groups R* (5 µm);
- *temperature*: 30 °C.

Mobile phase:
- *mobile phase A*: *acetonitrile R1, water for chromatography R* (40:60 *V/V*);
- *mobile phase B*: *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 50	100 → 20	0 → 80

Flow rate 0.7 mL/min.

Detection Spectrophotometer at 215 nm and, for impurity O, at 200 nm.

Injection 50 µL.

Identification of impurities Use the chromatograms supplied with *levonorgestrel for system suitability 1 CRS* and the chromatograms obtained with reference solution (a) at 215 nm to identify the peaks due to impurities A, H, K, M and S, and at 200 nm to identify the peak due to impurity O; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity B; use the

chromatogram obtained with reference solution (d) to identify the peak due to impurity U.

Relative retention With reference to levonorgestrel (retention time = about 20 min): impurity H = about 0.5; impurity U = about 0.8; impurity K = about 0.85; impurity A = about 0.91; impurity M = about 0.95; impurity O = about 1.16; impurity B = about 1.26; impurity S = about 1.9.

System suitability:

- *signal-to-noise ratio*: minimum 60 for the principal peak in the chromatogram obtained with reference solution (b);
- *peak-to-valley ratio*: minimum 3.0, where H_p = height above the baseline of the peak due to impurity M and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity A in the chromatogram obtained with reference solution (a).

Calculation of percentage contents:

- *correction factors*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.4; impurity M = 3.1; impurity O = 2.6;
- for impurity B, use the concentration of impurity B in reference solution (c);
- for impurity U, use the concentration of impurity U in reference solution (d);
- for impurities other than B and U, use the concentration of levonorgestrel in reference solution (b).

Limits:

- *impurities A, B, K*: for each impurity, maximum 0.3 per cent;
- *impurity O at 200 nm*: maximum 0.3 per cent;
- *impurities M, S, U*: for each impurity, maximum 0.2 per cent;
- *impurity H*: maximum 0.15 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *sum of impurities other than O*: maximum 1.0 per cent;
- *reporting threshold*: 0.05 per cent.

B. Impurities V and W. Liquid chromatography (*2.2.29*).

Solvent mixture *water R, acetonitrile R* (30:70 *V/V*).

Test solution Dissolve 10.0 mg of the substance to be examined in 7 mL of *acetonitrile R* using sonication and dilute to 10.0 mL with *water R*.

Reference solution (a) Dissolve 5 mg of *levonorgestrel for system suitability 2 CRS* (containing impurities V and W) in 3.5 mL of *acetonitrile R* using sonication and dilute to 5 mL with *water R*.

Reference solution (b) Dissolve 5.0 mg of *ethinylestradiol CRS* in 35 mL of *acetonitrile R* using sonication and dilute to 50.0 mL with *water R*. Dilute 3.0 mL of the solution to 100.0 mL with the solvent mixture.

Column:

- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography compatible with 100 per cent aqueous mobile phases R* (3 µm).

Mobile phase:

- *mobile phase A*: *acetonitrile R1, water for chromatography R* (40:60 *V/V*);
- *mobile phase B*: *water for chromatography R, acetonitrile R1* (10:90 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 1	92	8
1 - 3	92 → 82	8 → 18
3 - 6	82	18
6 - 16	82 → 60	18 → 40
16 - 21	60 → 0	40 → 100
21 - 32	0	100

Flow rate 1 mL/min.

Detection Spectrophotometer at 200 nm.

Injection 50 µL.

Identification of impurities Use the chromatogram supplied with *levonorgestrel for system suitability 2 CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities V and W.

Relative retention With reference to levonorgestrel (retention time = about 12 min): impurity W = about 0.9; impurity V = about 1.9.

System suitability Reference solution (a):

- *resolution*: minimum 2.8 between the peaks due to impurity W and levonorgestrel.

Calculation of percentage contents:

- for each impurity, use the concentration of ethinylestradiol in reference solution (b).

Limits:

- *impurity W*: maximum 0.3 per cent;
- *impurity V*: maximum 0.15 per cent.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 45 mL of *tetrahydrofuran R*. Add 10 mL of a 100 g/L solution of *silver nitrate R*. After 1 min, titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 mL of *0.1 M sodium hydroxide* is equivalent to 31.25 mg of $C_{21}H_{28}O_2$.

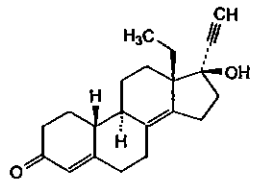
STORAGE

Protected from light.

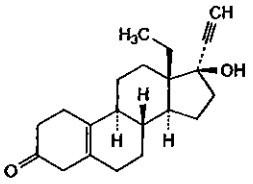
IMPURITIES

Specified impurities *A, B, H, K, M, O, S, U, V, W.*

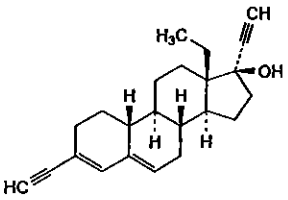
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C, D, G, I, J, L, N, P, Q, R, T.*



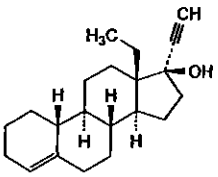
A. 13-ethyl-17-hydroxy-18,19-dinor-17α-pregna-4,8(14)-dien-20-yn-3-one,



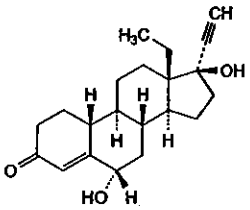
B. 13-ethyl-17-hydroxy-18,19-dinor-17α-pregn-5(10)-en-20-yn-3-one,



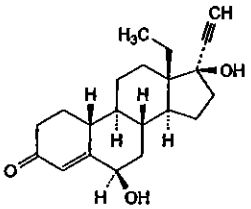
C. 13-ethyl-3-ethynyl-18,19-dinor-17α-pregna-3,5-dien-20-yn-17-ol,



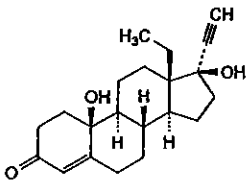
D. 13-ethyl-18,19-dinor-17α-pregn-4-en-20-yn-17-ol (3-deoxolevonorgestrel),



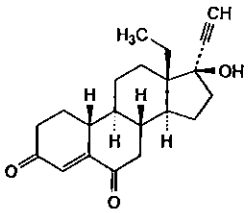
G. 13-ethyl-6α,17-dihydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one (6α-hydroxylevonorgestrel),



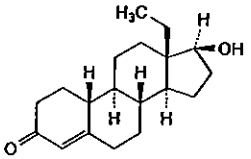
H. 13-ethyl-6β,17-dihydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one (6β-hydroxylevonorgestrel),



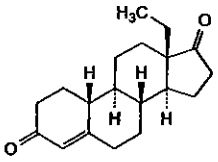
I. 13-ethyl-10,17-dihydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one (10-hydroxylevonorgestrel),



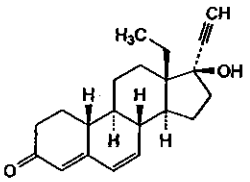
J. 13-ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yne-3,6-dione (6-oxolevonorgestrel),



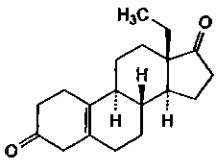
K. 13-ethyl-17β-hydroxygon-4-en-3-one (18-methylnandrolone),



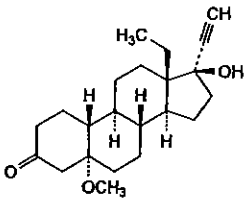
L. 13-ethylgon-4-ene-3,17-dione (levodione),



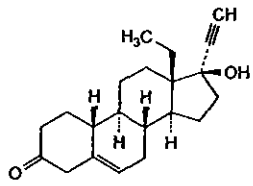
M. 13-ethyl-17-hydroxy-18,19-dinor-17α-pregna-4,6-dien-20-yn-3-one (Δ6-levonorgestrel),



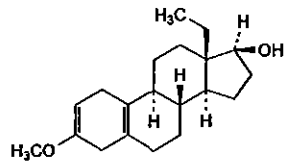
N. 13-ethylgon-5(10)-ene-3,17-dione (Δ5(10)-levodione),



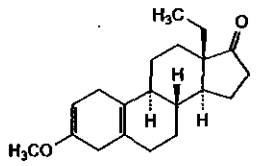
O. 13-ethyl-17-hydroxy-5α-methoxy-18,19-dinor-17α-pregn-20-yn-3-one (4,5-dihydro-5α-methoxylevonorgestrel),



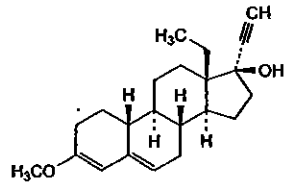
P. 13-ethyl-17-hydroxy-18,19-dinor-17α-pregn-5-en-20-yn-3-one (Δ5-levonorgestrel),



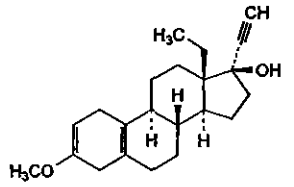
Q. 13-ethyl-3-methoxygona-2,5(10)-dien-17β-ol,



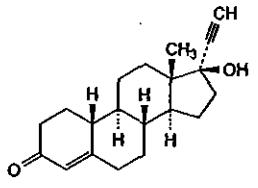
R. 13-ethyl-3-methoxygona-2,5(10)-dien-17-one,



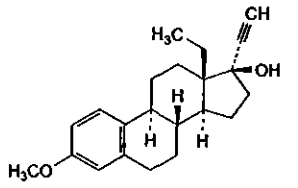
S. 13-ethyl-3-methoxy-18,19-dinor-17α-pregna-3,5-dien-20-yn-17-ol,



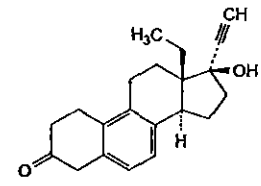
T. 13-ethyl-3-methoxy-18,19-dinor-17α-pregna-2,5(10)-dien-20-yn-17-ol,



U. 17-hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one (norethisterone),



V. 13-ethyl-3-methoxy-18,19-dinor-17α-pregna-1,3,5(10)-trien-20-yn-17-ol,

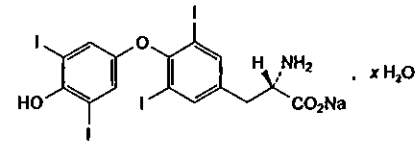


W. 13-ethyl-17-hydroxy-18,19-dinor-17α-pregna-5,7,9-trien-20-yn-3-one.

Ph Eur

Levothyroxine Sodium

(Ph. Eur. Monograph 0401)



$C_{15}H_{10}I_4NNaO_4,xH_2O$ ($x \approx 5$) 799 *25416-65-3*
(anhydrous substance)

Action and use
Thyroid hormone replacement.

Preparations
Levothyroxine Oral Solution
Levothyroxine Tablets

Ph Eur

DEFINITION
Sodium (2*S*)-2-amino-3-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]propanoate.

Content
97.0 per cent to 102.0 per cent (anhydrous substance).
It contains a variable quantity of water.

CHARACTERS
Appearance
Almost white or slightly brownish-yellow, fine, slightly hygroscopic, crystalline powder.

Solubility
Very slightly soluble in water, slightly soluble in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *levothyroxine sodium CRS.*

B. To 200 mg add 2 mL of *dilute sulfuric acid R.* Heat on a water-bath and then carefully over a naked flame, increasing the temperature gradually up to 600 ± 50 °C. Continue the ignition until most of the black particles have disappeared. Dissolve the residue in 2 mL of *water R.* The solution gives reaction (a) of sodium (*2.3.1*).

TESTS
Solution S
Dissolve 0.500 g in 23 mL of a gently boiling mixture of 1 volume of *1 M hydrochloric acid* and 4 volumes of *ethanol (96 per cent) R.* Cool and dilute to 25.0 mL with the same mixture of solvents.

Appearance of solution
Freshly prepared solution S is not more intensely coloured than reference solution BY_3 (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*)
+ 16 to + 20 (anhydrous substance), determined on freshly prepared solution S.

Related substances
Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Solvent mixture Mobile phase A, *ethanol (96 per cent) R* (1:2 *V/V*).

Test solution Dissolve 25.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture. Dilute 10.0 mL of the solution to 25.0 mL with the solvent mixture.

Reference solution (a) Dissolve 2.5 mg of *levothyroxine sodium CRS* and 2.5 mg of *liothyronine sodium CRS* (impurity A) in the solvent mixture and dilute to 25.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 50.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 25.0 mg of *levothyroxine sodium CRS* in the solvent mixture and dilute to 50.0 mL with the solvent mixture. Dilute 10.0 mL of the solution to 25.0 mL with the solvent mixture.

Reference solution (d) Dissolve 2.0 mg of *levothyroxine for peak identification CRS* (containing impurities F and G) in 10.0 mL of the solvent mixture and sonicate for 10 min.

Column:
— *size*: *l* = 0.15 m, Ø = 4.0 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
— *mobile phase A*: dissolve 1.97 g of *phosphoric acid R* in *water R* and dilute to 2 L with the same solvent;
— *mobile phase B*: dissolve 1.97 g of *phosphoric acid R* in *acetonitrile R1* and dilute to 2 L with the same solvent;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	70	30
10 - 40	70 → 20	30 → 80
40 - 50	20	80

Flow rate 1 mL/min.

Detection Spectrophotometer at 225 nm.

Injection 25 µL of the test solution and reference solutions (a), (b) and (d).

Identification of impurities Use the chromatogram supplied with *levothyroxine for peak identification CRS* and the chromatogram obtained with reference solution (d) to identify the peaks due to impurities F and G.

Relative retention With reference to levothyroxine (retention time = about 11 min): impurity A = about 0.5; impurity F = about 2.0; impurity G = about 2.4.

System suitability Reference solution (a):
— *resolution*: minimum 5.0 between the peaks due to impurity A and levothyroxine.

Limits:
— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
— *impurity F*: not more than 5 times the area of the peak due to levothyroxine in the chromatogram obtained with reference solution (b) (0.5 per cent);
— *impurity G*: not more than 3 times the area of the peak due to levothyroxine in the chromatogram obtained with reference solution (b) (0.3 per cent);
— *unspecified impurities*: for each impurity, not more than twice the area of the peak due to levothyroxine in the chromatogram obtained with reference solution (b) (0.2 per cent);
— *total*: maximum 2.0 per cent;
— *disregard limit*: 0.5 times the area of the peak due to levothyroxine in the chromatogram obtained with reference solution (b) (0.05 per cent).

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Water (*2.5.32*)
6.0 per cent to 12.0 per cent, determined on 0.100 g.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

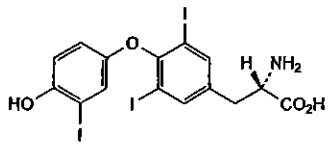
Injection Test solution and reference solution (c).

Calculate the percentage content of $C_{15}H_{10}I_4NNaO_4$ taking into account the assigned content of *levothyroxine sodium CRS*.

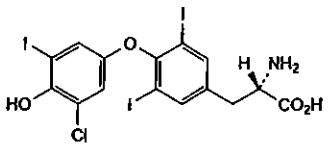
STORAGE
In an airtight container, protected from light, at a temperature of 2 °C to 8 °C.

IMPURITIES
Specified impurities *A, F, G.*

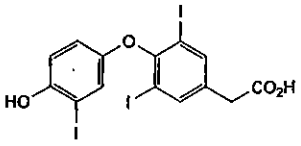
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D, E, H, I, J, K.*



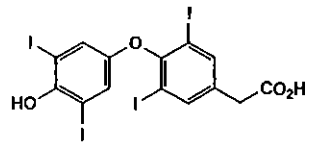
A. (2*S*)-2-amino-3-[4-(4-hydroxy-3-iodophenoxy)-3,5-diiodophenyl]propanoic acid (liothyronine),



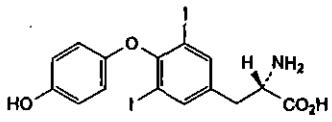
B. (2*S*)-2-amino-3-[4-(3-chloro-4-hydroxy-5-iodophenoxy)-3,5-diiodophenyl]propanoic acid,



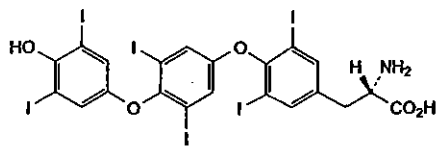
C. [4-(4-hydroxy-3-iodophenoxy)-3,5-diiodophenyl]acetic acid (triiodothyroacetic acid),



D. [4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]acetic acid (tetraiodothyroacetic acid),

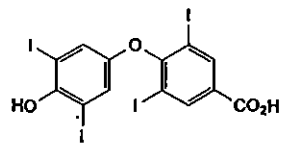


E. (2*S*)-2-amino-3-[4-(4-hydroxyphenoxy)-3,5-diiodophenyl]propanoic acid (diiodothyronine),

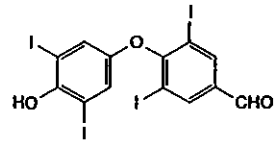


F. (2*S*)-2-amino-3-[4-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenoxy]-3,5-diiodophenyl]propanoic acid,

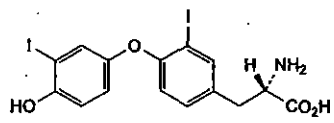
G. unknown structure,



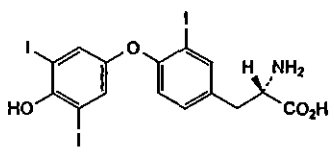
H. 4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodobenzoic acid,



I. 4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodobenzaldehyde,



J. (2*S*)-2-amino-3-[4-(4-hydroxy-3-iodophenoxy)-3-iodophenyl]propanoic acid,

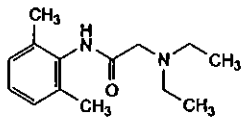


K. (2*S*)-2-amino-3-[4-(4-hydroxy-3,5-diiodophenoxy)-3-iodophenyl]propanoic acid.

Ph Eur

Lidocaine

(Ph. Eur. Monograph 0727)



$C_{14}H_{22}N_2O$ 234.3 *137-58-6*

Action and use
Local anaesthetic; Class I antiarrhythmic.

Preparation
Lidocaine Ointment

Ph Eur

DEFINITION
2-(Diethylamino)-*N*-(2,6-dimethylphenyl)acetamide.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, very soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION
First identification: A.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *lidocaine CRS.*

B. Melting point (*2.2.14*): 66 °C to 70 °C, determined without previous drying.

C. To about 5 mg add 0.5 mL of *fuming nitric acid R*. Evaporate to dryness on a water-bath, cool, and dissolve the residue in 5 mL of *acetone R*. Add 0.2 mL of *alcoholic potassium hydroxide solution R*. A green colour develops.

TESTS
Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *2,6-dimethylaniline R* (impurity A) in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 10.0 mL of this solution to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *2-chloro-N-(2,6-dimethylphenyl)acetamide R* (impurity H) in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (c) Dilute 1.0 mL of the test solution to 10.0 mL with the mobile phase.

Reference solution (d) Mix 1.0 mL of reference solution (a), 1.0 mL of reference solution (b) and 1.0 mL of reference solution (c), then dilute to 100.0 mL with the mobile phase.

Column:
— *size: l* = 0.15 m, Ø = 3.9 mm;
— *stationary phase: end-capped polar-embedded octadecylsilyl amorphous organosilica polymer R* (5 µm);
— *temperature*: 30 °C.

Mobile phase Mix 30 volumes of *acetonitrile for chromatography R* and 70 volumes of a 4.85 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 8.0 with *strong sodium hydroxide solution R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µL.

Run time 3.5 times the retention time of lidocaine.

Relative retention With reference to lidocaine (retention time = about 17 min): impurity H = about 0.37; impurity A = about 0.40.

System suitability Reference solution (d):
— *resolution*: minimum 1.5 between the peaks due to impurities H and A.

Limits:
— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.01 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the peak due to lidocaine in the chromatogram obtained with reference solution (d) (0.10 per cent);
— *total*: not more than 5 times the area of the peak due to lidocaine in the chromatogram obtained with reference solution (d) (0.5 per cent);
— *disregard limit*: 0.5 times the area of the peak due to lidocaine in the chromatogram obtained with reference solution (d) (0.05 per cent).

Chlorides (*2.4.4*)
Maximum 35 ppm.

Dissolve 1.4 g in a mixture of 3 mL of *dilute nitric acid R* and 12 mL of *water R*.

Sulfates (*2.4.13*)
Maximum 0.1 per cent.

Dissolve 0.2 g in 5 mL of *ethanol (96 per cent) R* and dilute to 20 mL with *distilled water R*.

Water (*2.5.12*)
Maximum 1.0 per cent, determined on 1.00 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

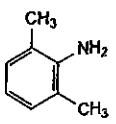
To 0.200 g add 50 mL of *anhydrous acetic acid R* and stir until dissolution is complete. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 23.43 mg of $C_{14}H_{22}N_2O$.

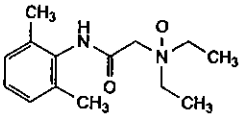
IMPURITIES

Specified impurities *A*.

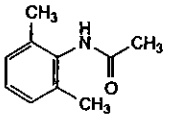
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D, E, F, G, H, I, J*.



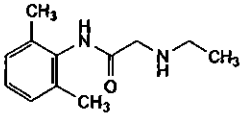
A. 2,6-dimethylaniline,



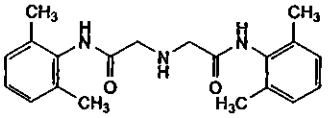
B. 2-(diethylazinoyl)-*N*-(2,6-dimethylphenyl)acetamide (lidocaine *N*-oxide),



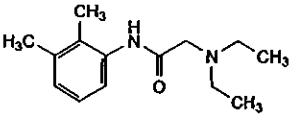
C. *N*-(2,6-dimethylphenyl)acetamide,



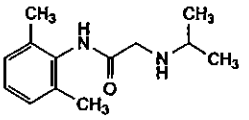
D. *N*-(2,6-dimethylphenyl)-2-(ethylamino)acetamide,



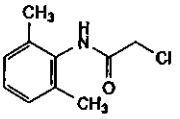
E. 2,2′-iminobis(*N*-(2,6-dimethylphenyl)acetamide),



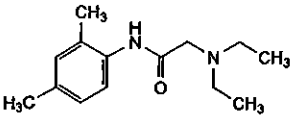
F. 2-(diethylamino)-*N*-(2,3-dimethylphenyl)acetamide,



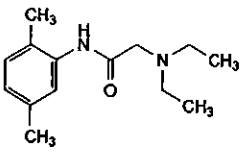
G. *N*-(2,6-dimethylphenyl)-2-((1-methylethyl)amino) acetamide,



H. 2-chloro-*N*-(2,6-dimethylphenyl)acetamide,



I. 2-(diethylamino)-*N*-(2,4-dimethylphenyl)acetamide,

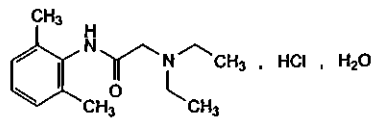


J. 2-(diethylamino)-*N*-(2,5-dimethylphenyl)acetamide.

Lidocaine Hydrochloride Monohydrate

Lidocaine Hydrochloride

(Ph. Eur. monograph 0227)



$C_{14}H_{23}ClN_2O,H_2O$ 288.8 *6108-05-0*

Action and use
Local anaesthetic; Class I antiarrhythmic.

Preparations
Lidocaine Gel

Lidocaine and Chlorhexidine Gel

Lidocaine Injection

Lidocaine and Adrenaline Injection/Lidocaine and Epinephrine Injection

Lidocaine Intraocular Injection

Lidocaine Sterile Solution

Ph Eur

DEFINITION

2-(Diethylamino)-*N*-(2,6-dimethylphenyl)acetamide hydrochloride monohydrate.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 74 °C to 79 °C, determined without previous drying.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *lidocaine hydrochloride CRS.*

C. To about 5 mg add 0.5 mL of *fuming nitric acid R*. Evaporate to dryness on a water-bath, cool and dissolve the residue in 5 mL of *acetone R*. Add 0.2 mL of *alcoholic potassium hydroxide solution R*. A green colour is produced.

D. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S
Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)
4.0 to 5.5.

Dilute 1 mL of solution S to 10 mL with *carbon dioxide-free water R*.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *2,6-dimethylaniline R* (impurity A) in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 10.0 mL of the solution to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve 5 mg of *2-chloro-N-(2,6-dimethylphenyl)acetamide R* (impurity H) in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (c) Dilute 1.0 mL of the test solution to 10.0 mL with the mobile phase.

Reference solution (d) Mix 1.0 mL of reference solution (a), 1.0 mL of reference solution (b) and 1.0 mL of reference solution (c), and dilute to 100.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.15 m, Ø = 3.9 mm;
— *stationary phase*: *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer R* (5 µm);
— *temperature*: 30 °C.

Mobile phase Mix 30 volumes of *acetonitrile for chromatography R* and 70 volumes of a 4.85 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 8.0 with *strong sodium hydroxide solution R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µL of the test solution and reference solutions (a), (b) and (d).

Run time 3.5 times the retention time of lidocaine.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity A; use the chromatogram obtained with reference solution (b) to identify the peak due to impurity H.

Relative retention With reference to lidocaine (retention time = about 17 min): impurity H = about 0.37; impurity A = about 0.40.

System suitability Reference solution (d):
— *resolution*: minimum 1.5 between the peaks due to impurities H and A.

Limits:
— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.01 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the peak due to lidocaine in the chromatogram obtained with reference solution (d) (0.10 per cent);
— *total*: not more than 5 times the area of the peak due to lidocaine in the chromatogram obtained with reference solution (d) (0.5 per cent);
— *disregard limit*: 0.5 times the area of the peak due to lidocaine in the chromatogram obtained with reference solution (d) (0.05 per cent); do not disregard the peak due to impurity A .

Water (*2.5.12*)
5.5 per cent to 7.0 per cent, determined on 0.25 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.220 g in 50 mL of *ethanol (96 per cent) R* and add 5.0 mL of *0.01 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 27.08 mg of $C_{14}H_{23}ClN_2O$.

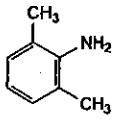
STORAGE

Protected from light.

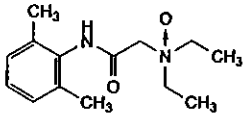
IMPURITIES

Specified impurities A.

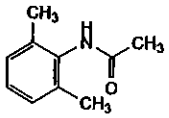
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) B, C, D, E, F, G, H, I, J, K.



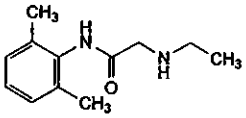
A. 2,6-dimethylaniline,



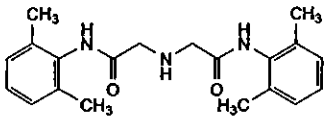
B. 2-(diethylazinoyl)-*N*-(2,6-dimethylphenyl)acetamide (lidocaine N^2-oxide),



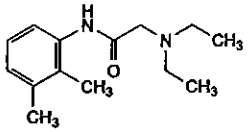
C. *N*-(2,6-dimethylphenyl)acetamide,



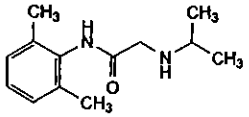
D. *N*-(2,6-dimethylphenyl)-2-(ethylamino)acetamide,



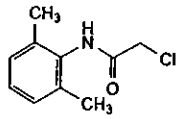
E. 2-2′-(azanediyl)bis[*N*-(2,6-dimethylphenyl)acetamide],



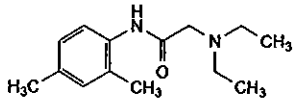
F. 2-(diethylamino)-*N*-(2,3-dimethylphenyl)acetamide,



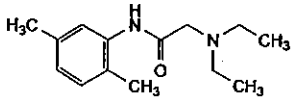
G. *N*-(2,6-dimethylphenyl)-2-[(1-methylethyl) amino]acetamide,



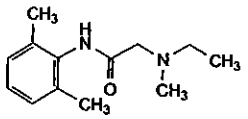
H. 2-chloro-*N*-(2,6-dimethylphenyl)acetamide,



I. 2-(diethylamino)-*N*-(2,4-dimethylphenyl)acetamide,



J. 2-(diethylamino)-*N*-(2,5-dimethylphenyl)acetamide,

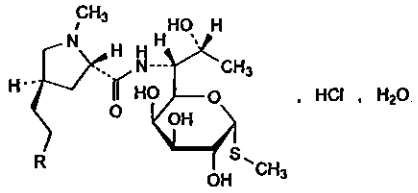


K. *N*-(2,6-dimethylphenyl)-2-(ethylmethylamino)acetamide.

Ph Eur

Lincomycin Hydrochloride

(Ph. Eur. monograph 0583)



Compound	R	Molecular formula	M_r
Lincomycin	CH_3	$C_{18}H_{35}ClN_2O_6S,H_2O$	461.0
Lincomycin B	H	$C_{17}H_{33}ClN_2O_6S,H_2O$	447.0

Lincomycin hydrochloride monohydrate *7179-49-9*

Action and use

Antibacterial.

Preparations

Lincomycin Capsules

Lincomycin Injection

Ph Eur

DEFINITION

Mixture of antibiotics produced by *Streptomyces lincolnensis* var. *lincolnensis* or obtained by any other means, the main component being methyl 6,8-dideoxy-6-[[[(2*S*,4*R*)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside (lincomycin) hydrochloride monohydrate.

Content

— *sum of the contents of lincomycin hydrochloride and lincomycin B hydrochloride*: 96.0 per cent to 102.0 per cent (anhydrous substance);

— *lincomycin B hydrochloride*: maximum 5.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Very soluble in water, slightly soluble in ethanol (96 per cent), very slightly soluble in acetone.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *lincomycin hydrochloride CRS.*

B. Dissolve 0.1 g in *water R* and dilute to 10 mL with the same solvent. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S
Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 20 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

pH (*2.2.3*)
3.5 to 5.5 for solution S.

Specific optical rotation (*2.2.7*)
+ 135 to + 150 (anhydrous substance).

Dissolve 1.000 g in *water R* and dilute to 25.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dissolve 25.0 mg of *lincomycin hydrochloride CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 5 mg of *lincomycin hydrochloride for system suitability CRS* (containing impurities A, B and C) in 2 mL of the mobile phase.

Reference solution (c) Dilute 2.0 mL of reference solution (a) to 100.0 mL with the mobile phase.

Reference solution (d) Dilute 1.0 mL of reference solution (c) to 20.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated end-capped octylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 50 °C.

Buffer solution pH 6.1 Dissolve 34 g of *phosphoric acid R* in 900 mL of *water for chromatography R*, adjust to pH 6.1 with *concentrated ammonia R* and dilute to 1000 mL with *water for chromatography R*.

Mobile phase *methanol R, acetonitrile R1,* buffer solution pH 6.1 (8:17:75 *V/V/V*).

Flow rate 1 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µL of the test solution and reference solutions (b), (c) and (d).

Run time 5.5 times the retention time of lincomycin.

Relative retention With reference to lincomycin (retention time = about 10 min): impurity C = about 0.4; lincomycin B = about 0.5; impurity A = about 0.7; impurity B = about 1.2 and 1.3.

System suitability Reference solution (b):
— *resolution*: minimum 1.8 between the peak due to lincomycin and the 1st peak due to impurity B.

Limits:
— *impurity A*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.5 per cent);
— *sum of the areas of the peaks due to impurity B*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.5 per cent);
— *impurity C*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (d) (0.2 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.10 per cent);
— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (2.0 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.05 per cent).

Water (*2.5.12*)
3.1 per cent to 4.6 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*)
Maximum 0.5 per cent, determined on 1.0 g.

Bacterial endotoxins (*2.6.14*)
Less than 0.50 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solutions (a) and (c).

Calculate the percentage content of $C_{18}H_{35}ClN_2O_6S$ (lincomycin) and $C_{17}H_{33}ClN_2O_6S$ (lincomycin B) taking into account the assigned content of $C_{18}H_{35}ClN_2O_6S$ in *lincomycin hydrochloride CRS*. Determine the content of lincomycin by comparing with the area of the peak due to lincomycin in the chromatogram obtained with reference solution (a). Determine the content of lincomycin B by comparing with the area of the peak due to lincomycin in the chromatogram obtained with reference solution (c).

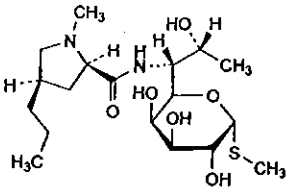
STORAGE

At a temperature not exceeding 30 °C. If the substance is sterile, store in a sterile, airtight, tamper-evident container.

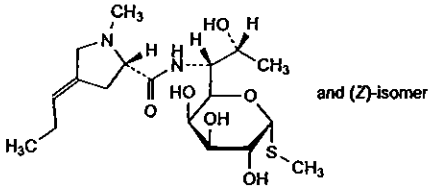
IMPURITIES

Specified impurities *A, B, C.*

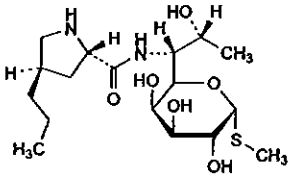
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *D, E, F.*



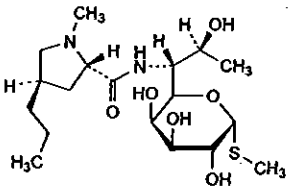
A. methyl 6,8-dideoxy-6-[[[(2*R*,4*R*)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside (α-amide epimer),



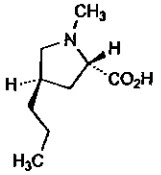
B. methyl 6,8-dideoxy-6-[[[(2*S*,4*EZ*)-1-methyl-4-propylidenepyrrolidin-2-yl]carbonyl]amino]-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside (propylidene analogues),



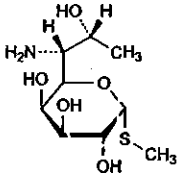
C. methyl 6,8-dideoxy-6-[[[(2*S*,4*R*)-4-propylpyrrolidin-2-yl]carbonyl]amino]-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside (*N*-desmethyl lincomycin),



D. methyl 6,8-dideoxy-6-[[[(2*S*,4*R*)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-1-thio-L-*threo*-α-D-*galacto*-octopyranoside (7-*epi*-lincomycin),



E. (2*S*,4*R*)-1-methyl-4-propylpyrrolidine-2-carboxylic acid (4-propyl hygric acid),



F. methyl 6-amino-6,8-dideoxy-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside (methyl-1-thiolincosaminide).

Ph Eur

Linoleoyl Macrogolglycerides

(Ph. Eur. monograph 1232)

Action and use
Excipient.

Ph Eur

DEFINITION

Mixtures of monoesters, diesters and triesters of glycerol and monoesters and diesters of macrogols.

They are obtained by partial alcoholysis of an unsaturated oil mainly containing triglycerides of linoleic ((9*Z*,12*Z*)-octadeca-9,12-dienoic) acid, using macrogol with a mean relative molecular mass between 300 and 400, or by esterification of glycerol and macrogol with unsaturated fatty acids, or by mixing glycerol esters and condensates of ethylene oxide with the fatty acids of this unsaturated oil.

CHARACTERS

Appearance
Amber, oily liquid which may give rise to a deposit after prolonged periods at 20 °C.

Solubility
Practically insoluble but dispersible in water, freely soluble in methylene chloride.

Relative density
About 0.95 at 20 °C.

Refractive index
About 1.47 at 20 °C.

Viscosity
About 35 mPa·s at 40 °C.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 1.0 g of the substance to be examined in *methylene chloride R* and dilute to 20 mL with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *hexane R, ether R* (30:70 *V/V*).

Application 10 µL.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with a 0.1 g/L solution of *rhodamine B R* in *ethanol (96 per cent) R* and examine in ultraviolet light at 365 nm.

Results The chromatogram shows a spot due to triglycerides with an R_F value of about 0.9 (R_{st} 1) and spots due to 1,3-diglycerides (R_{st} 0.7), to 1,2-diglycerides (R_{st} 0.6), to monoglycerides (R_{st} 0.1) and to esters of macrogol (R_{st} 0).

B. Hydroxyl value (see Tests).

C. Saponification value (see Tests).

D. Composition of fatty acids (see Tests).

TESTS

Acid value (*2.5.1*)
Maximum 2.0, determined on 2.0 g.

Hydroxyl value (*2.5.3, Method A*)
45 to 65, determined on 1.0 g.

Iodine value (*2.5.4, Method A*)
90 to 110.

Peroxide value (*2.5.5, Method A*)
Maximum 12.0, determined on 2.0 g.

Saponification value (*2.5.6*)
150 to 170, determined on 2.0 g.

Alkaline impurities
Introduce 5.0 g into a test tube and carefully add a mixture, neutralised if necessary with *0.01 M hydrochloric acid* or with *0.01 M sodium hydroxide*, of 0.05 mL of a 0.4 g/L solution of *bromophenol blue R* in *ethanol (96 per cent) R*, 0.3 mL of *water R* and 10 mL of *ethanol (96 per cent) R*. Shake and allow to stand. Not more than 1.0 mL of *0.01 M hydrochloric acid* is required to change the colour of the upper layer to yellow.

Free glycerol
Maximum 3.0 per cent.

Dissolve 1.20 g in 25.0 mL of *methylene chloride R*. Heat if necessary. After cooling, add 100 mL of *water R*. Shake and add 25.0 mL of *periodic acetic acid solution R*. Shake and allow to stand for 30 min. Add 40 mL of a 75 g/L solution of *potassium iodide R*. Allow to stand for 1 min. Add 1 mL of *starch solution R*. Titrate the iodine with *0.1 M sodium thiosulfate*. Carry out a blank titration.

1 mL of *0.1 M sodium thiosulfate* is equivalent to 2.3 mg of glycerol.

Composition of fatty acids (*2.4.22, Method A*)
Composition of the fatty-acid fraction of the substance:
— *palmitic acid*: 4.0 per cent to 20.0 per cent;
— *stearic acid*: maximum 6.0 per cent;
— *oleic acid*: 20.0 per cent to 35.0 per cent;
— *linoleic acid*: 50.0 per cent to 65.0 per cent;
— *linolenic acid*: maximum 2.0 per cent;
— *arachidic acid*: maximum 1.0 per cent;
— *eicosenoic acid*: maximum 1.0 per cent.

Ethylene oxide and dioxan (*2.4.25*)
Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Water (*2.5.12*)
Maximum 1.0 per cent, determined on 1.00 g. Use a mixture of 30 volumes of *anhydrous methanol R* and 70 volumes of *methylene chloride R* as solvent.

Total ash (*2.4.16*)
Maximum 0.1 per cent.

STORAGE
Protected from light.

LABELLING
The label states the type of macrogol used (mean relative molecular mass) or the number of units of ethylene oxide per molecule (nominal value).

FUNCTIONALITY-RELATED CHARACTERISTICS
This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for linoleoyl macrogolglycerides used as self-emulsifying agents and solubilisers.

Hydroxyl value
(see Tests).

Saponification value
(see Tests).

Composition of fatty acids
(see Tests).

Ph Eur

Virgin Linseed Oil

(Ph. Eur. monograph 1908)

Ph Eur

DEFINITION
Fatty oil obtained by cold expression from ripe seeds of *Linum usitatissimum* L. A suitable antioxidant may be added.

CHARACTERS

Appearance
Clear, yellow or brownish-yellow liquid, on exposure to air turning dark and gradually thickening. When cooled, it becomes a soft mass at about −20 °C.

Solubility
Very slightly soluble in ethanol (96 per cent), miscible with light petroleum.

Relative density
About 0.931.

Refractive index
About 1.480.

IDENTIFICATION
First identification: B, C.
Second identification: A, B.

A. Identification of fatty oils by thin-layer chromatography (*2.3.2*).

Results The chromatogram obtained is similar to the corresponding chromatogram shown in Figure 2.3.2.-1.

B. Iodine value (see Tests).

C. Composition of fatty acids (see Tests).

TESTS

Acid value (*2.5.1*)
Maximum 4.5.

Iodine value (*2.5.4*)
160 to 200.

Peroxide value (*2.5.5, Method A*)
Maximum 15.0.

Saponification value (*2.5.6*)
188 to 195; carry out the saponification for 1 h.

Unsaponifiable matter (*2.5.7*)
Maximum 1.5 per cent, determined on 5.0 g.

Composition of fatty acids
Gas chromatography (*2.4.22, Method C*). Use the calibration mixture in Table 2.4.22.-3.

Composition of the fatty-acid fraction of the oil:
— *fatty acids with a chain length less than C_{16}*: maximum 1.0 per cent,
— *palmitic acid*: 3.0 per cent to 8.0 per cent,
— *palmitoleic acid*: maximum 1.0 per cent,

— *stearic acid*: 2.0 per cent to 8.0 per cent,
— *oleic acid*: 11.0 per cent to 35.0 per cent,
— *linoleic acid*: 11.0 per cent to 24.0 per cent,
— *linolenic acid*: 35.0 per cent to 65.0 per cent,
— *arachidic acid*: maximum 1.0 per cent.

Cadmium
Maximum 0.5 ppm, determined as described in general method *2.4.27. Heavy metals in herbal drugs and herbal drug preparations.*

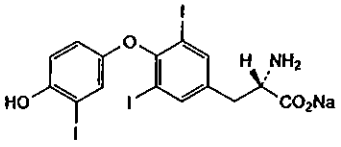
Water (*2.5.32*)
Maximum 0.1 per cent, determined on 1.00 g.

STORAGE
In an airtight container, protected from light.

Ph Eur

Liothyronine Sodium

(Ph. Eur. monograph 0728)



$C_{15}H_{11}I_3NNaO_4$ 673 *55-06-1*

Action and use
Thyroid hormone replacement.

Preparation
Liothyronine Tablets

Ph Eur

DEFINITION
Sodium (2*S*)-2-amino-3-[4-(4-hydroxy-3-iodophenoxy)-3,5-diiodophenyl]propanoate.

Content
95.0 per cent to 102.0 per cent (dried substance).

CHARACTERS
Appearance
White or slightly coloured, hygroscopic powder.

Solubility
Practically insoluble in water, slightly soluble in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION
First identification: A, C, E.
Second identification: A, B, D, E.

A. Specific optical rotation (see Tests).

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).
Test solution Dissolve 10.0 mg in *0.1 M sodium hydroxide* and dilute to 100.0 mL with the same solvent.
Spectral range 230-350 nm.
Absorption maximum At 319 nm.
Specific absorbance at the absorption maximum 63 to 69 (dried substance).

C. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *liothyronine sodium CRS.*

D. To about 50 mg in a porcelain dish add a few drops of *sulfuric acid R* and heat. Violet vapour is evolved.

E. To 200 mg add 2 mL of *dilute sulfuric acid R*. Heat on a water-bath and then carefully over a naked flame, increasing the temperature gradually up to about 600 °C. Continue the ignition until most of the particles have disappeared. Dissolve the residue in 2 mL of *water R*. The solution gives reaction (a) of sodium (*2.3.1*).

TESTS
Specific optical rotation (*2.2.7*)
+ 18.0 to + 22.0 (dried substance).
Dissolve 0.200 g in a mixture of 1 volume of *1 M hydrochloric acid* and 4 volumes of *ethanol (96 per cent) R* and dilute to 20.0 mL with the same mixture of solvents.

Related substances
Liquid chromatography (*2.2.29*). *Protect the solutions from light throughout the test.*
Solution A Mix 10 volumes of mobile phase A with 90 volumes of *methanol R.*
Solution B Mix 30 volumes of mobile phase B and 70 volumes of mobile phase A. Mix equal volumes of this solution with solution A.
Test solution Dissolve 20.0 mg of the substance to be examined in 20 mL of solution A. Dilute 4.0 mL of this solution to 20.0 mL with solution B.
Reference solution (a) Dissolve 2.5 mg of *levothyroxine sodium CRS* (impurity A) and 2.5 mg of *liothyronine sodium CRS* in solution A and dilute to 25 mL with the same solution. Dilute 1.0 mL of this solution to 50.0 mL with solution B.
Reference solution (b) Dilute 1.0 mL of reference solution (a) to 10.0 mL with solution B.
Reference solution (c) Dissolve the contents of a vial of *liothyronine for peak identification CRS* (containing impurities A, B, C, D and E) in solution B and dilute to 1.0 mL with the same solution.
Reference solution (d) Dissolve 20.0 mg of *liothyronine sodium CRS* in 20 mL of solution A. Dilute 4.0 mL of this solution to 20.0 mL with solution B.
Blank solution Solution B.
Column:
— *size*: *l* = 0.15 m, Ø = 4.0 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm).
Mobile phase:
— *mobile phase A*: dissolve 9.7 g of *sulfamic acid R* in *water R* and dilute to 2000 mL with the same solvent; add 1.5 g of *sodium hydroxide R* and adjust to pH 2.0 with *2 M sodium hydroxide*;
— *mobile phase B*: *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	75	25
3 - 4	75 → 70	25 → 30
4 - 14	70	30
14 - 44	70 → 20	30 → 80
44 - 54	20	80

Flow rate 1 mL/min.
Detection Spectrophotometer at 225 nm.
Injection 25 µL of the test solution and reference solutions (a), (b) and (c).

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, C, D and E.

— *relative retention* with reference to liothyronine (retention time = about 14 min): impurity B = about 0.2; impurity E = about 0.5; impurity A = about 1.4; impurity C = about 2; impurity D = about 2.4.

System suitability:

— *resolution*: minimum 5.0 between the peaks due to impurity A and liothyronine in the chromatogram obtained with reference solution (a).

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *impurity E*: not more than 5 times the area of the peak due to liothyronine in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *impurities B, C*: for each impurity, not more than 3 times the area of the peak due to liothyronine in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *impurity D*: not more than twice the area of the peak due to liothyronine in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the peak due to liothyronine in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than twice the area of the peak due to liothyronine in the chromatogram obtained with reference solution (a) (2.0 per cent);

— *disregard limit*: 0.5 times the area of the peak due to liothyronine in the chromatogram obtained with reference solution (b) (0.05 per cent).

Chlorides

Maximum 2.0 per cent, expressed as NaCl (dried substance).

Dissolve 0.500 g in a 2 g/L solution of *sodium hydroxide R* and dilute to 100 mL with the same solvent. Add 15 mL of *dilute nitric acid R* and titrate with *0.05 M silver nitrate*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.05 M silver nitrate* is equivalent to 2.93 mg of NaCl.

Loss on drying (*2.2.32*)

Maximum 4.0 per cent, determined on 0.500 g by drying *in vacuo* at 60 °C.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (d).

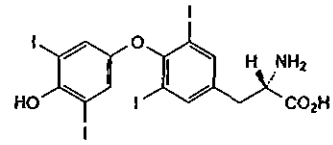
Calculate the percentage content of $C_{15}H_{11}I_3NNaO_4$ from the declared content of *liothyronine sodium CRS*.

STORAGE

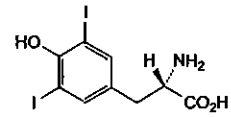
In an airtight container, protected from light, at a temperature between 2 °C and 8 °C.

IMPURITIES

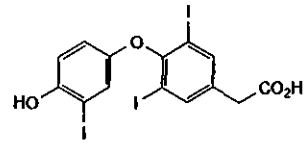
Specified impurities *A, B, C, D, E.*



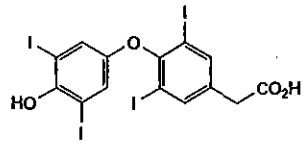
A. (2*S*)-2-amino-3-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]propanoic acid (levothyroxine),



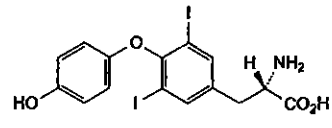
B. (2*S*)-2-amino-3-(4-hydroxy-3,5-diiodophenyl)propanoic acid (diiodotyrosine),



C. [4-(4-hydroxy-3-iodophenoxy)-3,5-diiodophenyl]acetic acid (triiodothyroacetic acid),



D. [4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]acetic acid (tetraiodothyroacetic acid),

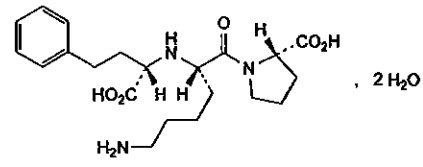


E. (2*S*)-2-amino-3-[4-(4-hydroxyphenoxy)-3,5-diiodophenyl] propanoic acid (diiodothyronine).

Ph Eur

Lisinopril Dihydrate

(Ph. Eur. monograph 1120)



$C_{21}H_{31}N_3O_5,2H_2O$ 441.5 *83915-83-7*

Action and use

Angiotensin converting enzyme inhibitor.

Preparations

Lisinopril Oral Solution

Lisinopril Tablets

Ph Eur

DEFINITION

(2*S*)-1-[(2*S*)-6-Amino-2-[[(1*S*)-1-carboxy-3-phenylpropyl]amino]hexanoyl]pyrrolidine-2-carboxylic acid dihydrate.

Content

98.5 per cent to 101.5 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Soluble in water, practically insoluble in anhydrous ethanol and in heptane.

IDENTIFICATION

A. Specific optical rotation (*2.2.7*): −47 to −43 (anhydrous substance).

Dissolve 0.5 g in *zinc acetate solution R* and dilute to 50.0 mL with the same solvent.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *lisinopril dihydrate CRS.*

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in mobile phase A and dilute to 10.0 mL with mobile phase A.

Reference solution (a) Dissolve 5 mg of *lisinopril for system suitability A CRS* (containing impurities A and E) in mobile phase A and dilute to 1 mL with mobile phase A.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

Reference solution (c) Dissolve the contents of a vial of *lisinopril impurity F CRS* in 1 mL of mobile phase A.

Reference solution (d) Dissolve 5 mg of *lisinopril for peak identification CRS* (containing impurity G) in mobile phase A and dilute to 1 mL with mobile phase A.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 50 °C.

Mobile phase:

- *mobile phase A*: mix 3 volumes of *acetonitrile R1* and 97 volumes of a 3.12 g/L solution of *sodium dihydrogen phosphate R* previously adjusted to pH 3.8 with *dilute phosphoric acid R*;
- *mobile phase B*: mix 20.5 volumes of *acetonitrile R1* and 79.5 volumes of a 3.12 g/L solution of *sodium dihydrogen phosphate R* previously adjusted to pH 3.5 with *dilute phosphoric acid R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	100	0
2 - 37	100 → 0	0 → 100
37 - 62	0	100

Flow rate 1.6 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 50 µL.

Identification of impurities Use the chromatogram supplied with *lisinopril for system suitability A CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A and E; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity F; use the chromatogram supplied with *lisinopril for peak identification CRS* and the chromatogram obtained with reference solution (d) to identify the peak due to impurity G.

Relative retention With reference to lisinopril (retention time = about 14 min): impurity A = about 0.7; impurity E = about 1.2; impurity F = about 1.9; impurity G = about 2.9.

System suitability:

- *resolution*: minimum 1.5 between the peaks due to lisinopril and impurity E in the chromatogram obtained with reference solution (a);
- *signal-to-noise ratio*: minimum 45 for the principal peak in the chromatogram obtained with reference solution (b).

Calculation of percentage contents:

- *correction factor*: multiply the peak area of impurity F by 2.1;
- for each impurity, use the concentration of lisinopril dihydrate in reference solution (b).

Limits:

- *impurities A, E, F*: for each impurity, maximum 0.2 per cent;
- *impurity G*: maximum 0.15 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.5 per cent;
- *reporting threshold*: 0.05 per cent; disregard any peak with a retention time less than 3 min.

Water (*2.5.12*)

8.0 per cent to 9.5 per cent, determined on 0.200 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

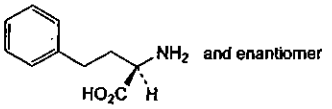
Dissolve 0.350 g in 50 mL of *water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*) at the 1st point of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 40.55 mg of $C_{21}H_{31}N_3O_5$.

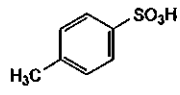
IMPURITIES

Specified impurities *A, E, F, G.*

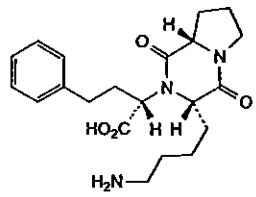
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D, H, I, J.*



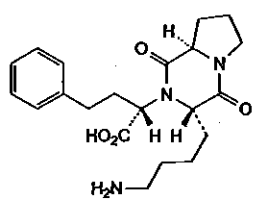
A. (2*RS*)-2-amino-4-phenylbutanoic acid,



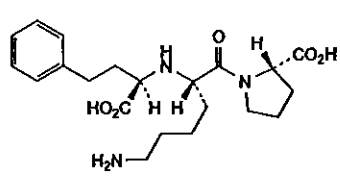
B. 4-methylbenzenesulfonic acid,



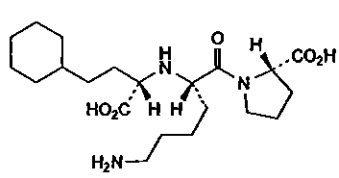
C. (2*S*)-2-[(3*S*,8a*S*)-3-(4-aminobutyl)-1,4-dioxohexahydropyrrolo[1,2-*a*]pyrazin-2(1*H*)-yl]-4-phenylbutanoic acid (*S,S,S*-diketopiperazine),



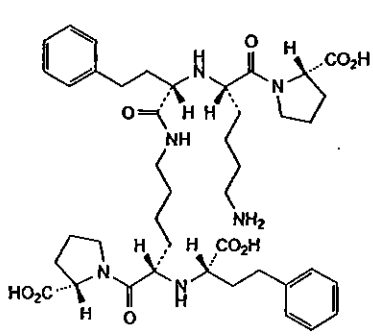
D. (2*S*)-2-[(3*S*,8a*R*)-3-(4-aminobutyl)-1,4-dioxohexahydropyrrolo[1,2-*a*]pyrazin-2(1*H*)-yl]-4-phenylbutanoic acid (*R,S,S*-diketopiperazine),



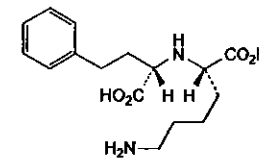
E. (2*S*)-1-[(2*S*)-6-amino-2-[[(1*R*)-1-carboxy-3-phenylpropyl]amino]hexanoyl]pyrrolidine-2-carboxylic acid (lisinopril *R,S,S*-isomer),



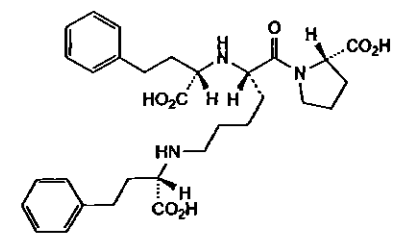
F. (2*S*)-1-[(2*S*)-6-amino-2-[[(1*S*)-1-carboxy-3-cyclohexylpropyl]amino]hexanoyl]pyrrolidine-2-carboxylic acid (cyclohexyl analogue),



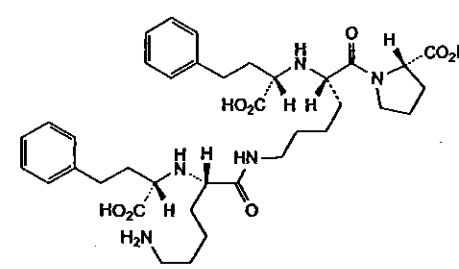
G. (2*S*)-1-[(2*S*)-6-amino-2-[[(2*S*)-1-[[(5*S*)-5-[[(1*S*)-1-carboxy-3-phenylpropyl]amino]-6-[(2*S*)-2-carboxypyrrolidin-1-yl]-6-oxohexyl]amino]-1-oxo-4-phenylbutan-2-yl]amino]hexanoyl]pyrrolidine-2-carboxylic acid (lisinopril dimer),



H. (2*S*)-6-amino-2-[[(1*S*)-1-carboxy-3-phenylpropyl]amino]hexanoic acid,



I. (2*S*)-1-[(2*S*)-2,6-bis[[(1*S*)-1-carboxy-3-phenylpropyl]amino]hexanoyl]pyrrolidine-2-carboxylic acid,



J. (2*S*)-1-[(2*S*)-6-[(2*S*)-6-amino-2-[[(1*S*)-1-carboxy-3-phenylpropyl]amino]hexanamido]-2-[[(1*S*)-1-carboxy-3-phenylpropyl]amino]hexanoyl]pyrrolidine-2-carboxylic acid.

Ph Eur

Lithium Carbonate

(Ph. Eur. monograph 0228)

Li_2CO_3	73.9	*554-13-2*

Action and use
Prophylaxis of affective disorders.

Preparations
Lithium Carbonate Tablets
Lithium Carbonate Prolonged-release Tablets

Ph Eur

DEFINITION

Content
98.5 per cent to 100.5 per cent.

CHARACTERS

Appearance
White or almost white powder.

Solubility
Slightly soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. When moistened with *hydrochloric acid R*, it gives a red colour to a non-luminous flame.

B. Dissolve 0.2 g in 1 mL of *hydrochloric acid R*. Evaporate to dryness on a water-bath. The residue dissolves in 3 mL of *ethanol (96 per cent) R*.

C. It gives the reaction of carbonates (*2.3.1*).

TESTS

Solution S

Suspend 10.0 g in 30 mL of *distilled water R* and dissolve by the addition of 22 mL of *nitric acid R*. Add *dilute sodium hydroxide solution R* until the solution is neutral and dilute to 100 mL with *distilled water R*.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Chlorides (*2.4.4*)

Maximum 200 ppm.

Dilute 2.5 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)

Maximum 200 ppm.

Disperse 1.25 g in 5 mL of *distilled water R* and dissolve by adding 5 mL of *hydrochloric acid R1*. Boil for 2 min. Cool and add *dilute sodium hydroxide solution R* until neutral. Dilute to 25 mL with *distilled water R*.

Arsenic (*2.4.2, Method A*)

Maximum 2 ppm, determined on 0.5 g.

Calcium (*2.4.3*)

Maximum 200 ppm.

Dilute 5 mL of solution S to 15 mL with *distilled water R*.

Iron (*2.4.9*)

Maximum 20 ppm.

Dilute 5 mL of solution S to 10 mL with *water R*.

Magnesium (*2.4.6*)

Maximum 150 ppm.

Dilute 1 mL of solution S to 10 mL with *water R*. Dilute 6.7 mL of this solution to 10 mL with *water R*.

Potassium

Maximum 300 ppm.

Atomic emission spectrometry (*2.2.22, Method I*).

Test solution Dissolve 1.0 g in 10 mL of *hydrochloric acid R1* and dilute to 50.0 mL with *water R*.

Reference solutions Prepare the reference solutions using a solution of *potassium chloride R* containing 500 µg of K per millilitre, diluted as necessary with *water R*.

Wavelength 766.5 nm.

Sodium

Maximum 300 ppm.

Atomic emission spectrometry (*2.2.22, Method I*).

Test solution Dissolve 1.0 g in 10 mL of *hydrochloric acid R1* and dilute to 50.0 mL with *water R*.

Reference solutions Prepare the reference solutions using a solution of *sodium chloride R* containing 500 µg of Na per millilitre, diluted as necessary with *water R*.

Wavelength 589 nm.

ASSAY

Dissolve 0.250 g in 50 mL of *carbon dioxide-free water R*. Titrate with *1 M hydrochloric acid*, determining the end-point potentiometrically (*2.2.20*). Read the volume added at the 2nd point of inflection.

1 mL of *1 M hydrochloric acid* is equivalent to 36.95 mg of Li_2CO_3.

Ph Eur

Lithium Citrate

(Ph. Eur. monograph 0621)

$C_6H_5Li_3O_7,4H_2O$ 282.0

Action and use

Prophylaxis of affective disorders.

Preparation

Lithium Citrate Oral Solution

Ph Eur

DEFINITION

Trilithium 2-hydroxypropane-1,2,3-tricarboxylate tetrahydrate.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, fine crystalline powder.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. When moistened with *hydrochloric acid R*, it gives a red colour to a non-luminous flame.

B. Dilute 3 mL of solution S (see Tests) to 10 mL with *water R*. Add 3 mL of *potassium ferriperiodate solution R*. A white or yellowish-white precipitate is formed.

C. To 1 mL of solution S add 4 mL of *water R*. The solution gives the reaction of citrates (*2.3.1*).

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 10 mL of solution S add 0.1 mL of *phenolphthalein solution R*. Not more than 0.2 mL of *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Readily carbonisable substances

To 0.20 g of the powdered substance to be examined add 10 mL of *sulfuric acid R* and heat in a water-bath at 90 ± 1 °C for 60 min. Cool rapidly. The solution is not more intensely coloured than reference solution Y_2 or GY_2 (*2.2.2, Method II*).

Chlorides (*2.4.4*)

Maximum 100 ppm.

Dilute 5 mL of solution S to 15 mL with *water R*.

Oxalates

Maximum 300 ppm, calculated as anhydrous oxalate ion.

Dissolve 0.50 g in 4 mL of *water R*, add 3 mL of *hydrochloric acid R* and 1 g of *zinc R* in granules and heat on a water-bath for 1 min. Allow to stand for 2 min, decant the liquid into a test-tube containing 0.25 mL of a 10 g/L solution of *phenylhydrazine hydrochloride R* and heat to boiling. Cool rapidly, transfer to a graduated cylinder and add an equal volume of *hydrochloric acid R* and 0.25 mL of *potassium ferricyanide solution R*. Shake and allow to stand for 30 min. Any pink colour in the solution is not more intense than that

in a standard prepared at the same time and in the same manner using 4 mL of a 0.05 g/L solution of *oxalic acid R*.

Sulfates (*2.4.13*)
Maximum 500 ppm.

To 3 mL of solution S add 2 mL of *hydrochloric acid R1* and dilute to 17 mL with *distilled water R*. Prepare the reference solution using 15 mL of a mixture of 2 mL of *hydrochloric acid R1* and 15 mL of *sulfate standard solution (10 ppm SO_4) R* and compare the opalescence after 15 min.

Water (*2.5.12*)
24.0 per cent to 27.0 per cent, determined on 0.100 g. After adding the substance to be examined, stir for 15 min before titrating. Carry out a blank titration.

ASSAY
Dissolve 80.0 mg in 50 mL of *anhydrous acetic acid R*, heating to about 50 °C. Allow to cool. Titrate with *0.1 M perchloric acid*, using 0.25 mL of *naphtholbenzein solution R* as indicator, until the colour changes from yellow to green.

1 mL of *0.1 M perchloric acid* is equivalent to 7.00 mg of $C_6H_5Li_3O_7$.

STORAGE
In an airtight container.

Ph Eur

Live Biotherapeutic Products for Human Use

(Ph. Eur. monograph 3053)

Ph Eur

DEFINITION
Live biotherapeutic products (LBP) are medicinal products containing live micro-organisms (bacteria or yeasts) for human use.

LBP are administered orally or vaginally and are available in different pharmaceutical forms.

LBP may contain one or multiple microbial strains from the same or different species of micro-organisms. The quantity of living bacteria or yeasts is determined by counting viable and culturable micro-organisms of the intended strain(s).

Faecal microbiota transplantation (FMT) and products intended as gene therapy agents are excluded from this monograph.

PRODUCTION

GENERAL PROVISIONS
The production method for a given product must have been shown to yield consistently an LBP comparable with the batch(es) of proven clinical efficacy and safety in man. Suitable in-process controls ensure that the production process is under control and consistently produces LBP of defined quality.

LBP are produced using a seed-lot system. The methods of preparation are designed to maintain the viability of the micro-organisms. Stabilisers and other excipients of controlled microbiological quality may be added.

Where LBP are produced using substances of human or animal origin, the requirements of general chapter *5.1.7. Viral safety* apply. The production process is optimised to consistently minimise or remove adventitious agents and toxic or other impurities.

In the production of a final lot of LBP, the number of subcultures of a bacterium or yeast, from the master seed lot, shall not exceed that used for production of the LBP shown to be satisfactory in clinical trials with respect to quality, safety and efficacy.

Consistency of production is important to ensure the quality of LBP. Limits for process parameters and for tests carried out during production and on the final lot may be in the form of maximum values, minimum values, or tolerances around a given value. Limits are based on the results found for batches tested clinically and those used to demonstrate consistency of production. These limits may subsequently be refined on a statistical basis in the light of production data.

CHARACTERISATION OF THE MICRO-ORGANISM (S)
The characterisation of the micro-organism(s) is carried out at the strain level. The strain used for master seed lots is identified by historical records that include information on its origin, subsequent manipulation and the tests used to characterise the strain. Characterisation includes determination of the phenotype and genotype of the strain, using methods such as macroscopic and microscopic methods, biochemical tests, molecular genetic tests, sequencing or mass spectrometry. The strain must be phenotypically and genotypically stable. Antimicrobial resistance is determined and any resistance transferable from the micro-organism to the relevant microbiota is investigated and excluded. The presence of virulence factors must be investigated and evaluated with respect to safety.

For products to be administered orally, survivability of the micro-organism(s) in the human gut is demonstrated by *in vitro* gastric acid and bile resistance testing.

SEED-LOT SYSTEM
A seed-lot system is used. Suitable measures are taken to ensure that adventitious agents and toxic or other impurities are not present in master or working seed-lots. When a master seed-lot is replaced, the new master seed-lot must be fully characterised and the critical steps in the production process revalidated to the extent required to demonstrate that no adverse change has occurred in the quality, safety and efficacy of the product.

CULTURE MEDIA
Culture media are sterile and, as far as possible, free from ingredients known to cause toxic, allergic or other undesirable reactions in humans; if inclusion of such ingredients is necessary, it is demonstrated that the residual amount in the final lot does not adversely affect product safety.

GROWTH AND HARVESTING
Each strain is cultured and the culture is examined for purity by suitable tests. These tests may include inoculation into suitable media, examination of colony morphology and microscopic examination. The culture is harvested under defined conditions and may be stored under appropriate conditions.

INTERMEDIATE PRODUCTS
When intermediate products are stored, a storage period justified by stability data is established for each intermediate.

Test for microbial contamination
Each intermediate is shown to comply with defined in-process limits using a suitable method.

FINAL BULK

The final bulk may contain a single strain or a mixture of 2 or more strains. Stabilisers and other excipients may be added depending on the dosage form.

FINAL LOT

When the final lot is an aqueous suspension, it is distributed into sterile, tamper-evident containers (*3.2*).

The final lot complies with the requirements under Identification, Tests and Assay. Certain tests prescribed for the final lot may be carried out on the final bulk, provided it has been demonstrated that subsequent manufacturing operations do not affect compliance.

IDENTIFICATION

Each micro-organism is identified by a suitable method.

TESTS

Appearance

It complies with the established specification.

Microbial contamination

Microbiological examination of LBP is performed according to the methods given in general chapters *2.6.36* and *2.6.38*.

When an acceptance criterion for microbial contamination is prescribed it is interpreted as follows:

— 10^1 CFU: maximum acceptable count = 20;
— 10^2 CFU: maximum acceptable count = 200;
— 10^3 CFU: maximum acceptable count = 2000.

Acceptance criteria based upon the aerobic microbial contamination count (AMCC) and the combined yeasts/moulds contamination count (YMCC) are given in Table 3053.-1.

In addition to the micro-organisms listed in Table 3053.-1, tests for other pathogens may be carried out based on a risk assessment (e.g. specific environmental contaminants, anaerobic contaminants (e.g. Clostridia)).

The significance of other micro-organisms recovered is evaluated in terms of:

— intended recipient: risk may differ for neonates, infants, the debilitated;
— use of immunosuppressive agents, corticosteroids;
— presence of disease, wounds, organ damage.

pH (*2.2.3*)

It complies with the limit approved for the particular product.

Water (*2.5.12*), Loss on drying (*2.2.32*) or Water activity (*2.9.39*)

It complies with the limit approved for the particular product.

ASSAY

Number of live micro-organisms

The number of micro-organisms, expressed in CFU, is determined by a suitable microbial enumeration test, for example by the pour-plate method or surface-spread method.

The potency of each strain is expressed in CFU/g, CFU/mL, or CFU/unit; it is not less than the stated value or it is within the stated range.

The potency may be expressed in number of viable cells/mL based on viable-cell assay, in agreement with the competent authority.

Table 3053.-1. – *Acceptance criteria for microbiological quality of LBP*

Route of administration	AMCC* (CFU/g or CFU/mL)	YMCC** (CFU/g or CFU/mL)	Specified micro-organisms***
Non-aqueous preparations for oral use	10^3	10^2	Absence of *Escherichia coli***** (1 g or 1 mL)
Aqueous preparations for oral use	10^2	10^1	Absence of *Escherichia coli***** (1 g or 1 mL)
Vaginal use	10^2	10^1	Absence of *Pseudomonas aeruginosa* (1 g or 1 mL) Absence of *Staphylococcus aureus* (1 g or 1 mL) Absence of *Candida albicans* (1 g or 1 mL)

* If it is not possible to determine the AMCC due to the absence of a suitable method for the LBP, then test for the absence of the following specified micro-organisms using the methods in general chapter *2.6.38*: bile-tolerant Gram-negative bacteria(1) (1 g or 1 mL), *P. aeruginosa* (1 g or 1 mL), *S. aureus* (1 g or 1 mL), *Salmonella* spp. (10 g or 10 mL). Tests for other pathogens may be carried out based on a risk assessment (e.g. specific environmental contaminants).

(1) If the LBP consists of *E. coli*, test for the absence of Enterobacteriaceae and other Gram-negative bacteria using suitable culture media instead of testing for the absence of bile-tolerant Gram-negative bacteria.

** If it is not possible to determine the YMCC due to the absence of a suitable method for the LBP, then test for the absence of *C. albicans* and test for pathogenic moulds based on a risk assessment.

*** If the LBP consists of *Bacillus* spp. spores, test for the absence of *Bacillus cereus* in addition to the tests described in the table.

**** If the LBP consists of *E. coli*, the test for *E. coli* should be replaced by another test for the absence of faecal contaminants by testing for *Enterococcus faecalis* and *Enterococcus faecium* as indicators. A search for other pathogens may be carried out based on a risk assessment (e.g. pathogenic *E. coli*).

STORAGE

Liquid LBP are not to be frozen.

LABELLING

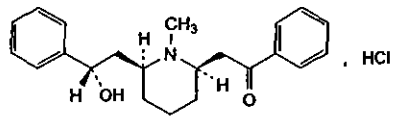
The label states:

— the name of the strain(s);
— the route of administration;
— the storage conditions;
— the expiry date;
— the potency of each strain expressed in CFU/mL, CFU/g, CFU/unit or viable cells/mL;
— the name of any stabilisers and other excipients;
— where applicable, for freeze-dried preparations to be reconstituted before use:
  — the name, composition and volume of the reconstituting liquid to be added;
  — the storage conditions and the period of time within which the LBP is to be used after reconstitution.

Ph Eur

Lobeline Hydrochloride

(Ph. Eur. monograph 1988)



$C_{22}H_{28}ClNO_2$ 373.9 *134-63-4*

Action and use
Respiratory stimulant.

Ph Eur

DEFINITION
2-[(2*R*,6*S*)-6-[(2*S*)-2-Hydroxy-2-phenylethyl]-1-methylpiperidin-2-yl]-1-phenylethanone hydrochloride.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, microcrystalline powder.

Solubility
Sparingly soluble in water, freely soluble in ethanol (96 per cent), soluble in methylene chloride.

IDENTIFICATION
First identification: A, B.
Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *lobeline hydrochloride CRS.*

B. Solution S (see Tests) gives reaction (a) of chlorides (*2.3.1*).

C. Examine the chromatograms obtained in the test for foreign alkaloids.
Results The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (b).

TESTS

Solution S
Dissolve 0.250 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 25.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)
4.6 to 6.4 for solution S.

Specific optical rotation (*2.2.7*)
−55 to −59 (dried substance), determined on solution S.

Foreign alkaloids
Thin-layer chromatography (*2.2.27*).
Test solution (a) Dissolve 0.10 g of the substance to be examined in *methanol R* and dilute to 5.0 mL with the same solvent.
Test solution (b) Dilute 1 mL of test solution (a) to 10 mL with *methanol R.*
Reference solution (a) Dilute 0.1 mL of test solution (a) to 10 mL with *methanol R.*
Reference solution (b) Dissolve 10 mg of *lobeline hydrochloride CRS* in *methanol R* and dilute to 5 mL with the same solvent.
Plate *TLC silica gel GF_{254} plate R.*
Mobile phase *diethylamine R, cyclohexane R* (10:90 *V/V*).
Application 10 µL.
Development Over 2/3 of the plate.
Drying At 120 °C.
Detection Examine in ultraviolet light at 254 nm.
Limits In the chromatogram obtained with test solution (a):
— *any impurity:* any spot, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (1 per cent).

Related substances
Liquid chromatography (*2.2.29*).
Test solution Dissolve 10.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.
Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.
Reference solution (b) Dissolve 5 mg of *phenytoin CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase. To 1 mL of the solution add 0.1 mL of the test solution and dilute to 25 mL with the mobile phase.
Column:
— *size:* *l* = 0.25 m, Ø = 4 mm,
— *stationary phase:* spherical *end-capped octylsilyl silica gel for chromatography R* (5 µm).
Mobile phase Dissolve 1.0 g of *sodium methanesulfonate R* and 2.50 g of *disodium hydrogen phosphate dihydrate R* in a mixture of 3 volumes of a 6.7 per cent *V/V* solution of *phosphoric acid R*, 29 volumes of *acetonitrile R* and 70 volumes of *water R* and dilute to 1000 mL with the same mixture of solvents.
Flow rate 1.5 mL/min.
Detection Spectrophotometer at 210 nm.
Injection 10 µL.
Run time 2 times the retention time of lobeline which is about 17 min.
System suitability Reference solution (b):
— *resolution:* minimum 4.0 between the peaks due to phenytoin and to lobeline.
Limits:
— *any impurity:* not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
— *total:* maximum of 2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent),
— *disregard level:* 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Sulfates (*2.4.13*)
Maximum 0.1 per cent, determined on solution S.

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g *in vacuo.*

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on the residue obtained in the test for loss on drying.

ASSAY
Dissolve 0.300 g in 50 mL of *ethanol (96 per cent) R.* Add 5 mL of *0.01 M hydrochloric acid.* Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium*

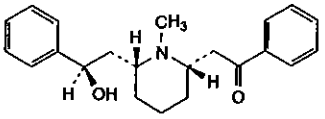
hydroxide. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 37.39 mg of $C_{22}H_{28}ClNO_2$.

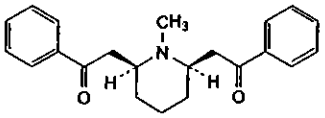
STORAGE

Protected from light.

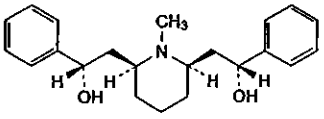
IMPURITIES



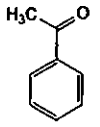
A. 2-[(2*S*,6*R*)-6-[(2*R*)-2-hydroxy-2-phenylethyl]-1-methylpiperidin-2-yl]-1-phenylethanone ((+)-lobeline),



B. 2,2′-[(2*R*,6*S*)-1-methylpiperidine-2,6-diyl]bis(1-phenylethanone) (lobelanine),



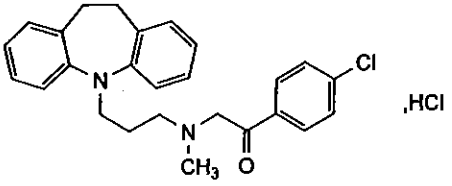
C. *meso*-(1*R*,1′*S*)-2,2′-[(2*R*,6*S*)-1-methylpiperidine-2,6-diyl]bis(1-phenylethanol) (lobelanidine),



D. acetophenone.

Ph Eur

Lofepramine Hydrochloride



$C_{26}H_{27}ClN_2O,HCl$ 455.4 *26786-32-3*

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

Preparation

Lofepramine Tablets

DEFINITION

Lofepramine Hydrochloride is 5-(3-[*N*-(4-chlorophenacyl)-*N*-methylamino]propyl)10,11-dihydro-5*H*-dibenz[*b*,*f*]azepine hydrochloride. It contains not less than 98.5% and not more than 101.0% of $C_{26}H_{27}ClN_2O,HCl$, calculated with reference to the dried substance.

CHARACTERISTICS

A fine, yellowish white to greenish yellow powder.

Very soluble in *ethanol (96%)* and in *methanol*; slightly soluble in *acetone*; very slightly soluble in *water*.

It exhibits polymorphism.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the reference spectrum of *lofepramine hydrochloride (form A) (RS 399A)*.

B. 2 mL of a 1% w/v solution in *ethanol (96%)* complies with the test for *chlorides*, Appendix VI.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.1% w/v of the substance being examined in the mobile phase. For solution (2) dilute 1 volume of solution (1) to 200 volumes with the mobile phase. Solution (3) contains 0.0002% w/v each of *desipramine hydrochloride BPCRS* and *imipramine hydrochloride BPCRS* in the mobile phase.

Inject 20 µL of solutions (1) and (2). Inject 20 µL of solution (3) and allow the chromatography to continue for 4 times the retention time of the principal peak.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *base-deactivated end-capped octylsilyl silica gel for chromatography* (5 µm) (Lichrospher 60 RP-select B is suitable) maintained at 50°, (b) as the mobile phase with a flow rate of 1.5 mL per minute a 0.09% w/v solution of *sodium dodecyl sulfate* in a mixture of 550 volumes of *acetonitrile*, 325 volumes of *water* and 125 volumes of a buffer solution of pH 1.0 containing 0.015% w/v of *glycine*, 0.018% w/v of *sodium chloride* and 0.44% w/v of *hydrochloric acid* and (c) a detection wavelength of 254 nm.

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the two principal peaks is at least 0.9.

In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of any *secondary peaks* is not greater than twice the area of the peak in the chromatogram obtained with solution (2) (1%).

Loss on drying

When dried at a temperature of 100° at a pressure not exceeding 0.2 kPa, loses not more than 0.5% of its weight. Use 1 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Solution (1) contains 0.02% w/v of the substance being examined in the mobile phase. Solution (2) contains 0.02% w/v of *lofepramine hydrochloride BPCRS* in the mobile phase.

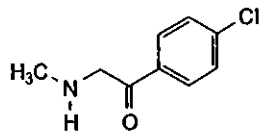
The chromatographic procedure described under the test for Related substances may be used.

Inject 20 µL of each solution.

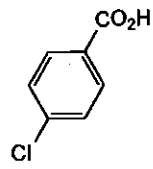
Calculate the content of $C_{26}H_{27}ClN_2O,HCl$ from the chromatograms obtained and using the declared content of $C_{26}H_{27}ClN_2O,HCl$ in *lofepramine hydrochloride BPCRS*.

STORAGE
Lofepramine Hydrochloride should be kept in an airtight container and protected from light.

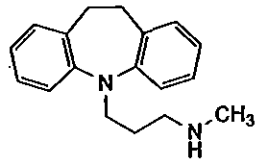
IMPURITIES



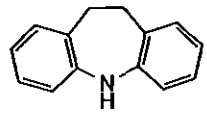
A. 1-methylamino-4′-chloroacetophenone,



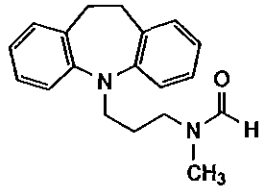
B. 4-chlorobenzoic acid,



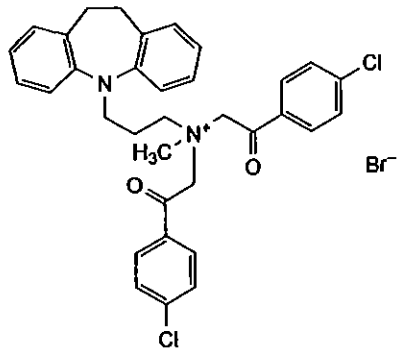
C. desipramine,



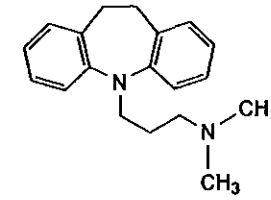
D. iminodibenzyl,



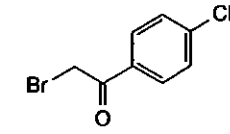
E. *N*-formyldesipramine,



F. *N*,*N*-bis(4′-chlorophenacyl)desipramine bromide,



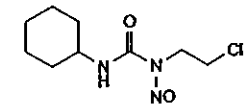
G. imipramine,



H. 2-bromo-4′-chloroacetophenone.

Lomustine

(Ph. Eur. monograph 0928)



$C_9H_{16}ClN_3O_2$ 233.7 *13010-47-4*

Action and use
Cytotoxic alkylating agent.

Preparation
Lomustine Capsules

Ph Eur

DEFINITION
1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
Yellow, crystalline powder.

Solubility
Practically insoluble in water, freely soluble in acetone and in methylene chloride, soluble in ethanol (96 per cent).

Carry out the tests protected from light and prepare all the solutions immediately before use.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *lomustine CRS.*

TESTS
Related substances
Liquid chromatography (*2.2.29*).

Phosphate buffer solution Dissolve 1.36 g of *potassium dihydrogen phosphate R* in 900 mL of *water R*, adjust to pH 3.0 with *dilute phosphoric acid R* and dilute to 1000 mL with *water R*.

Test solution Dissolve 50.0 mg of the substance to be examined in *acetonitrile R1* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dissolve 5.0 mg of *dicyclohexylurea R* (impurity C) in *methanol R* and dilute to 10.0 mL with the

same solvent. Dilute 1.0 mL of the solution to 50.0 mL with *acetonitrile R1*. Mix 1.0 mL of this solution and 1.0 mL of the test solution.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *acetonitrile R1*. Dilute 1.0 mL of this solution to 10.0 mL with *acetonitrile R1*.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: *acetonitrile R1*, phosphate buffer solution (20:80 *V/V*);
— *mobile phase B*: phosphate buffer solution, *acetonitrile R1* (24:76 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	75	25
2 - 17	75 → 40	25 → 60
17 - 34	40 → 30	60 → 70
34 - 42	30	70

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 205 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity C.

Relative retention With reference to lomustine (retention time = about 23 min): impurity C = about 0.7.

System suitability Reference solution (a):
— *resolution*: minimum 5.0 between the peaks due to impurity C and lomustine.

Calculation of percentage contents:
— for each impurity, use the concentration of lomustine in reference solution (b).

Limits:
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.2 per cent;
— *reporting threshold*: 0.05 per cent.

Chlorides (*2.4.4*)
Maximum 500 ppm.

Dissolve 0.24 g in 4 mL of *methanol R* and add 20 mL of *water R*. Allow to stand for 20 min and filter. To 10 mL of the filtrate, add 5 mL of *methanol R*. When preparing the standard, replace the 5 mL of *water R* with 5 mL of *methanol R*.

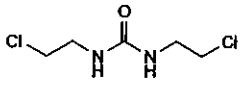
Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying in a desiccator at a pressure not exceeding 0.7 kPa for 24 h.

ASSAY
Dissolve 0.200 g in about 3 mL of *ethanol (96 per cent) R* and add 20 mL of a 200 g/L solution of *potassium hydroxide R* and boil under a reflux condenser for 2 h. Add 75 mL of *water R* and 4 mL of *nitric acid R*. Cool and titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

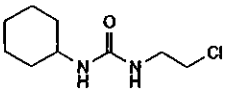
1 mL of *0.1 M silver nitrate* is equivalent to 23.37 mg of $C_9H_{16}ClN_3O_2$.

STORAGE
Protected from light.

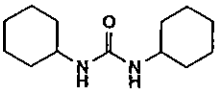
IMPURITIES
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C.*



A. 1,3-bis(2-chloroethyl)urea,



B. 1-(2-chloroethyl)-3-cyclohexylurea,

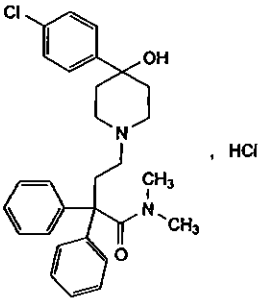


C. 1,3-dicyclohexylurea.

Ph Eur

Loperamide Hydrochloride

(Ph. Eur. monograph 0929)



$C_{29}H_{34}Cl_2N_2O_2$ 513.5 *34552-83-5*

Action and use
Opioid receptor agonist; antidiarrhoeal.

Preparations
Loperamide Capsules
Loperamide Oral Solution
Loperamide Oral Suspension
Loperamide Tablets
Loperamide Orodispersible Tablets

Ph Eur

DEFINITION
4-[4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl]-*N*,*N*-dimethyl-2,2-diphenylbutanamide hydrochloride.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Slightly soluble in water, freely soluble in ethanol (96 per cent) and in methanol.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *loperamide hydrochloride CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *methylene chloride R*, evaporate to dryness and record new spectra using the residues.

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dissolve 10.0 mg of *loperamide for system suitability CRS* (containing impurities A, B, D, E, G and H) in *methanol R* and dilute to 1.0 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *methanol R*. Dilute 1.0 mL of this solution to 10.0 mL with *methanol R*.

Column:

— *size*: *l* = 0.10 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (3 µm);
— *temperature*: 35 °C.

Mobile phase:

— *mobile phase A*: 17.0 g/L solution of *tetrabutylammonium hydrogen sulfate R1*;
— *mobile phase B*: *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	90 → 30	10 → 70
15 - 17	30	70

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µL.

Identification of impurities Use the chromatogram supplied with *loperamide for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, D, E, G and H.

Relative retention With reference to loperamide (retention time = about 6 min): impurity D = about 0.8; impurity G = about 1.20; impurity H = about 1.25; impurity E = about 1.45; impurity A = about 1.50; impurity B = about 1.9.

System suitability Reference solution (a):

— *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity H; minimum 1.5, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity A.

Calculation of percentage contents:

— *correction factor*: multiply the peak area of impurity D by 1.7;
— for each impurity, use the concentration of loperamide hydrochloride in reference solution (b).

Limits:

— *impurities B, D*: for each impurity, maximum 0.2 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.3 per cent;
— *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.400 g in 50 mL of *ethanol (96 per cent) R* and add 5.0 mL of *0.01 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 51.35 mg of $C_{29}H_{34}Cl_2N_2O_2$.

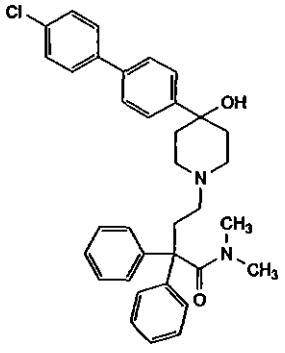
STORAGE

Protected from light.

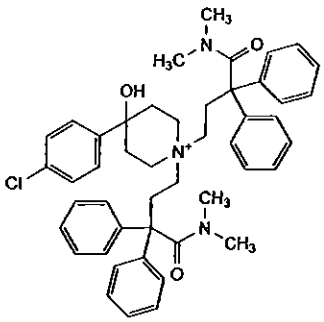
IMPURITIES

Specified impurities *B, D.*

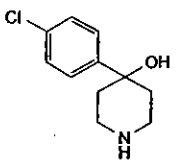
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, C, E, F, G, H.*



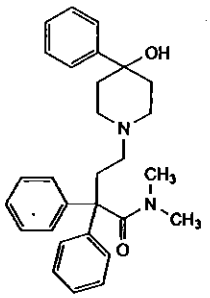
A. 4-[4-(4′-chloro-[1,1′-biphenyl]-4-yl)-4-hydroxypiperidin-1-yl]-*N*,*N*-dimethyl-2,2-diphenylbutanamide,



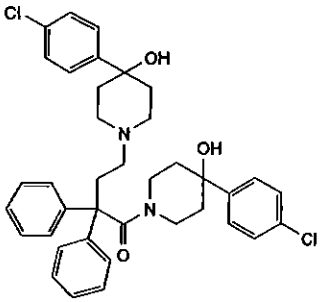
B. 4-(4-chlorophenyl)-1,1-bis[4-(dimethylamino)-4-oxo-3,3-diphenylbutyl]-4-hydroxypiperidin-1-ium,



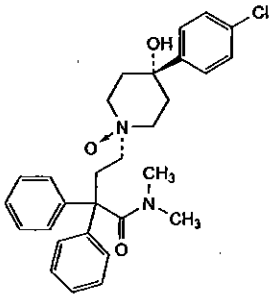
C. 4-(4-chlorophenyl)piperidin-4-ol,



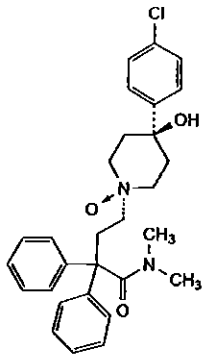
D. 4-(4-hydroxy-4-phenylpiperidin-1-yl)-*N*,*N*-dimethyl-2,2-diphenylbutanamide,



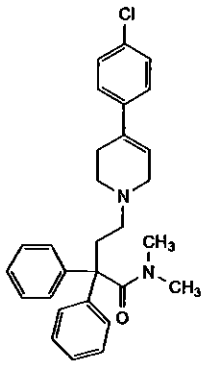
E. 1,4-bis[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]-2,2-diphenylbutan-1-one,



F. (1*r*,4*r*)-4-(4-chlorophenyl)-1-[4-(*N*,*N*-dimethylamino)-3,3-diphenyl-4-oxobutyl]-4-hydroxypiperazine 1-oxide (loperamide oxide),



G. (1*s*,4*s*)-4-(4-chlorophenyl)-1-[4-(*N*,*N*-dimethylamino)-3,3-diphenyl-4-oxobutyl]-4-hydroxypiperazine 1-oxide,

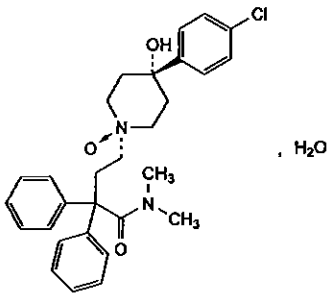


H. 4-[4-(4-chlorophenyl)-3,6-dihydropyridin-1(2*H*)-yl]-*N*,*N*-dimethyl-2,2-diphenylbutanamide.

Ph Eur

Loperamide Oxide Monohydrate

(Ph. Eur. monograph 1729)



$C_{29}H_{33}ClN_2O_3,H_2O$ 511.1

Action and use
Opioid receptor agonist; antidiarrhoeal.

Ph Eur

DEFINITION
4-[*trans*-4-(4-Chlorophenyl)-4-hydroxy-1-oxidopiperidin-1-yl]-*N*,*N*-dimethyl-2,2-diphenylbutanamide monohydrate.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white powder, slightly hygroscopic.

Solubility
Practically insoluble in water, freely soluble in alcohol and in methylene chloride.

mp
About 152 °C, with decomposition.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *loperamide oxide monohydrate CRS.*

TESTS
Related substances
Liquid chromatography (*2.2.29*).
Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 mL with the same solvent.
Reference solution (a) Dissolve 5.0 mg of *loperamide hydrochloride CRS* in *methanol R*, add 0.5 mL of the test solution and dilute to 100.0 mL with *methanol R*.
Reference solution (b) Dilute 1.0 mL of the test solution to 20.0 mL with *methanol R*. Dilute 1.0 mL of this solution to 25.0 mL with *methanol R*.
Column:
— *size*: *l* = 0.10 m, Ø = 4.6 mm,
— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm),
— *temperature*: 35 °C.
Mobile phase:
— *mobile phase A*: 17.0 g/L solution of *tetrabutylammonium hydrogen sulfate R1*,
— *mobile phase B*: *acetonitrile R*,

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	90 → 30	10 → 70
15 - 17	30	70

Flow rate 1.5 mL/min.
Detection Spectrophotometer at 220 nm.
Injection 10 µL.
Relative retention With reference to loperamide oxide (retention time = about 7 min): impurity A = about 0.9; impurity B = about 1.11; impurity C = about 1.13.
System suitability Reference solution (a):
— *resolution*: minimum 3.8 between the peaks due to loperamide oxide and impurity A.
Limits:
— *impurities A, B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent),
— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent),
— *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent),
— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

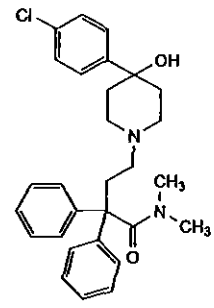
Water (*2.5.12*)
3.4 per cent to 4.2 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

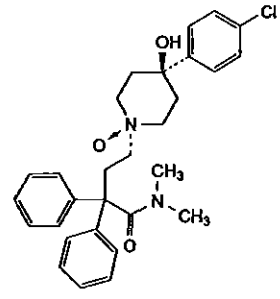
ASSAY
Dissolve 0.350 g in 50 mL of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Titrate with *0.1 M perchloric acid* using 0.2 mL of *naphtholbenzein solution R* as indicator.
1 mL of *0.1 M perchloric acid* is equivalent to 49.30 mg of $C_{29}H_{33}ClN_2O_3$.

STORAGE
In an airtight container, protected from light.

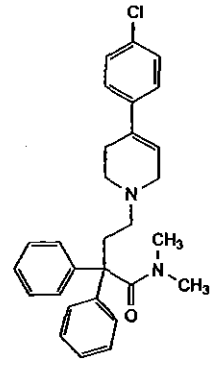
IMPURITIES
Specified impurities *A, B, C.*



A. 4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]-*N*,*N*-dimethyl-2,2-diphenylbutanamide (loperamide),



B. 4-[*cis*-4-(4-chlorophenyl)-4-hydroxy-1-oxidopiperidin-1-yl]-*N*,*N*-dimethyl-2,2-diphenylbutanamide,

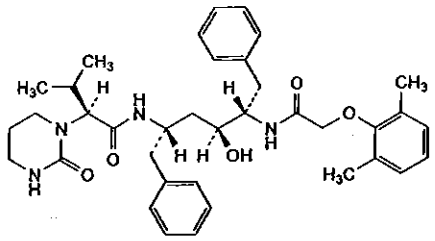


C. 4-[4-(4-chlorophenyl)-3,6-dihydropyridin-1(2*H*)-yl]-*N*,*N*-dimethyl-2,2-diphenylbutanamide.

Ph Eur

Lopinavir

(Ph. Eur. monograph 2615)



$C_{37}H_{48}N_4O_5$ 629 *192725-17-0*

Action and use
Protease inhibitor; antiviral (HIV).

Ph Eur

DEFINITION

(2*S*)-*N*-[(1*S*,3*S*,4*S*)-1-Benzyl-4-[[2-(2,6-dimethylphenoxy)acetyl]amino]-3-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or yellowish-white, slightly hygroscopic powder.

Solubility
Practically insoluble in water, very soluble in methanol and in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *lopinavir CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

TESTS

Specific optical rotation (*2.2.7*)
−27.0 to −22.0 (anhydrous substance).

Dissolve 0.200 g in *methanol R* and dilute to 25.0 mL with the same solvent.

Related substances
A. Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R1*, *water R* (50:50 *V/V*).

Phosphate buffer solution Dissolve 0.9 g of *dipotassium hydrogen phosphate R* and 2.7 g of *potassium dihydrogen phosphate R* in 900 mL of *water R* and mix well. Adjust to pH 6.0 with *phosphoric acid R*, dilute to 1000 mL with *water R* and filter.

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Test solution (b) Dilute 5.0 mL of test solution (a) to 100.0 mL with the solvent mixture.

Reference solution (a) Dissolve 50.0 mg of *lopinavir CRS* in the solvent mixture and dilute to 100.0 mL with the solvent mixture. Dilute 5.0 mL of the solution to 100.0 mL with the solvent mixture.

Reference solution (b) Dilute 5.0 mL of test solution (b) to 250.0 mL with the solvent mixture.

Reference solution (c) Dissolve 2.5 mg of *lopinavir for system suitability CRS* (containing impurities A, B, C, F, G, I, N, Q, R, S and T) in the solvent mixture and dilute to 5.0 mL with the solvent mixture.

Reference solution (d) Dissolve 2.5 mg of *lopinavir for peak identification CRS* (containing impurities D and O) in the solvent mixture and dilute to 5.0 mL with the solvent mixture.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (4 µm);
- *temperature*: 50 °C.

Mobile phase:
- *mobile phase A*: *acetonitrile R1*, phosphate buffer solution (45:55 *V/V*);
- *mobile phase B*: phosphate buffer solution, *acetonitrile R1* (25:75 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 60	100	0
60 - 61	100 → 0	0 → 100
61 - 81	0	100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µL of test solution (a) and reference solutions (b), (c) and (d).

Identification of impurities Use the chromatogram supplied with *lopinavir for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, C, F, G, I and N; use the chromatogram supplied with *lopinavir for peak identification CRS* and the chromatogram obtained with reference solution (d) to identify the peak due to impurity D.

Relative retention r (not r_G) with reference to lopinavir (retention time = about 37 min): impurity A = about 0.03; impurity B = about 0.07; impurity C = about 0.10; impurity D = about 0.13; impurity F = about 0.59; impurity G = about 0.62; impurity I = about 1.1; impurity N = about 1.4.

System suitability Reference solution (c):
- *resolution*: minimum 1.5 between the peaks due to impurities F and G.

Calculation of percentage contents:
- for impurity A, multiply the peak area by the correction factor 1.6;
- for impurity B, multiply the peak area by the correction factor 1.3;
- for impurity C, multiply the peak area by the correction factor 1.5;
- for impurity D, multiply the peak area by the correction factor 1.3;
- for each impurity, use the concentration of lopinavir in reference solution (b).

Limits:
- *impurities B, I*: for each impurity, maximum 0.2 per cent;
- *impurities A, C, D, F, G*: for each impurity, maximum 0.15 per cent;

— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *reporting threshold*: 0.05 per cent; disregard any peak eluting after impurity N.

B. Liquid chromatography (*2.2.29*) as described in test A for related substances with the following modifications.

Mobile phase Mobile phase A, mobile phase B (30:70 *V/V*).

Run time 8.3 times the retention time of lopinavir.

Identification of impurities Use the chromatogram supplied with *lopinavir for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities Q, R, S and T; use the chromatogram supplied with *lopinavir for peak identification CRS* and the chromatogram obtained with reference solution (d) to identify the peak due to impurity O.

Relative retention r (not r_G) with reference to lopinavir (retention time = about 6 min): impurity N = about 1.4; impurity O = about 1.5; impurity Q = about 4.4; impurity R = about 6.0; impurity S = about 7.1; impurity T = about 8.5.

System suitability Reference solution (c):
— *resolution*: minimum 3.0 between the peaks due to impurities S and T.

Calculation of percentage contents:
— for impurity O, multiply the peak area by the correction factor 1.3;
— for impurity Q, multiply the peak area by the correction factor 0.7;
— for each impurity, use the concentration of lopinavir in reference solution (b).

Limits:
— *impurities O, Q, R, T*: for each impurity, maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *reporting threshold*: 0.05 per cent; disregard any peak eluting before and including impurity N;
— *total of all impurities eluting before and including impurity N in test A and after impurity N in test B*: maximum 0.7 per cent.

Water

(*2.5.12*): maximum 4.4 per cent, determined on 0.250 g.

Sulfated ash (*2.4.14*)

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in test A for related substances with the following modifications.

Mobile phase Mobile phase A.

Injection Test solution (b) and reference solution (a).

Run time 1.6 times the retention time of lopinavir.

Calculate the percentage content of $C_{37}H_{48}N_4O_5$ taking into account the assigned content of *lopinavir CRS*.

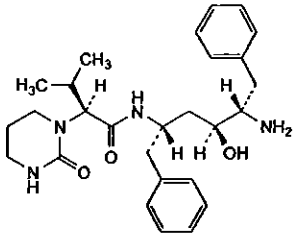
STORAGE

In an airtight container.

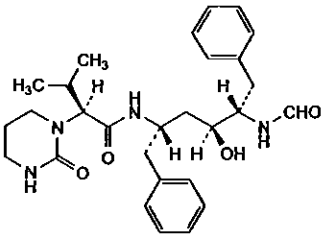
IMPURITIES

Specified impurities *A, B, C, D, F, G, I, O, Q, R, T.*

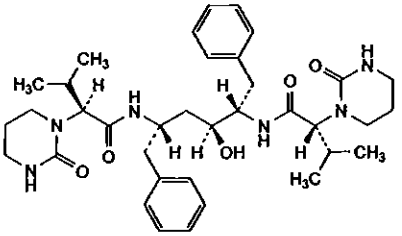
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *E, H, J, K, L, M, N, P, S.*



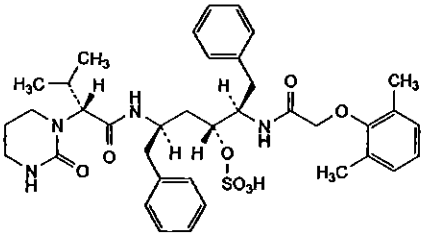
A. (2*S*)-*N*-[(1*S*,3*S*,4*S*)-1-benzyl-4-amino-3-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide,



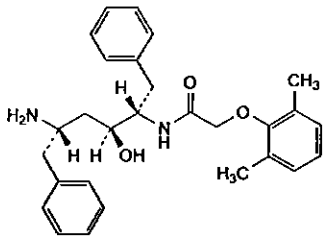
B. (2*S*)-*N*-[(1*S*,3*S*,4*S*)-1-benzyl-4-(formylamino)-3-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide,



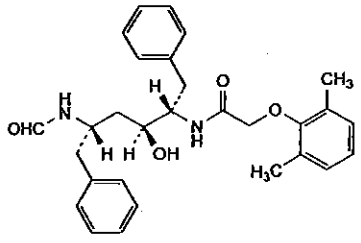
C. (2*R*)-*N*-[(1*S*,2*S*,4*S*)-1-benzyl-2-hydroxy-4-[[(2*S*)-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanoyl]amino]-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide,



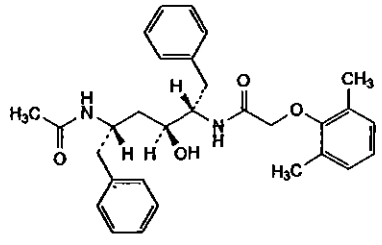
D. (1*R*,3*R*)-1-[(1*R*)-1-[[2-(2,6-dimethylphenoxy)acetyl]amino]-2-phenylethyl]-3-[[(2*R*)-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanoyl]amino]-4-phenylbutyl hydrogen sulfate,



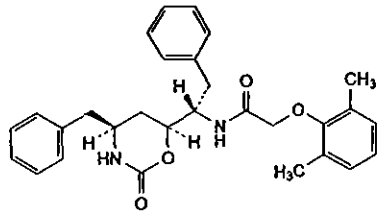
E. *N*-[(1*S*,2*S*,4*S*)-4-amino-1-benzyl-2-hydroxy-5-phenylpentyl]-2-(2,6-dimethylphenoxy)acetamide,



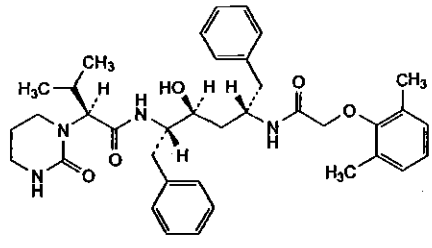
F. *N*-[(1*S*,2*S*,4*S*)-1-benzyl-4-(formylamino)-2-hydroxy-5-phenylpentyl]-2-(2,6-dimethylphenoxy)acetamide,



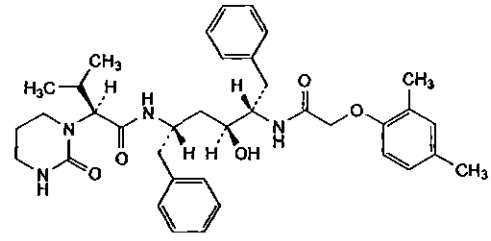
G. *N*-[(1*S*,2*S*,4*S*)-(4-acetylamino)-1-benzyl-2-hydroxy-5-phenylpentyl]-2-(2,6-dimethylphenoxy)acetamide,



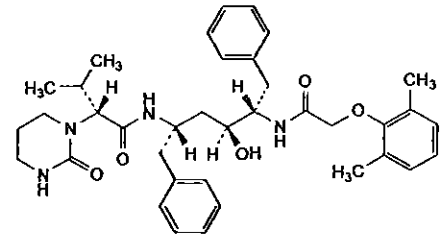
H. *N*-[(1*S*)-1-[(4*S*,6*S*)-4-benzyl-2-oxo-1,3-oxazinan-6-yl]-2-phenylethyl]-2-(2,6-dimethylphenoxy)acetamide,



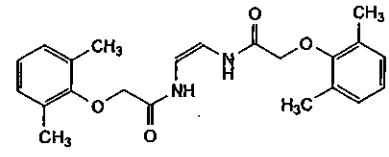
I. (2*S*)-*N*-[(1*S*,2*S*,4*S*)-1-benzyl-4-[[2-(2,6-dimethylphenoxy)acetyl]amino]-2-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide,



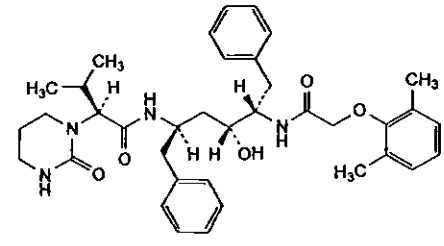
J. (2*S*)-*N*-[(1*S*,3*S*,4*S*)-1-benzyl-4-[[2-(2,4-dimethylphenoxy)acetyl]amino]-3-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide,



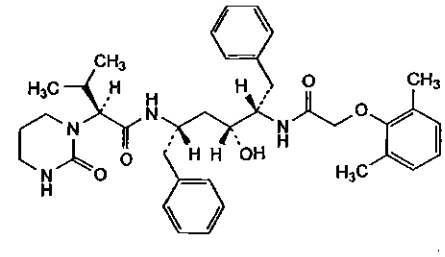
K. (2*R*)-*N*-[(1*S*,3*S*,4*S*)-1-benzyl-4-[[2-(2,6-dimethylphenoxy)acetyl]amino]-3-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide,



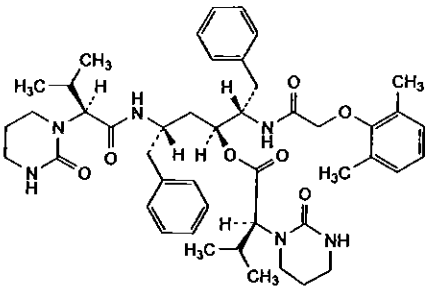
L. *N*,*N*′-(*Z*)-ethene-1,2-diylbis[2-(2,6-dimethylphenoxy)acetamide],



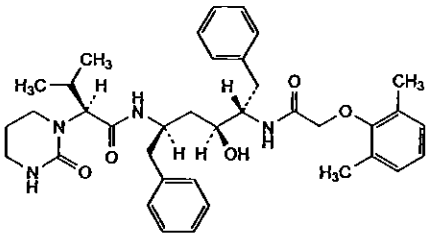
M. (2*S*)-*N*-[(1*R*,3*R*,4*S*)-1-benzyl-4-[[2-(2,6-dimethylphenoxy)acetyl]amino]-3-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide,



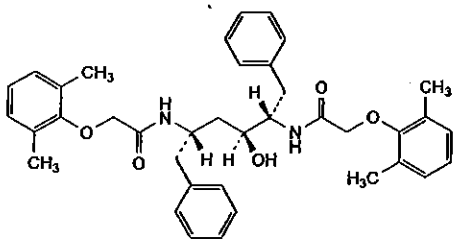
N. (2*S*)-*N*-[(1*S*,3*R*,4*S*)-1-benzyl-4-[[2-(2,6-dimethylphenoxy)acetyl]amino]-3-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide,



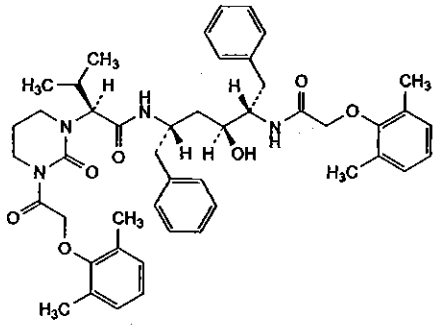
O. (1*S*,3*S*)-1-[(1*S*)-1-[[2-(2,6-dimethylphenoxy)acetyl]amino]-2-phenylethyl]-3-[[(2*S*)-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanoyl]amino]-4-phenylbutyl (2*S*)-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanoate,



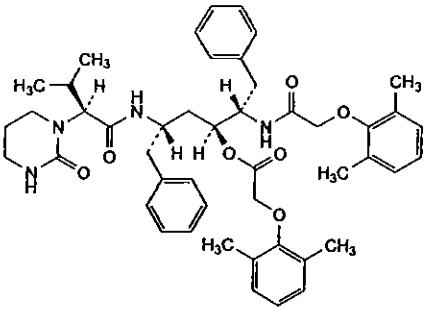
P. (2*S*)-*N*-[(1*R*,3*S*,4*S*)-1-benzyl-4-[[2-(2,6-dimethylphenoxy)acetyl]amino]-3-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide,



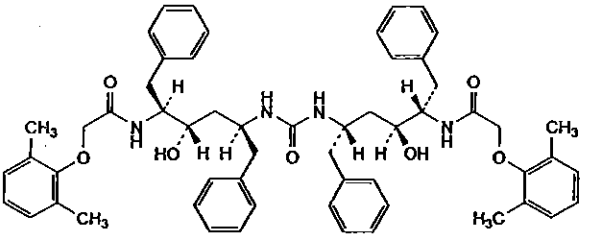
Q. *N*-[(1*S*,2*S*,4*S*)-1-benzyl-4-[[2-(2,6-dimethylphenoxy)acetyl]amino]-2-hydroxy-5-phenylpentyl]-2-(2,6-dimethylphenoxy)acetamide,



R. (2*S*)-*N*-[(1*S*,3*S*,4*S*)-1-benzyl-4-[[2-(2,6-dimethylphenoxy)acetyl]amino]-3-hydroxy-5-phenylpentyl]-2-[3-[2-(2,6-dimethylphenoxy)acetyl]-2-oxotetrahydropyrimidin-1(2*H*)-yl]-3-methylbutanamide,



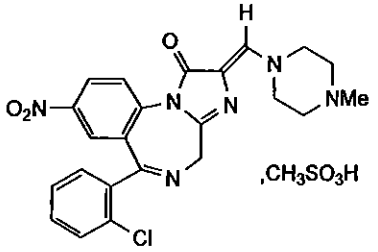
S. (1*S*,3*S*)-1-[(1*S*)-1-[[2-(2,6-dimethylphenoxy)acetyl]amino]-2-phenylethyl]-3-[[(2*S*)-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanoyl]amino]-4-phenylbutyl 2-(2,6-dimethylphenoxy)acetate,



T. *N*,*N*′-bis[(1*S*,3*S*,4*S*)-1-benzyl-4-[[2-(2,6-dimethylphenoxy)acetyl]amino]-3-hydroxy-5-phenylpentyl]urea.

Ph Eur

Loprazolam Mesilate



$C_{23}H_{21}ClN_6O_3,CH_4O_3S,H_2O$ 579.1 *70111-54-5*

Action and use
Benzodiazepine.

Preparation
Loprazolam Tablets

DEFINITION
Loprazolam Mesilate is (*Z*)-6-(2-chlorophenyl)-2,4-dihydro-2-(4-methylpiperazin-1-ylmethylene)-8-nitroimidazo[1,2-*a*][1,4]benzodiazepin-1-one methanesulfonate mono-hydrate. It contains not less than 98.5% and not more than 101.0% of $C_{23}H_{21}ClN_6O_3,CH_4O_3S$, calculated with reference to the dried substance.

PRODUCTION
Risk assessment should be used to evaluate the potential for genotoxic methanesulfonate esters to be formed in the presence of low molecular weight alcohols. If a risk of methanesulfonate ester formation is identified through risk assessment, these impurities should not exceed the threshold of toxicological concern.

CHARACTERISTICS

A yellow, crystalline powder.

Slightly soluble in *water* and in *ethanol (96%)*; very slightly soluble in *ether*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of loprazolam mesilate *(RS 205)*.

B. The *light absorption*, Appendix II B, in the range 210 to 370 nm of a 0.001% w/v solution in *ethanol (96%)* exhibits a maximum at 330 nm and a shoulder at 240 nm.
The *absorbance* at the maximum is about 0.7.

TESTS

N-*methylpiperazine*

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in a mixture of equal volumes of *chloroform* and *methanol*.

(1) 2.0% w/v of the substance being examined.

(2) 0.0050% w/v of N-*methylpiperazine*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, heat at 110° for 15 minutes and spray with a mixture of 50 volumes of a 2% w/v solution of *potassium iodide* and 1 volume of a 10% w/v solution of *chloroplatinic(IV) acid*.

MOBILE PHASE

2 volumes of 13.5M *ammonia*, 20 volumes of *methanol* and 80 volumes of *chloroform*.

LIMITS

Any spot corresponding to N-*methylpiperazine* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.25%).

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in a mixture of 8 volumes of *water*, 46 volumes of *chloroform* and 46 volumes of *methanol*.

(1) 2.0% w/v of the substance being examined.

(2) 0.0020% w/v of the substance being examined.

(3) 0.010% w/v of *6-(2-chlorophenyl)-2,4-dihydro-2-[(dimethylamino)methylene]-8-nitroimidazo[1,2-a][1,4]benzodiazepin-1-one BPCRS* (impurity A).

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable). Before use, stand the plate in *methanol*, allowing the solvent front to ascend 17 cm, allow to dry in air, heat the plate at 100° to 105° for 1 hour and use with the flow of mobile phase in the same direction as that used for the pretreatment.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, spray the plate with a 5% w/v solution of *titanium(III) chloride* in a solution of hydrochloric acid containing 10% w/v of HCl and then spray with a solution containing 0.4 g of *4-dimethylaminocinnamaldehyde* in a mixture of 20 mL of *6M hydrochloric acid* and 100 mL of *ethanol (96%)*. Heat at 100° until spots appear (about 10 minutes).

MOBILE PHASE

20 volumes of *methanol* and 80 volumes of *chloroform*.

LIMITS

Any spot corresponding to impurity A in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.1%).

Loss on drying

When dried at 100° to 105° for 3 hours, loses 2.5 to 4.5% of its weight. Use 1 g.

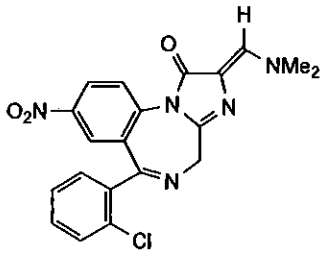
Sulfated ash

Not more than 0.1%, Appendix IX A.

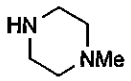
ASSAY

Dissolve 0.25 g in 60 mL of a 50% v/v solution of *propan-2-ol* and titrate with *0.05M sodium hydroxide VS* determining the end point potentiometrically. Each mL of *0.05M sodium hydroxide VS* is equivalent to 28.05 mg of $C_{23}H_{21}ClN_6O_3$, CH_4O_3S.

IMPURITIES



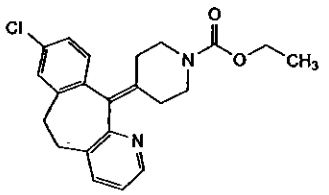
A. 6-(2-chlorophenyl)-2,4-dihydro-2-[(dimethylamino) methylene]-8-nitroimidazo[1,2-*a*][1,4]benzodiazepin-1-one; dimethylamino analogue.



B. *N*-methylpiperazine.

Loratadine

(Ph. Eur. monograph 2124)



$C_{22}H_{23}ClN_2O_2$ 382.9 *79794-75-5*

Action and use

Histamine H_1 receptor antagonist; antihistamine.

Preparation

Loratadine Tablets

Ph Eur

DEFINITION

Ethyl 4-(8-chloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-ylidene)piperidine-1-carboxylate.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in acetone and in methanol.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *loratadine CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *acetone R*, evaporate to dryness and record new spectra using the residues.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

Dissolve 1.0 g in *methanol R* and dilute to 20.0 mL with the same solvent.

Impurity H

Gas chromatography (*2.2.28*).

Internal standard solution Dissolve 25 mg of *isoamyl benzoate R* in *methylene chloride R* and dilute to 100 mL with the same solvent. Dilute 5.0 mL of this solution to 50 mL with *methylene chloride R*.

Test solution Dissolve 25.0 mg of the substance to be examined in *methylene chloride R*, add 1.0 mL of reference solution (a) and 1.0 mL of the internal standard solution and dilute to 5.0 mL with *methylene chloride R*.

Reference solution (a) Dissolve 25.0 mg of *loratadine impurity H CRS* in *methylene chloride R* and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of this solution to 50.0 mL with *methylene chloride R*.

Reference solution (b) To 1.0 mL of reference solution (a) add 1.0 mL of the internal standard solution and dilute to 5.0 mL with *methylene chloride R*.

Column:
- *material*: fused silica;
- *size*: *l* = 25 m, Ø = 0.32 mm;
- *stationary phase*: *methylpolysiloxane R* (film thickness 0.52 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.0 mL/min.

Split ratio 1:30.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 1	80
	1 - 23	80 → 300
	23 - 33	300
Injection port		260
Detector		300

Detection Flame ionisation.

Injection 1 µL of the test solution and reference solution (b).

Relative retention With reference to loratadine (retention time = about 32 min): impurity H = about 0.33; isoamyl benzoate = about 0.37.

System suitability Reference solution (b):
- *resolution*: minimum 2.0 between the peaks due to impurity H and isoamyl benzoate;
- *signal-to-noise ratio*: minimum 10 for the peak due to impurity H.

Limit:
- *impurity H*: calculate the ratio (*R*) of the area of the peak due to impurity H to the area of the peak due to isoamyl benzoate from the chromatogram obtained with reference solution (b); from the chromatogram obtained with the test solution, calculate the ratio of the area of the peak due to impurity H to the area of the peak due to isoamyl benzoate: this ratio is not greater than twice *R* (0.1 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 mL with the mobile phase.

Reference solution (a) Dissolve 5 mg of *loratadine impurity F CRS* in the mobile phase and dilute to 25 mL with the mobile phase. Dilute 1 mL of this solution to 10 mL with the mobile phase.

Reference solution (b) Dissolve 5 mg of *loratadine for system suitability CRS* (containing impurities A and E) in the mobile phase, add 0.5 mL of reference solution (a) and dilute to 5 mL with the mobile phase.

Reference solution (c) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm) with very low silanol activity;
- *temperature*: 40 °C.

Mobile phase Mix 30 volumes of *methanol R*, 35 volumes of a 6.8 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 2.80 ± 0.05 with *phosphoric acid R* and 40 volumes of *acetonitrile R*.

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µL of the test solution and reference solutions (b) and (c).

Run time 5 times the retention time of loratadine.

Identification of impurities Use the chromatogram supplied with *loratadine for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and E.

Relative retention With reference to loratadine (retention time = about 12 min): impurity D = about 0.2; impurity B = about 0.4; impurity F = about 0.9; impurity E = about 1.1; impurity A = about 2.4; impurity C = about 2.7.

System suitability Reference solution (b):
- *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the

curve separating this peak from the peak due to loratadine.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.7; impurity F = 1.6; impurity E = 1.9;

— *impurity F*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

— *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Sulfates (*2.4.13*)

Maximum 150 ppm.

Ignite 1.33 g at 800 ± 25 °C and take up the residue with 20 mL of *distilled water R*. Filter, if necessary, through paper free from sulfates. Repeat the filtration with new paper filters until the filtrate is no longer turbid.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

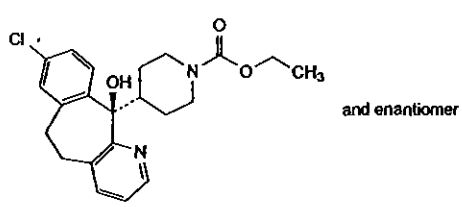
Dissolve 0.300 g in 50 mL of *glacial acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 38.29 mg of $C_{22}H_{23}ClN_2O_2$.

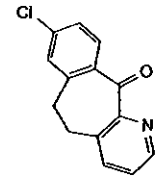
IMPURITIES

Specified impurities *A, B, C, D, E, F, H.*

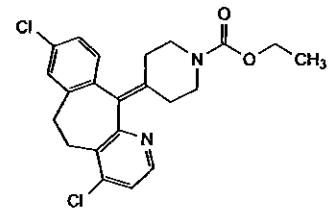
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *G.*



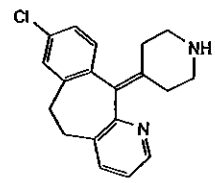
A. ethyl 4-[(11*RS*)-8-chloro-11-hydroxy-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl]piperidine-1-carboxylate,



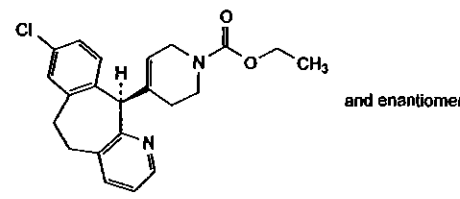
B. 8-chloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-one,



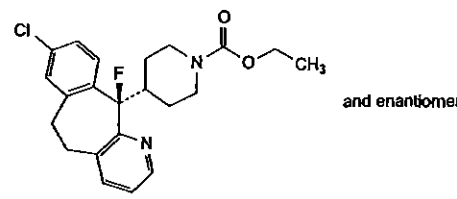
C. ethyl 4-(4,8-dichloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-ylidene)piperidine-1-carboxylate,



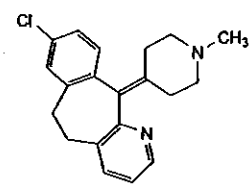
D. 8-chloro-11-(piperidin-4-ylidene)-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine,



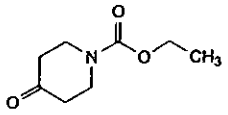
E. ethyl 4-[(11*RS*)-8-chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl]-3,6-dihydropyridine-1(2*H*)-carboxylate,



F. ethyl 4-[(11*RS*)-8-chloro-11-fluoro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl]piperidine-1-carboxylate,



G. 8-chloro-11-(1-methylpiperidin-4-ylidene)-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine,

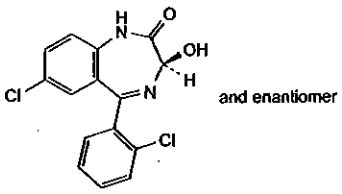


H. ethyl 4-oxopiperidine-1-carboxylate.

Ph Eur

Lorazepam

(Ph. Eur. monograph 1121)



and enantiomer

$C_{15}H_{10}Cl_2N_2O_2$ 321.2 *846-49-1*

Action and use
Benzodiazepine.

Preparations
Lorazepam Injection
Lorazepam Oral Solution
Lorazepam Oral Suspension
Lorazepam Tablets

Ph Eur

DEFINITION
(3*RS*)-7-Chloro-5-(2-chlorophenyl)-3-hydroxy-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.

Content
98.5 per cent to 102.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, sparingly soluble in ethanol (96 per cent), sparingly soluble or slightly soluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Spectral range 600-2000 cm^{-1}.

Comparison *lorazepam CRS*.

TESTS
Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 40.0 mg of the substance to be examined in 25 mL of *acetonitrile R1* and dilute to 50.0 mL with *water R*.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with a mixture of equal volumes of *acetonitrile R1* and *water R*. Dilute 1.0 mL of this solution to 10.0 mL with a mixture of equal volumes of *acetonitrile R1* and *water R*.

Reference solution (b) Dissolve the contents of a vial of *lorazepam for system suitability CRS* (containing impurities B and D) in 1 mL of a mixture of equal volumes of *acetonitrile R1* and *water R*.

Reference solution (c) Dissolve 4.0 mg of *lorazepam impurity D CRS* in 25 mL of *acetonitrile R1* and dilute to 50.0 mL with *water R*. Dilute 1.0 mL of this solution to 100.0 mL with a mixture of equal volumes of *acetonitrile R1* and *water R*.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography with extended pH range R* (5 µm).

Mobile phase:
— *mobile phase A*: dissolve 3.48 g of *dipotassium hydrogen phosphate R* in a mixture of 50 mL of *acetonitrile R1* and 850 mL of *water for chromatography R*; adjust the apparent pH to 10.5 with a 40 g/L solution of *sodium hydroxide R* and dilute to 1000 mL with *water for chromatography R*;
— *mobile phase B*: *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	80	20
5 - 35	80 → 30	20 → 70
35 - 50	30	70
50 - 60	30 → 80	70 → 20

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 235 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram supplied with *lorazepam for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B and D.

Relative retention With reference to lorazepam (retention time = about 17 min): impurity D = about 0.9; impurity B = about 1.1.

System suitability Reference solution (b):
— *resolution*: minimum 4.5 between the peaks due to impurity D and lorazepam;
— *peak-to-valley ratio*: minimum 5.0, where H_p = height above the baseline of the peak due to impurity B and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to lorazepam.

Limits:
— *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *impurity D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 105 °C at a pressure not exceeding 0.1 kPa.

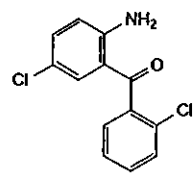
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.250 g in 30 mL of *dimethylformamide R*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Protect the solution from atmospheric carbon dioxide throughout the titration.

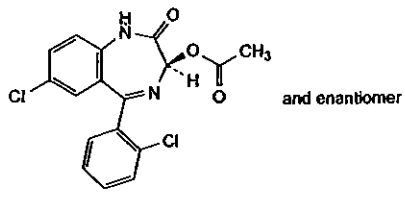
1 mL of *0.1 M tetrabutylammonium hydroxide* is equivalent to 32.12 mg of $C_{15}H_{10}Cl_2N_2O_2$.

IMPURITIES
Specified impurities *B, D.*

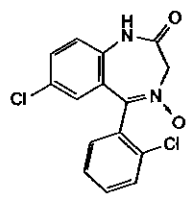
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, C, E.*



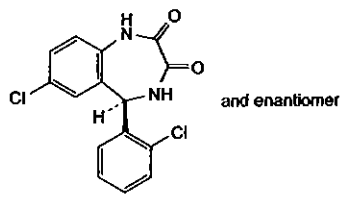
A. (2-amino-5-chlorophenyl)(2-chlorophenyl)methanone,



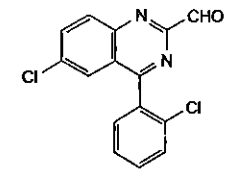
B. (3*RS*)-7-chloro-5-(2-chlorophenyl)-2-oxo-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl acetate,



C. 7-chloro-5-(2-chlorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one 4-oxide,



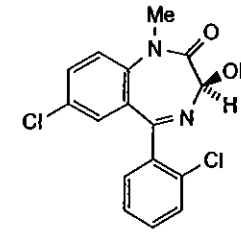
D. (5*RS*)-7-chloro-5-(2-chlorophenyl)-4,5-dihydro-1*H*-1,4-benzodiazepine-2,3-dione,



E. 6-chloro-4-(2-chlorophenyl)quinazoline-2-carbaldehyde.

Ph Eur

Lormetazepam



and enantiomer

$C_{16}H_{12}Cl_2N_2O_2$ 335.2 *848-75-9*

Action and use
Benzodiazepine.

Preparation
Lormetazepam Tablets

DEFINITION
Lormetazepam is (*RS*)-7-chloro-5-(2-chlorophenyl)-1,3-dihydro-3-hydroxy-1-methyl-1,4-benzodiazepin-2-one.
It contains not less than 99.0% and not more than 101.0% of $C_{16}H_{12}Cl_2N_2O_2$, calculated with reference to the dried substance.

CHARACTERISTICS
A white, crystalline powder.

Practically insoluble in *water*; soluble in *ethanol (96%)* and in *methanol*.

IDENTIFICATION
A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of lormetazepam *(RS 207)*.

B. In the test for Related substances, the chromatogram obtained with solution (2) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (4).

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *methanol (70%)*.

(1) 0.25% w/v of the substance being examined.

(2) 0.0005% w/v of the substance being examined.

(3) 0.00025% w/v of the substance being examined.

(4) 0.0005% w/v of *lormetazepam BPCRS*.

(5) 0.00025% w/v each of *lormetazepam BPCRS* and *lorazepam BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 230 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

48 volumes of *methanol* and 52 volumes of a phosphate buffer prepared by dissolving 4.91 g of *sodium dihydrogen orthophosphate* and 0.633 g of *disodium hydrogen orthophosphate* in sufficient *water* to produce 1000 mL.

SYSTEM SUITABILITY

The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (5) is at least 4.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than that of the principal peak in the chromatogram obtained with solution (2) (0.2%);

not more than two such peaks have an area greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%);

the sum of the areas of all such peaks is not greater than 2.5 times the area of the principal peak obtained with solution (2) (0.5%).

Loss on drying

When dried to constant weight at 105° for 3 hours, loses not more 1.0% of its weight. Use 1 g.

Sulfated ash

Not more than 0.1%, Appendix IX A.

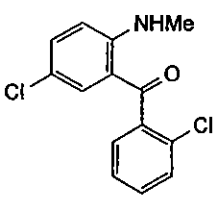
ASSAY

Dissolve 0.5 g in 50 mL of *nitroethane* and carry out Method I for *non-aqueous titration*, Appendix VIII A, determining the end point potentiometrically. Each mL of *0.1M perchloric acid VS* is equivalent to 33.52 mg of $C_{16}H_{12}Cl_2N_2O_2$.

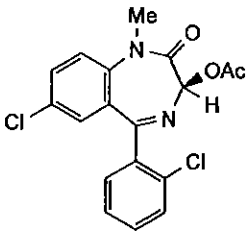
STORAGE

Lormetazepam should be protected from light.

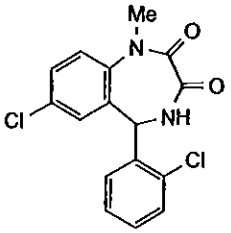
IMPURITIES



A. 2-methylamino-2′,5-dichlorobenzophenone,



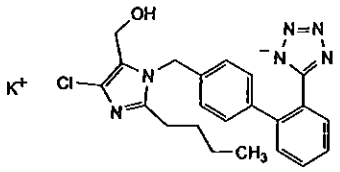
B. O^3acetyl-lormetazepam,



C. 7-chloro-1-methyl-5-(2-chlorophenyl)-4,5-dihydro-2*H*-1,4-benzodiazepin-2,3-(1*H*)-dione.

Losartan Potassium

(Ph. Eur. monograph 2232)



$C_{22}H_{22}ClKN_6O$ 461.0 *124750-99-8*

Action and use

Angiotensin II (AT1) receptor antagonist.

Preparation

Losartan Potassium Tablets

Ph Eur

DEFINITION

Potassium 5-[4′-[[2-butyl-4-chloro-5-(hydroxymethyl)-1*H*-imidazol-1-yl]methyl]biphenyl-2-yl]tetrazol-1-ide.

Content

98.5 per cent to 101.5 per cent (dried substance).

PRODUCTION

As *N*-nitrosamines are classified as probable human carcinogens, their presence in losartan potassium should be avoided or limited as much as possible. For this reason, manufacturers of losartan potassium for human use are expected to perform an assessment of the risk of *N*-nitrosamine formation and contamination during their manufacturing process; if this assessment identifies a potential risk, the manufacturing process should be modified to minimise contamination and a control strategy implemented to detect and control *N*-nitrosamine impurities in losartan potassium. The general chapter *2.5.42. N-Nitrosamines in active substances* is available to assist manufacturers.

CHARACTERS

Appearance

White or almost white, crystalline powder, hygroscopic.

Solubility

Freely soluble in water and in methanol, slightly soluble in acetonitrile.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *losartan potassium CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

B. Dissolve 25 mg in 3 mL of *water R*. The solution gives reaction (a) of potassium (*2.3.1*).

TESTS

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 30.0 mg of the substance to be examined in *methanol R* and dilute to 100.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *methanol R*. Dilute 1.0 mL of this solution to 10.0 mL with *methanol R*.

Reference solution (b) Dissolve 6 mg of *triphenylmethanol R* (impurity G) in 100 mL of *methanol R*. Dilute 1 mL of the solution to 100 mL with *methanol R*. Use 1 mL of this solution to dissolve the contents of a vial of *losartan for system suitability CRS* (containing impurities J, K, L and M) and sonicate for 5 min.

Reference solution (c) Dissolve 3.0 mg of *losartan impurity D CRS* in *methanol R* and dilute to 100.0 mL with the same solvent. Dilute 1.5 mL of this solution to 100.0 mL with *methanol R*.

Column:
- *size*: l = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 35 °C.

Mobile phase:
- *mobile phase A*: dilute 1.0 mL of *phosphoric acid R* to 1000 mL with *water for chromatography R*;
- *mobile phase B*: *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	75	25
5 - 30	75 → 10	25 → 90
30 - 40	10	90

Flow rate 1.3 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µL.

Identification of impurities Use the chromatogram supplied with *losartan for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities G, J, K, L and M; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity D.

Relative retention With reference to losartan (retention time = about 14 min): impurity D = about 0.9; impurity J = about 1.4; impurity K = about 1.5; impurity L = about 1.6; impurity M = about 1.75; impurity G = about 1.8.

System suitability Reference solution (b):
- *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity M and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity G.

Limits:
- *impurity D*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.15 per cent);
- *impurities J, K, L, M*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.200 g in 75 mL of *anhydrous acetic acid R* and sonicate for 10 min. Carry out a potentiometric titration (*2.2.20*) using *0.1 M perchloric acid*.

1 mL of *0.1 M perchloric acid* is equivalent to 23.05 mg of $C_{22}H_{22}ClKN_6O$.

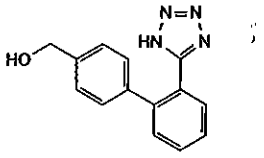
STORAGE

In an airtight container.

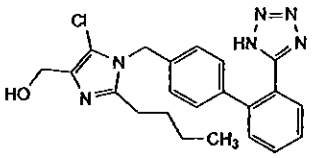
IMPURITIES

Specified impurities *D, J, K, L, M.*

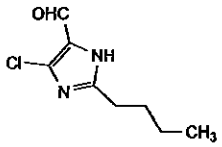
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, E, F, G, H, I.*



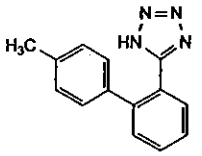
B. [2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methanol,



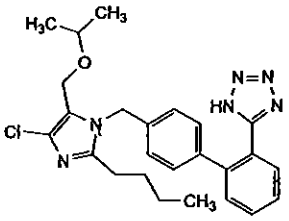
C. [2-butyl-5-chloro-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl] methyl]-1*H*-imidazol-4-yl]methanol,



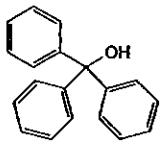
D. 2-butyl-4-chloro-1*H*-imidazole-5-carbaldehyde,



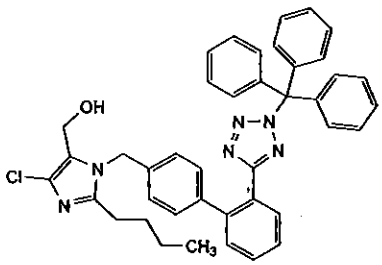
E. 5-(4′-methylbiphenyl-2-yl)-1*H*-tetrazole,



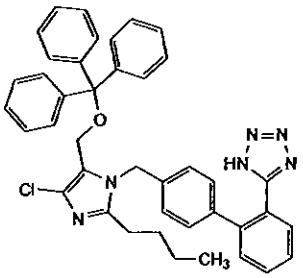
F. 5-[4′-[[2-butyl-4-chloro-5-[[(1-methylethyl)oxy]methyl]-1*H*-imidazol-1-yl]methyl]biphenyl-2-yl]-1*H*-tetrazole,



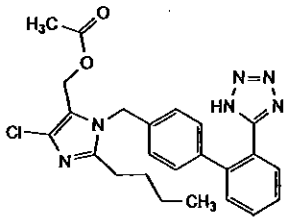
G. triphenylmethanol,



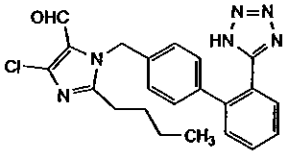
H. [2-butyl-4-chloro-1-[[2′-[2-(triphenylmethyl)-2*H*-tetrazol-5-yl]biphenyl-4-yl]methyl]-1*H*-imidazol-5-yl]methanol,



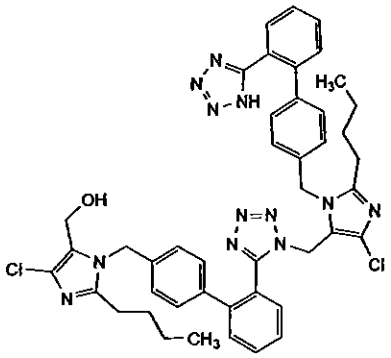
I. 5-[4′-[[2-butyl-4-chloro-5-[[(triphenylmethyl]oxy]methyl]-1*H*-imidazol-1-yl]methyl]biphenyl-2-yl]-1*H*-tetrazole,



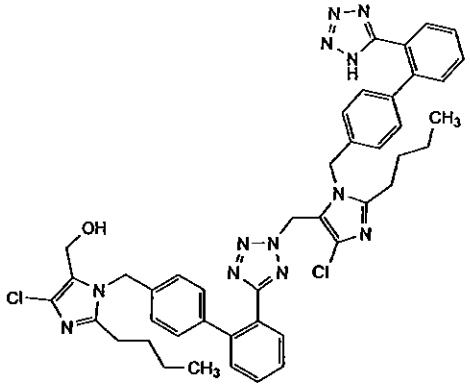
J. [2-butyl-4-chloro-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl] methyl]-1*H*-imidazol-5-yl]methyl acetate,



K. 2-butyl-4-chloro-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl] methyl]-1*H*-imidazol-5-carbaldehyde,



L. [2-butyl-1-[[2′-[1-[[2-butyl-4-chloro-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-imidazol-5-yl]methyl]-1*H*-tetrazol-5-yl]biphenyl-4-yl]methyl]-4-chloro-1*H*-imidazol-5-yl]methanol,

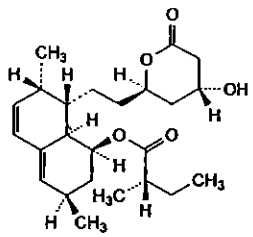


M. [2-butyl-1-[[2′-[2-[[2-butyl-4-chloro-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-imidazol-5-yl]methyl]-2*H*-tetrazol-5-yl]biphenyl-4-yl]methyl]-4-chloro-1*H*-imidazol-5-yl]methanol.

Ph Eur

Lovastatin

(Ph. Eur Monograph 1538)



$C_{24}H_{36}O_5$ 404.5 *75330-75-5*

Action and use
HMG Co-A reductase inhibitor; lipid-regulating drug.

Ph Eur

DEFINITION
(1*S*,3*R*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-hydroxy-6-oxooxan-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl (2*S*)-2-methylbutanoate.

Content
97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, soluble in acetone, sparingly soluble in anhydrous ethanol.

IDENTIFICATION
A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *lovastatin CRS.*

TESTS
Specific optical rotation (*2.2.7*)
+ 325 to + 340 (anhydrous substance).

Dissolve 0.125 g in *acetonitrile R* and dilute to 25.0 mL with the same solvent.

Impurity E
Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in *acetonitrile R* and dilute to 25.0 mL with the same solvent.

Reference solution (a) Dilute 5.0 mL of the test solution to 100.0 mL with *acetonitrile R*. Dilute 5.0 mL of this solution to 50.0 mL with *acetonitrile R*.

Reference solution (b) Dissolve 4 mg of *lovastatin for peak identification CRS* (containing impurities A, B, C, D, E and F) in *acetonitrile R* and dilute to 10 mL with the same solvent.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated octylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 40 °C.

Mobile phase 1.1 g/L solution of *phosphoric acid R*, *acetonitrile R1* (35:65 *V/V*).

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 200 nm.

Injection 10 µL.

Run time 3 times the retention time of lovastatin.

Identification of impurities Use the chromatogram supplied with *lovastatin for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity E.

Relative retention With reference to lovastatin (retention time = about 5 min): impurity E = about 1.3.

System suitability Reference solution (b):
— *resolution*: minimum 5.0 between the peaks due to lovastatin and impurity E.

Limit:
— *correction factor*: for the calculation of content, multiply the peak area of impurity E by 1.6;
— *impurity E*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent).

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in *acetonitrile R* and dilute to 50.0 mL with the same solvent.

Reference solution (a) Dissolve 20.0 mg of *lovastatin CRS* in *acetonitrile R* and dilute to 50.0 mL with the same solvent.

Reference solution (b) Dilute 5.0 mL of the test solution to 100.0 mL with *acetonitrile R*. Dilute 5.0 mL of this solution to 50.0 mL with *acetonitrile R*.

Reference solution (c) Dissolve 4 mg of *lovastatin for peak identification CRS* (containing impurities A, B, C, D, E and F) in *acetonitrile R* and dilute to 10 mL with the same solvent.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: 0.1 per cent *V/V* solution of *phosphoric acid R*;
— *mobile phase B*: *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 7	40	60
7 - 9	40 → 35	60 → 65
9 - 15	35 → 10	65 → 90
15 - 20	10	90

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 238 nm.

Injection 10 µL of the test solution and reference solutions (b) and (c).

Identification of impurities Use the chromatogram supplied with *lovastatin for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, C, D and F.

Relative retention With reference to lovastatin (retention time = about 7 min): impurity B = about 0.6; impurity A = about 0.8; impurity F = about 0.9; impurity C = about 1.6; impurity D = about 2.3.

System suitability Reference solution (c):

— *peak-to-valley ratio*: minimum 3.0, where H_p = height above the baseline of the peak due to impurity F and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to lovastatin.

Limits:

— *impurities A, B, C, D*: for each impurity, not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *impurity F*: not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);

— *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.12*)

Maximum 0.5 per cent, determined on 1.00 g.

Sulfated ash (*2.4.14*)

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

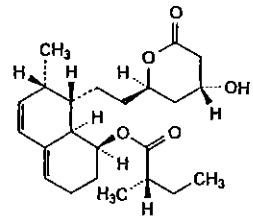
Calculate the percentage content of $C_{24}H_{36}O_5$ taking into account the assigned content of *lovastatin CRS*.

STORAGE

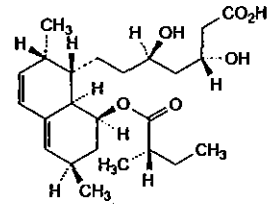
Under nitrogen, at a temperature of 2 °C to 8 °C.

IMPURITIES

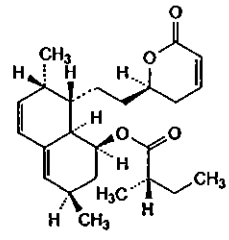
Specified impurities *A, B, C, D, E, F.*



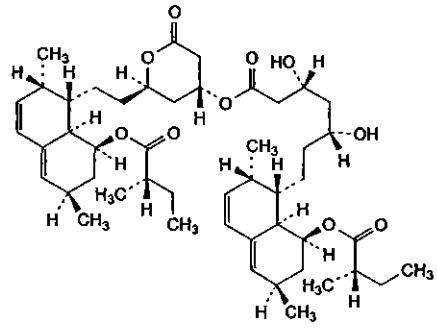
A. (1*S*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-hydroxy-6-oxooxan-2-yl]ethyl]-7-methyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl (2*S*)-2-methylbutanoate (mevastatin),



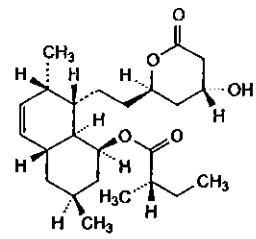
B. (3*R*,5*R*)-7-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-2,6-dimethyl-8-[[(2*S*)-2-methylbutanoyl]oxy]-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]-3,5-dihydroxyheptanoic acid (hydroxyacid lovastatin),



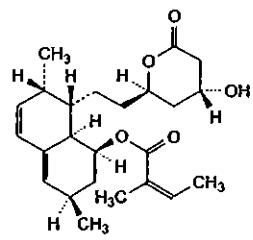
C. (1*S*,3*R*,7*S*,8*S*,8a*R*)-3,7-dimethyl-8-[2-[(2*R*)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl]ethyl]-1,2,3,7,8,8a-hexahydronaphthalen-1-yl (2*S*)-2-methylbutanoate (dehydrolovastatin),



D. (2*R*,4*R*)-2-[2-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-2,6-dimethyl-8-[[(2*S*)-2-methylbutanoyl]oxy]-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]ethyl]-6-oxooxan-4-yl (3*R*,5*R*)-7-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-2,6-dimethyl-8-[[(2*S*)-2-methylbutanoyl]oxy]-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]-3,5-dihydroxyheptanoate (lovastatin dimer),



E. (1*S*,3*S*,4a*R*,7*S*,8*S*,8a*S*)-8-[2-[(2*R*,4*R*)-4-hydroxy-6-oxooxan-2-yl]ethyl]-3,7-dimethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalen-1-yl (2*S*)-2-methylbutanoate (4,4a-dihydrolovastatin),

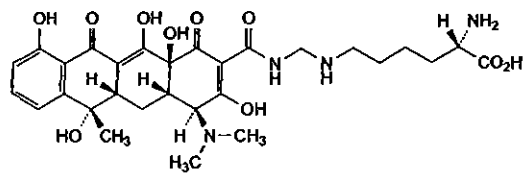


F. (1*S*,3*R*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-hydroxy-6-oxooxan-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl (2*Z*)-2-methylbut-2-enoate.

Ph Eur

Lymecycline

(Ph. Eur. monograph 1654)



$C_{29}H_{38}N_4O_{10}$ 603 *992-21-2*

Action and use
Tetracycline antibacterial.

Preparation
Lymecycline Capsules

Ph Eur

DEFINITION

(2*S*)-2-Amino-6-[[[[[(4*S*,4a*S*,5a*S*,6*S*,12a*S*)-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracen-2-yl]carbonyl]amino]methyl]amino]hexanoic acid (reaction product of formaldehyde, lysine and tetracycline).

Semi-synthetic product derived from a fermentation product.

Content
81.0 per cent to 102.0 per cent (equivalent to 60.0 per cent to 75.0 per cent of tetracycline) (anhydrous substance).

CHARACTERS

Appearance
Yellow, hygroscopic powder.

Solubility
Very soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 5 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dissolve 5 mg of *tetracycline hydrochloride CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 5 mg of *tetracycline hydrochloride CRS*, 5 mg of *demeclocycline hydrochloride R* and 5 mg of *oxytetracycline hydrochloride R* in *methanol R* and dilute to 10 mL with the same solvent.

Plate *TLC octadecylsilyl silica gel F_{254} plate R* (2-10 µm).

Mobile phase Mix 20 volumes of *acetonitrile R*, 20 volumes of *methanol R* and 60 volumes of a 63 g/L solution of *oxalic acid R* previously adjusted to pH 2.0 with *concentrated ammonia R*.

Application 2 µL.

Development Over half of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):
— the chromatogram shows 3 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 50 mg of the substance to be examined in 50 mL of *water R*.

Reference solution (a) Dissolve 10 mg of *lysine hydrochloride CRS* in *water R* and dilute to 50 mL with the same solvent.

Reference solution (b) Dissolve 10 mg of *arginine CRS* and 10 mg of *lysine hydrochloride CRS* in *water R* and dilute to 25 mL with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *concentrated ammonia R, 2-propanol R* (30:70 *V/V*).

Application 5 µL.

Development Over 3/4 of the plate.

Drying At 100-105 °C until the ammonia disappears completely.

Detection Spray with *ninhydrin solution R* and heat at 100-105 °C for 15 min.

System suitability Reference solution (b):
— the chromatogram shows 2 clearly separated principal spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 0.2 g in 5 mL of *water R*, add 0.3 mL of *phosphoric acid R* and distil. To 1 mL of the distillate add 10 mL of *chromotropic acid-sulfuric acid solution R*. A violet colour is produced.

D. Specific optical rotation (see Tests).

TESTS

pH (*2.2.3*)
7.8 to 8.2.

Dissolve 0.1 g in 10 mL of *carbon dioxide-free water R*.

Specific optical rotation (*2.2.7*)
−180 to −210 (anhydrous substance).

Dissolve 0.250 g in *water R* and dilute to 50.0 mL with the same solvent.

Free tetracycline (impurity H)
Maximum 2.5 per cent (anhydrous and methanol-free substance).

To 0.5 g add 50 mL of *butyl acetate R* and allow to stand at 25 °C for 1 h. Filter and extract the filtrate with 2 quantities, each of 25 mL, of *0.1 M hydrochloric acid*. Combine the extracts and dilute to 50.0 mL with *0.1 M hydrochloric acid*. Dilute 10.0 mL of this solution to 100.0 mL with *0.1 M hydrochloric acid*. The absorbance (*2.2.25*) measured at 355 nm is not greater than 0.64.

Light-absorbing impurities
The absorbance (*2.2.25*) is not greater than 0.50 at 430 nm (anhydrous and methanol-free substance).

Dissolve 25.0 mg in *0.01 M hydrochloric acid* and dilute to 10.0 mL with the same acid.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 0.125 g of the substance to be examined in 5.0 mL of *water R*. Add 1.0 mL of a 40 g/L solution of *sodium metabisulfite R* and allow to stand in the dark at 20-25 °C for 16-24 h, without stirring. Add 50 mL of *0.05 M hydrochloric acid*, shake to dissolve the precipitate and dilute to 100.0 mL with *water R*.

Reference solution (a) Dissolve 25.0 mg of *tetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 mL with the same acid.

Reference solution (b) Dissolve 12.5 mg of *4-epitetracycline hydrochloride CRS* (impurity A) in *0.01 M hydrochloric acid* and dilute to 50.0 mL with the same acid.

Reference solution (c) Dissolve 10.0 mg of *anhydrotetracycline hydrochloride CRS* (impurity C) in *0.01 M hydrochloric acid* and dilute to 100.0 mL with the same acid.

Reference solution (d) Dissolve 10.0 mg of *4-epianhydrotetracycline hydrochloride CRS* (impurity D) in *0.01 M hydrochloric acid* and dilute to 50.0 mL with the same acid.

Reference solution (e) Mix 1 mL of reference solution (a), 2 mL of reference solution (b) and 5 mL of reference solution (d) and dilute to 25 mL with *0.01 M hydrochloric acid*.

Reference solution (f) Mix 40.0 mL of reference solution (b), 20.0 mL of reference solution (c) and 5.0 mL of reference solution (d) and dilute to 200.0 mL with *0.01 M hydrochloric acid*.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *styrene-divinylbenzene copolymer R* (8 µm) with a pore size of 10 nm;
— *temperature*: 60 °C.

Mobile phase Weigh 80.0 g of *2-methyl-2-propanol R* and transfer to a 1000 mL volumetric flask with the aid of 200 mL of *water R*; add 100 mL of a 35 g/L solution of *dipotassium hydrogen phosphate R* adjusted to pH 8.0 with *dilute phosphoric acid R*, 200 mL of a 10 g/L solution of *tetrabutylammonium hydrogen sulfate R* adjusted to pH 8.0 with *dilute sodium hydroxide solution R*, and 10 mL of a 40 g/L solution of *sodium edetate R* adjusted to pH 8.0 with *dilute sodium hydroxide solution R*; dilute to 1000.0 mL with *water R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL of the test solution and reference solutions (e) and (f).

Run time 5 times the retention time of the principal peak in the chromatogram obtained with the test solution.

Relative retention With reference to tetracycline (retention time = about 8 min): impurity E = about 0.50; impurity A = about 0.6; impurity F = about 0.68; impurity B (eluting on the tail of the principal peak) = about 1.2; impurity D = about 1.45; impurity G = about 1.45; impurity C = about 2.95.

System suitability Reference solution (e):
— *resolution*: minimum 3.0 between the 1st peak (impurity A) and the 2nd peak (tetracycline) and minimum 5.0 between the 2nd peak and the 3rd peak (impurity D); adjust the concentration of 2-methyl-2-propanol in the mobile phase if necessary;
— *symmetry factor*: maximum 1.25 for the peak due to tetracycline.

Limits:
— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (5.0 per cent),
— *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (1.0 per cent),
— *impurities B, E, F*: for each impurity, not more than 0.1 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (f) (0.5 per cent),
— *sum of impurities D and G*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (0.5 per cent),
— *any other impurity*: for each impurity, not more than 0.04 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (f) (0.2 per cent),
— *total*: not more than 1.6 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (f) (8.0 per cent),
— *disregard limit*: 0.02 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (f) (0.1 per cent).

Methanol (*2.4.24, System A*)
Maximum 1.5 per cent.

Water (*2.5.12*)
Maximum 5.0 per cent, determined on 0.20 g.

Sulfated ash (*2.4.14*)
Maximum 0.5 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (a).

System suitability:
— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections of reference solution (a).

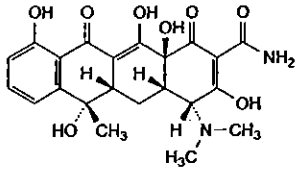
Calculate the percentage content of tetracycline and multiply it by 1.356 to obtain the percentage content of lymecycline.

STORAGE

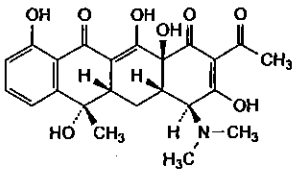
In an airtight container, protected from light.

IMPURITIES

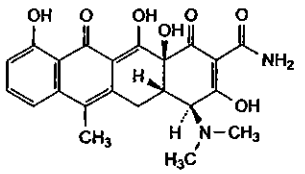
Specified impurities *A, B, C, D, E, F, G, H.*



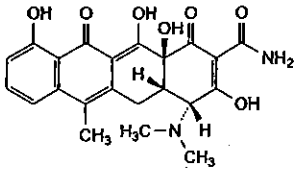
A. (4*R*,4a*S*,5a*S*,6*S*,12a*S*)-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epitetracycline),



B. (4*S*,4a*S*,5a*S*,6*S*,12a*S*)-2-acetyl-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-4a,5a,6,12a-tetrahydrotetracene-1,11(4*H*,5*H*)-dione (2-acetyl-2-decarbamoyltetracycline),



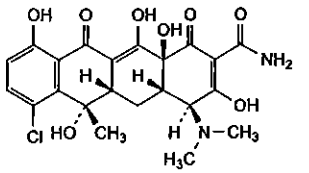
C. (4*S*,4a*S*,12a*S*)-4-(dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide (anhydrotetracycline),



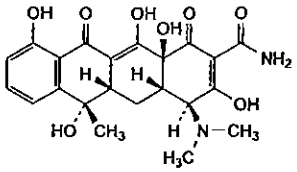
D. (4*R*,4a*S*,12a*S*)-4-(dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide (4-epianhydrotetracycline),

E. unknown structure,

F. unknown structure,



G. (4*S*,4a*S*,5a*S*,6*S*,12a*S*)-7-chloro-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (chlortetracycline),

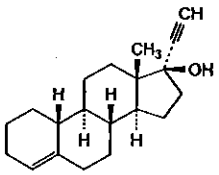


H. (4*S*,4a*S*,5a*S*,6*S*,12a*S*)-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (tetracycline).

Ph Eur

Lynestrenol

(Ph. Eur. monograph 0558)



$C_{20}H_{28}O$ 284.4 *52-76-6*

Action and use

Progestogen.

Ph Eur

DEFINITION

19-Nor-17α-pregn-4-en-20-yn-17-ol.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, soluble in acetone and in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *lynestrenol CRS.*

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 0.2 g in *ethanol (96 per cent) R* and dilute to 10 mL with the same solvent.

Specific optical rotation (*2.2.7*)

−9.5 to −11 (dried substance).

Dissolve 0.900 g in *ethanol (96 per cent) R* and dilute to 25.0 mL with the same solvent.

Related substances

Gas chromatography (*2.2.28*).

Test solution Dissolve 0.250 g of the substance to be examined in *ethyl acetate R* and dilute to 25.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *ethyl acetate R*. Dilute 1.0 mL of this solution to 10.0 mL with *ethyl acetate R*.

Reference solution (b) Dissolve 10 mg of *lynestrenol for peak identification CRS* (containing impurities A, B and C) in 1.0 mL of *ethyl acetate R*.

Column:
— *material*: fused silica;
— *size*: *l* = 50 m, Ø = 0.32 mm;
— *stationary phase*: *phenyl(5)methyl(95)polysiloxane R* (film thickness 1.0 µm).

Carrier gas *helium for chromatography R*.

Flow rate 3.0 mL/min.

Split ratio 1:34.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 30	80 → 230
	30 - 32	230 → 310
	32 - 42	310
Injection port		150
Detector		300

Detection Flame ionisation.

Injection 1.0 µL.

Identification of impurities Use the chromatogram supplied with *lynestrenol for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B and C.

Relative retention With reference to lynestrenol (retention time = about 38 min): artefact degradation peak = about 0.97; impurity A = about 0.99; impurity B = about 1.005; impurity C = about 1.01.

System suitability Reference solution (b):
— *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to impurity B and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to lynestrenol.

Limits:
— *impurity A*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
— *impurity C*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent). Disregard the artefact peak, which may be generated in the injection system.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 0.500 g by drying in an oven at 105 °C.

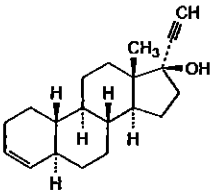
ASSAY
Dissolve 0.150 g in 40 mL of *tetrahydrofuran R* and add 5.0 mL of a 100 g/L solution of *silver nitrate R*. Titrate with *0.1 M sodium hydroxide*. Determine the end-point potentiometrically (*2.2.20*), using a glass indicator electrode and as comparison electrode a silver-silver chloride double-junction electrode with a saturated solution of *potassium nitrate R* as junction liquid. Carry out a blank titration.

1 mL of *0.1 M sodium hydroxide* is equivalent to 28.44 mg of $C_{20}H_{28}O$.

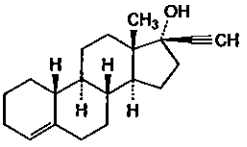
STORAGE
Protected from light.

IMPURITIES
Specified impurities *A, C.*

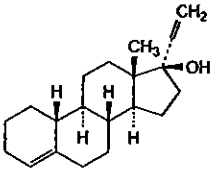
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B.*



A. 19-nor-5α,17α-pregn-3-en-20-yn-17-ol,



B. 19-norpregn-4-en-20-yn-17-ol,

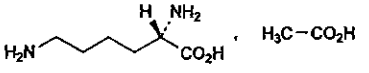


C. 19-nor-17α-pregna-4,20-dien-17-ol.

Ph Eur

Lysine Acetate

(Ph. Eur. monograph 2114)



$C_8H_{18}N_2O_4$ 206.2 *57282-49-2*

Action and use
Amino acid.

Ph Eur

DEFINITION
(2*S*)-2,6-Diaminohexanoic acid acetate.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder or colourless crystals.

Solubility
Freely soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: A, B, E.

Second identification: A, C, D, E.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *lysine acetate CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *water R*, evaporate to dryness at 60 °C and record new spectra using the residues.

C. Examine the chromatograms obtained in the test for ninhydrin-positive substances.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To 0.1 mL of solution S (see Tests) add 2 mL of *water R* and 1 mL of a 50 g/L solution of *phosphomolybdic acid R*. A yellowish-white precipitate is formed.

E. It gives reaction (a) of acetates (*2.3.1*).

TESTS

Solution S
Dissolve 5.0 g in *distilled water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*)
+ 8.5 to + 10.0 (dried substance), determined on solution S.

Ninhydrin-positive substances
Thin-layer chromatography (*2.2.27*).

Test solution (a) Dissolve 0.10 g of the substance to be examined in *water R* and dilute to 10 mL with the same solvent.

Test solution (b) Dilute 1.0 mL of test solution (a) to 50 mL with *water R*.

Reference solution (a) Dissolve 10 mg of *lysine acetate CRS* in *water R* and dilute to 50 mL with the same solvent.

Reference solution (b) Dilute 5 mL of test solution (b) to 20 mL with *water R*.

Reference solution (c) Dissolve 10 mg of *lysine acetate CRS* and 10 mg of *arginine CRS* in *water R* and dilute to 25 mL with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *concentrated ammonia R, 2-propanol R* (30:70 *V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying At 100-105 °C until the ammonia has evaporated.

Detection Spray with *ninhydrin solution R* and heat at 100-105 °C for 15 min.

System suitability Reference solution (c):
— the chromatogram shows 2 clearly separated spots.

Limits Test solution (a):
— *any impurity*: any spot, apart from the principal spot, is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Chlorides (*2.4.4*)
Maximum 200 ppm.

Dilute 2.5 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 300 ppm.

Dilute 5 mL of solution S to 15 mL with *distilled water R*.

Ammonium (*2.4.1, Method B*)
Maximum 200 ppm, determined on 50 mg.

Prepare the standard using 0.1 mL of *ammonium standard solution (100 ppm NH_4) R*.

Iron (*2.4.9*)
Maximum 30 ppm.

In a separating funnel, dissolve 0.33 g in 10 mL of *dilute hydrochloric acid R*. Shake with 3 quantities, each of 10 mL, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 mL of *water R* and shake for 3 min. The aqueous layer complies with the test.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 60 °C for 3 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 80.0 mg in 3 mL of *anhydrous formic acid R*. Add 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

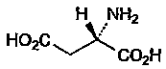
1 mL of *0.1 M perchloric acid* is equivalent to 10.31 mg of $C_8H_{18}N_2O_4$.

STORAGE

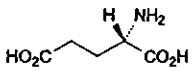
Protected from light.

IMPURITIES

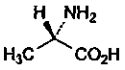
Specified impurities *A, B, C, D, E, F.*



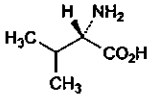
A. (2*S*)-2-aminobutanedioic acid (aspartic acid),



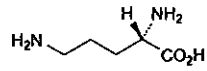
B. (2*S*)-2-aminopentanedioic acid (glutamic acid),



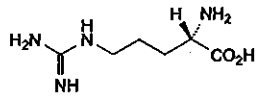
C. (*S*)-2-aminopropanoic acid (alanine),



D. (*S*)-2-amino-3-methylbutanoic acid (valine),



E. (2*S*)-2,5-diaminopentanoic acid (ornithine),

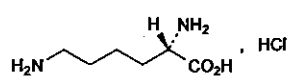


F. (*S*)-2-amino-5-guanidinopentanoic acid (arginine).

Ph Eur

Lysine Hydrochloride

(Ph. Eur. monograph 0930)



$C_6H_{15}ClN_2O_2$ 182.7 *657-27-2*

Action and use
Amino acid.

Ph Eur

DEFINITION

(2*S*)-2,6-diaminohexanoic acid hydrochloride.

Product of fermentation or of protein hydrolysis.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder or colourless crystals.

Solubility
Freely soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: A, B, E.

Second identification: A, C, D, E.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *lysine hydrochloride CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *water R*, evaporate to dryness at 60 °C and record new spectra using the residues.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *water R* and dilute to 50 mL with the same solvent.

Reference solution Dissolve 10 mg of *lysine hydrochloride CRS* in *water R* and dilute to 50 mL with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *concentrated ammonia R, 2-propanol R* (30:70 *V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying At 105 °C until the ammonia disappears completely.

Detection Spray with *ninhydrin solution R* and heat at 105 °C for 15 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. To 0.1 mL of solution S (see Tests) add 2 mL of *water R* and 1 mL of a 50 g/L solution of *phosphomolybdic acid R*. A yellowish-white precipitate is formed.

E. To 0.1 mL of solution S add 2 mL of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S
Dissolve 5.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_7 or GY_7 (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*)
+ 21.0 to + 22.5 (dried substance).

Dissolve 2.00 g in *hydrochloric acid R1* and dilute to 25.0 mL with the same acid.

Ninhydrin-positive substances
Amino acid analysis (*2.2.56*). For analysis, use Method 1.

The concentrations of the test solution and the reference solutions may be adapted according to the sensitivity of the equipment used. The concentrations of all solutions are adjusted so that the system suitability requirements described in general chapter *2.2.46* are fulfilled, keeping the ratios of concentrations between all solutions as described.

Solution A *water R* or a sample preparation buffer suitable for the apparatus used.

Test solution Dissolve 30.0 mg of the substance to be examined in solution A and dilute to 50.0 mL with solution A.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with solution A. Dilute 2.0 mL of this solution to 10.0 mL with solution A.

Reference solution (b) Dissolve 30.0 mg of *proline R* in solution A and dilute to 100.0 mL with solution A. Dilute 1.0 mL of the solution to 250.0 mL with solution A.

Reference solution (c) Dilute 6.0 mL of *ammonium standard solution (100 ppm NH_4) R* to 50.0 mL with solution A. Dilute 1.0 mL of this solution to 100.0 mL with solution A.

Reference solution (d) Dissolve 30 mg of *isoleucine R* and 30 mg of *leucine R* (impurity A) in solution A and dilute to 50.0 mL with solution A. Dilute 1.0 mL of the solution to 200.0 mL with solution A.

Blank solution Solution A.

Inject suitable, equal amounts of the test solution, blank solution and reference solutions (a), (b) and (d) into the amino acid analyser. Run a program suitable for the determination of physiological amino acids.

System suitability Reference solution (d):
— *resolution*: minimum 1.5 between the peaks due to isoleucine and impurity A.

Calculation of percentage contents:
— for any ninhydrin-positive substance detected at 570 nm, use the concentration of lysine hydrochloride in reference solution (a);
— for any ninhydrin-positive substance detected at 440 nm, use the concentration of proline in reference solution (b);

if a peak is above the reporting threshold at both wavelengths, use the result obtained at 570 nm for quantification.

Limits:
— *any ninhydrin-positive substance*: for each impurity, maximum 0.2 per cent;
— *total*: maximum 1.0 per cent;
— *reporting threshold*: 0.05 per cent.

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Sulfates (*2.4.13*)
Maximum 300 ppm.

Dilute 5 mL of solution S to 15 mL with *distilled water R*.

Ammonium
Amino acid analysis (*2.2.56*) as described in the test for ninhydrin-positive substances with the following modifications.

Injection Test solution, reference solution (c) and blank solution.

Limit:
— *ammonium at 570 nm*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.02 per cent), taking into account the peak due to ammonium in the chromatogram obtained with the blank solution.

Iron (*2.4.9*)
Maximum 30 ppm.

In a separating funnel, dissolve 0.33 g in 10 mL of *dilute hydrochloric acid R*. Shake with 3 quantities, each of 10 mL, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 mL of *water R* and shake for 3 min. Use the aqueous layer.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

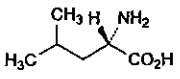
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.150 g in 5 mL of *anhydrous formic acid R*. Add 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

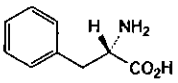
1 mL of *0.1 M perchloric acid* is equivalent to 18.27 mg of $C_6H_{15}ClN_2O_2$.

STORAGE
Protected from light.

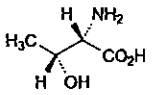
IMPURITIES
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C.*



A. (2*S*)-2-amino-4-methylpentanoic acid (leucine),



B. (2*S*)-2-amino-3-phenylpropanoic acid (phenylalanine),



C. (2*S*,3*R*)-2-amino-3-hydroxybutanoic acid (threonine).

Ph Eur

Macrogols

(Ph. Eur. monograph 1444)

Action and use
Non-ionic surfactant.

Ph Eur

DEFINITION
Mixtures of polymers with the general formula H-[OCH_2-CH_2]$_n$-OH where *n* represents the average number of oxyethylene groups. The type of macrogol is defined by a number that indicates the average relative molecular mass. A suitable stabiliser may be added.

CHARACTERS

Type of macrogol	Appearance	Solubility
300 400 600	clear, viscous, colourless or almost colourless, hygroscopic liquid	miscible with water, very soluble in acetone, in alcohol and in methylene chloride, practically insoluble in fatty oils and in mineral oils
1000	white or almost white, hygroscopic solid with a waxy or paraffin-like appearance	very soluble in water, freely soluble in alcohol and in methylene chloride, practically insoluble in fatty oils and in mineral oils
1500	white or almost white solid with a waxy or paraffin-like appearance	very soluble in water and in methylene chloride, freely soluble in alcohol, practically insoluble in fatty oils and in mineral oils
3000 3350	white or almost white solid with a waxy or paraffin-like appearance	very soluble in water and in methylene chloride, very slightly soluble in alcohol, practically insoluble in fatty oils and in mineral oils
4000 6000 8000	white or almost white solid with a waxy or paraffin-like appearance	very soluble in water and in methylene chloride, practically insoluble in alcohol, in fatty oils and in mineral oils
20 000 35 000	white or almost white solid with a waxy or paraffin-like appearance	very soluble in water, soluble in methylene chloride, practically insoluble in alcohol, in fatty oils and in mineral oils

IDENTIFICATION
A. Viscosity (see Tests).

B. To 1 g in a test-tube add 0.5 mL of *sulfuric acid R*, close the test-tube with a stopper fitted with a bent delivery tube and heat until white fumes are evolved. Collect the fumes via the delivery tube into 1 mL of *mercuric chloride solution R*. An abundant, white, crystalline precipitate is formed.

C. To 0.1 g add 0.1 g of *potassium thiocyanate R* and 0.1 g of *cobalt nitrate R* and mix thoroughly with a glass rod. Add 5 mL of *methylene chloride R* and shake. The liquid phase becomes blue.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Dissolve 12.5 g in *water R* and dilute to 50 mL with the same solvent.

Acidity or alkalinity

Dissolve 5.0 g in 50 mL of *carbon dioxide-free water R* and add 0.15 mL of *bromothymol blue solution R1*. The solution is yellow or green. Not more than 0.1 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator to blue.

Viscosity (*2.2.9*)

The viscosity is calculated using a density given in Table 1444.-1.

For macrogols with a relative molecular mass greater than 400, determine the viscosity on a 50 per cent *m/m* solution of the substance to be examined.

Freezing point (*2.2.18*)

See Table 1444.-2.

Hydroxyl value

Introduce *m* g (see Table 1444.-3) into a dry conical flask fitted with a reflux condenser. Add 25.0 mL of *phthalic anhydride solution R*, swirl to dissolve and boil under a reflux condenser on a hot plate for 60 min. Allow to cool. Rinse the condenser first with 25 mL of *pyridine R* and then with 25 mL of *water R*, add 1.5 mL of *phenolphthalein solution R* and titrate with *1 M sodium hydroxide* until a faint pink colour is obtained (n_1 mL). Carry out a blank test (n_2 mL). Calculate the hydroxyl value using the following expression:

$$\frac{56.1 \times (n_2 - n_1)}{m}$$

For macrogols with a relative molecular mass greater than 1000, if the water content is more than 0.5 per cent, dry a sample of suitable mass at 100-105 °C for 2 h and carry out the determination of the hydroxyl value on the dried sample.

Table 1444.-1

Type of macrogol	Kinematic viscosity ($mm^2 \cdot s^{-1}$)	Dynamic viscosity (mPa·s)	Density* (g/mL)
300	71 - 94	80 - 105	1.120
400	94 - 116	105 - 130	1.120
600	13.9 - 18.5	15 - 20	1.080
1000	20.4 - 27.7	22 - 30	1.080
1500	31 - 46	34 - 50	1.080
3000	69 - 93	75 - 100	1.080
3350	76 - 110	83 - 120	1.080
4000	102 - 158	110 - 170	1.080
6000	185 - 250	200 - 270	1.080
8000	240 - 472	260 - 510	1.080
20 000	2500 - 3200	2700 - 3500	1.080
35 000	10 000 - 13 000	11 000 - 14 000	1.080

*Density of the substance for macrogols 300 and 400. Density of the 50 per cent *m/m* solution for the other macrogols.

Table 1444.-2

Type of macrogol	Freezing point (°C)
600	15 - 25
1000	35 - 40
1500	42 - 48
3000	50 - 56
3350	53 - 57
4000	53 - 59
6000	55 - 61
8000	55 - 62
20 000	minimum 57
35 000	minimum 57

Table 1444.-3

Type of macrogol	Hydroxyl value	*m* (g)
300	340 - 394	1.5
400	264 - 300	1.9
600	178 - 197	3.5
1000	107 - 118	5.0
1500	70 - 80	7.0
3000	34 - 42	12.0
3350	30 - 38	12.0
4000	25 - 32	14.0
6000	16 - 22	18.0
8000	12 - 16	24.0
20 000	-	-
35 000	-	-

Reducing substances

Dissolve 1 g in 1 mL of a 10 g/L solution of *resorcinol R* and warm gently if necessary. Add 2 mL of *hydrochloric acid R*. After 5 min the solution is not more intensely coloured than reference solution R_3 (*2.2.2, Method I*).

Formaldehyde

Maximum 30 ppm.

Test solution To 1.00 g add 0.25 mL of *chromotropic acid, sodium salt solution R*, cool in iced water and add 5.0 mL of *sulfuric acid R*. Allow to stand for 15 min and dilute slowly to 10 mL with *water R*.

Reference solution Dilute 0.860 g of *formaldehyde solution R* to 100 mL with *water R*. Dilute 1.0 mL of this solution to 100 mL with *water R*. In a 10 mL flask, mix 1.00 mL of this solution and 0.25 mL of *chromotropic acid, sodium salt solution R*, cool in iced water and add 5.0 mL of *sulfuric acid R*. Allow to stand for 15 min and dilute slowly to 10 mL with *water R*.

Blank solution In a 10 mL flask mix 1.00 mL of *water R* and 0.25 mL of *chromotropic acid, sodium salt solution R*, cool in iced water and add 5.0 mL of *sulfuric acid R*. Dilute slowly to 10 mL with *water R*.

Determine the absorbance (*2.2.25*) of the test solution at 567 nm, against the blank solution. It is not higher than that of the reference solution.

If the use of macrogols with a higher content of formaldehyde may have adverse effects, the competent authority may impose a limit of not more than 15 ppm.

Ethylene glycol and diethylene glycol

carry out this test only if the macrogol has a relative molecular mass below 1000.

Gas chromatography (*2.2.28*).

Test solution Dissolve 5.00 g of the substance to be examined in *acetone R* and dilute to 100.0 mL with the same solvent.

Reference solution Dissolve 0.10 g of *ethylene glycol R* and 0.50 g of *diethylene glycol R* in *acetone R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with *acetone R*.

Column:
- *material*: glass;
- *size*: *l* = 1.8 m, Ø = 2 mm;
- *stationary phase*: *silanised diatomaceous earth for gas chromatography R*, impregnated with 5 per cent *m/m* of *macrogol 20 000 R*.

Carrier gas *nitrogen for chromatography R*.

Flow rate 30 mL/min.

Temperature:
- *column*: if necessary, precondition the column by heating at 200 °C for about 15 h; adjust the initial temperature of the column to obtain a retention time of 14-16 min for diethylene glycol; raise the temperature of the column by about 30 °C at a rate of 2 °C/min but without exceeding 170 °C;
- *injection port and detector*: 250 °C.

Detection Flame ionisation.

Injection 2 µL.

Carry out 5 replicate injections to check the repeatability of the response.

Limit Maximum 0.4 per cent, calculated as the sum of the contents of ethylene glycol and diethylene glycol.

Ethylene oxide and dioxan (*2.4.25*)
Maximum 1 ppm of ethylene oxide and 10 ppm of dioxan.

Water (*2.5.12*)
Maximum 2.0 per cent for macrogols with a relative molecular mass not greater than 1000 and maximum 1.0 per cent for macrogols with a relative molecular mass greater than 1000, determined on 2.00 g.

Sulfated ash (*2.4.14*)
Maximum 0.2 per cent, determined on 1.0 g.

STORAGE
In an airtight container.

LABELLING
The label states:
- the type of macrogol;
- the content of formaldehyde.

FUNCTIONALITY-RELATED CHARACTERISTICS
This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristic may be relevant for macrogols used as solvent.

Viscosity
(see Tests).

The following characteristics may be relevant for macrogols used as suspension stabiliser and thickener.

Viscosity
(see Tests).

The following characteristic may be relevant for macrogols used as lubricant in tablets.

Particle-size distribution (*2.9.31*)
The following characteristics may be relevant for macrogols used as suppository base and for macrogols used in hydrophilic ointments.

Viscosity
(see Tests).

Melting point (*2.2.15*)

Ph Eur

High-molecular-mass Macrogols

(Ph. Eur. monograph 2444)

Ph Eur

DEFINITION
Mixtures of high-molecular-mass macrogols (also known as polyethylene oxides). The type of high-molecular-mass macrogol is defined by a number that indicates the nominal average molecular mass in the range of 100 000 to 7 000 000. A suitable stabiliser (e.g. butylhydroxytoluene) and a suitable flowability agent (e.g. silicon dioxide) may be added.

CHARACTERS
Appearance
White or almost white, free-flowing powder.

Solubility
Freely soluble in water.

mp
Minimum 65 °C.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *high-molecular-mass macrogol CRS*.

B. Viscosity (see Tests).

TESTS
Solution S
Prepare a solution of the substance to be examined in a mixture of *carbon dioxide-free water R* and *2-propanol R* according to Table 2444.-1.

pH (*2.2.3*)
6.0 to 10.0 for solution S.

Viscosity (*2.2.10*)
Determine the dynamic viscosity of solution S at 25 °C using a rotating viscometer and a suitable spindle.

Ethylene oxide (*2.4.25, Method A*)
Maximum 1 ppm.

Carry out the test with the following modifications.

Stock solution Dilute 0.5 mL of *ethylene oxide stock solution R2* to 10.0 mL with *propanol R*. Dilute 1.0 mL of this solution to 50.0 mL with *propanol R*.

Table 2444.-1. – *Solution S and viscosity*

Nominal average molecular mass	Composition of solution S (g of substance / mL of *carbon dioxide-free water R* + mL of *2-propanol R*)	Dynamic viscosity (mPa·s)	Rotation speed (r/min)
100 000	30 g / 570 + 125	12 - 50	50
200 000	30 g / 570 + 125	65 - 115	50
300 000	30 g / 570 + 125	600 - 1200	10
400 000	30 g / 570 + 125	2250 - 4500	2
600 000	30 g / 570 + 125	4500 - 8800	2
900 000	30 g / 570 + 125	8800 - 17 600	2
1 000 000	12 g / 588 + 125	400 - 800	10
2 000 000	12 g / 588 + 125	2000 - 4000	10
4 000 000	6 g / 594 + 125	1650 - 5500	2
5 000 000	6 g / 594 + 125	5500 - 7500	2
7 000 000	6 g / 594 + 125	7500 - 10 000	2

Test solution Weigh 0.50 g (M_T) of the substance to be examined into a 10 mL vial (other sizes may be used depending on the operating conditions). Close and allow to stand at 70 °C for 30 min.

Reference solution (a) Weigh 0.50 g (M_R) of the substance to be examined into an identical 10 mL vial and add 10 µL of the stock solution. Close and homogenise, and allow to stand at 70 °C for 30 min.

Reference solution (b) To 0.50 mL of *ethylene oxide solution R3* in a 10 mL vial add 0.1 mL of a freshly prepared 10 mg/L solution of *acetaldehyde R*. Close and homogenise, and allow to stand at 70 °C for 30 min.

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 45 min.

Sulfated ash
Maximum 5.0 per cent, determined on 1.00 g.

Heat a silica crucible to redness for 30 min, allow to cool in a desiccator and weigh. Evenly distribute 1.00 g of the substance to be examined in the crucible and weigh. Dry at 100-105 °C for 1 h and ignite in a muffle furnace at 600 ± 25 °C, until the substance is thoroughly charred. Carry out the test for sulfated ash (*2.4.14*) on the residue obtained, starting from "Moisten the substance to be examined...".

STORAGE
In an airtight container.

LABELLING
The label states the type of high-molecular-mass macrogol.

Ph Eur

Macrogol Cetostearyl Ether

(Ph. Eur. monograph 1123)

Action and use
Non-ionic surfactant.

Ph Eur

DEFINITION
Mixture of ethers of mixed macrogols with linear fatty alcohols, mainly cetostearyl alcohol. It may contain some free macrogols and it contains various amounts of free cetostearyl alcohol. The number of moles of ethylene oxide reacted per mole of cetostearyl alcohol is 2 to 33 (nominal value).

CHARACTERS
Appearance
White or yellowish-white, waxy, unctuous mass, pellets, microbeads or flakes.

Solubility
— macrogol cetostearyl ether with low numbers of moles of ethylene oxide reacted per mole: practically insoluble in water, soluble in ethanol (96 per cent) and in methylene chloride;
— macrogol cetostearyl ether with higher numbers of moles of ethylene oxide reacted per mole: dispersible or soluble in water, soluble in ethanol (96 per cent) and in methylene chloride.

It solidifies at 32 °C to 52 °C.

IDENTIFICATION
A. Hydroxyl value (see Tests).

B. Iodine value (see Tests).

C. Saponification value (see Tests).

D. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve the prescribed amount of substance to be examined (see table below) in a mixture of 1 volume of *water R* and 9 volumes of *methanol R* and dilute to 75 mL with the same mixture of solvents.

Number of moles of ethylene oxide reacted per mole	Amount to be dissolved (g)
2 - 6	5.0
10 - 22	10.0
25 - 33	15.0

Add 60 mL of *hexane R* and shake for 3 min. The formation of foam can be reduced by the addition of some drops of *ethanol (96 per cent) R*. Filter the upper layer through *anhydrous sodium sulfate R*, wash the filter with 3 quantities, each of 10 mL, of *hexane R* and evaporate the combined filtrates to dryness. Dissolve 0.05 g of the residue in 10 mL of *methanol R* (the solution may be opalescent).

Reference solution Dissolve 25 mg of *stearyl alcohol CRS* in *methanol R* and dilute to 25 mL with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *ethyl acetate R.*

Application 20 µL.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with vanillin-sulfuric acid reagent prepared as follows: dissolve 0.5 g of *vanillin R* in 50 mL of *ethanol (96 per cent) R* and dilute to 100 mL with *sulfuric acid R*; allow to dry in air; heat at about 130 °C for 15 min and allow to cool in air.

Results The chromatogram obtained with the test solution shows several spots; one of these spots corresponds to the principal spot in the chromatogram obtained with the reference solution.

E. Dissolve or disperse 0.1 g in 5 mL of *ethanol (96 per cent) R*, add 2 mL of *water R*, 10 mL of *dilute hydrochloric acid R*, 10 mL of *barium chloride solution R1* and 10 mL of a 100 g/L solution of *phosphomolybdic acid R*. A precipitate is formed.

TESTS

Appearance of solution

The solution is not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

Dissolve 5.0 g in *ethanol (96 per cent) R* and dilute to 50 mL with the same solvent.

Alkalinity

Dissolve 2.0 g in a hot mixture of 10 mL of *ethanol (96 per cent) R* and 10 mL of *water R*. Add 0.1 mL of *bromothymol blue solution R1*. Not more than 0.5 mL of *0.1 M hydrochloric acid* is required to change the colour of the indicator to yellow.

Acid value (*2.5.1*)

Maximum 1.0, determined on 5.0 g.

Hydroxyl value (*2.5.3, Method A*)

Number of moles of ethylene oxide reacted per mole (nominal value)	Hydroxyl value
2	150 - 180
3	135 - 155
5 - 6	100 - 134
10	75 - 90
12	67 - 77
15	58 - 67
20 - 22	40 - 55
25	36 - 46
30 - 33	32 - 40

Iodine value (*2.5.4, Method A*)

Maximum 2.0.

Saponification value (*2.5.6*)

Maximum 3.0, determined on 10.0 g.

Ethylene oxide and dioxan (*2.4.25*)

Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Water (*2.5.12*)

Maximum 3.0 per cent, determined on 2.00 g.

Total ash (*2.4.16*)

Maximum 0.2 per cent, determined on 2.0 g.

STORAGE

In an airtight container.

LABELLING

The label states the number of moles of ethylene oxide reacted per mole of cetostearyl alcohol (nominal value).

Ph Eur

Macrogol 30 Dipolyhydroxystearate

(Ph. Eur. monograph 2584)

Ph Eur

DEFINITION

Mixture of mainly diesters of polymerised 12-hydroxystearic ((12Ξ)-hydroxyoctadecanoic) acid and *Macrogols (1444)* obtained by esterification of macrogol with 12-hydroxystearic acid. The average number of moles of ethylene oxide reacted per mole of substance is 30.

CHARACTERS

Appearance

Brownish-red, waxy mass.

Solubility

Practically insoluble in water, very soluble in methylene chloride and soluble in most aliphatic and aromatic hydrocarbons.

mp

30 °C to 40 °C.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison macrogol 30 dipolyhydroxystearate CRS.

B. Hydroxyl value (see Tests).

C. Saponification value (see Tests).

TESTS

Acid value (*2.5.1*)

Maximum 10.0.

Hydroxyl value (*2.5.3, Method A*)

12 to 30.

Iodine value (*2.5.4, Method A*)

Maximum 10.0.

Peroxide value (*2.5.5*)

Maximum 5.0.

Saponification value (*2.5.6*)

125 to 145, determined on 2.0 g.

Use 30.0 mL of *0.5 M alcoholic potassium hydroxide*, heat under reflux for 60 min and add 50 mL of *anhydrous ethanol R* before carrying out the titration.

Water (*2.5.12*)

Maximum 1.0 per cent, determined on 0.50 g.

Sulfated ash (*2.4.14*)

Maximum 0.5 per cent.

Heat a silica crucible to redness for 30 min, allow to cool in a desiccator and weigh. Evenly distribute 1.0 g in the crucible and weigh. Dry at 100-105 °C for 1 h and ignite in a muffle furnace at 600 ± 25 °C, until the substance is thoroughly charred. Carry out the test for sulfated ash (*2.4.14*) on the residue obtained, starting from "Moisten the substance to be examined...".

STORAGE

In an airtight container.

Ph Eur

Macrogol 6 Glycerol Caprylocaprate

(Ph. Eur. monograph 1443)

Action and use
Non-ionic surfactant.

Ph Eur

DEFINITION
Mixture of mainly mono- and diesters of polyoxyethylene glycerol ethers mainly with caprylic (octanoic) and capric (decanoic) acids. The average number of moles of ethylene oxide reacted per mole of substance is 6.

Macrogol 6 glycerol caprylocaprate may be obtained by ethoxylation of glycerol and esterification with distilled coconut or palm kernel fatty acids, or by ethoxylation of mono- and diglycerides of caprylic and capric acids.

CHARACTERS

Appearance
Pale yellow liquid.

Solubility
Partly soluble in water, freely soluble in castor oil, in glycerol, in isopropanol and in propylene glycol.

Viscosity
About 145 mPa·s.

IDENTIFICATION

A. Dissolve 1.0 g in 99 g of a mixture of 10 volumes of *2-propanol R* and 90 volumes of *water R*. Heat the solution obtained to about 40 °C. A turbidity is produced. Allow to cool until the turbidity disappears. The cloud point is between 15 °C and 35 °C.

B. Saponification value (see Tests).

C. Composition of fatty acids (see Tests).

TESTS

Appearance
The substance to be examined is clear (*2.2.1*) and not more intensely coloured than reference solution Y_2 (*2.2.2, Method I*).

Alkalinity
Dissolve 2.0 g in a hot mixture of 10 mL of *ethanol (96 per cent) R* and 10 mL of *water R*. Add 0.1 mL of *bromothymol blue solution R1*. Not more than 0.5 mL of *0.1 M hydrochloric acid* is required to change the colour of the indicator to yellow.

Acid value (*2.5.1*)
Maximum 5.0, determined on 5.0 g.

Hydroxyl value (*2.5.3, Method A*)
165 to 225.

Saponification value (*2.5.6*)
85 to 105, determined on 2.0 g.

Composition of fatty acids
Gas chromatography (*2.4.22, Method A*).

Composition of the fatty-acid fraction of the substance:
— *caproic acid*: maximum 2.0 per cent;
— *caprylic acid*: 50.0 per cent to 80.0 per cent;
— *capric acid*: 20.0 per cent to 50.0 per cent;
— *lauric acid*: maximum 3.0 per cent;
— *myristic acid*: maximum 1.0 per cent.

Ethylene oxide and dioxan (*2.4.25*)
Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Water (*2.5.12*)
Maximum 1.0 per cent, determined on 1.00 g.

Total ash (*2.4.16*)
Maximum 0.3 per cent.

Ph Eur

Macrogol Glycerol Cocoates

(Macrogoglycerol Cocoates, Ph. Eur. monograph 1122)

Action and use
Pharmaceutical aids.

Ph Eur

DEFINITION
Mixtures of mono-, di- and triesters of ethoxylated glycerol with fatty acids of vegetable origin having a composition corresponding to the fatty acid composition of the oil extracted from the hard, dried fraction of the endosperm of *Cocos nucifera* L. The average number of moles of ethylene oxide reacted per mole of substance (nominal value) is either 7 (macrogol 7 glycerol cocoate) or 23 (macrogol 23 glycerol cocoate).

CHARACTERS

Appearance
Clear, yellowish, oily liquid.

Solubility
Soluble in water and in ethanol (96 per cent) and practically insoluble in light petroleum (bp: 50-70 °C) for macrogol 7 glycerol cocoate and macrogol 23 glycerol cocoate.

Relative density
About 1.05 for macrogol 7 glycerol cocoate; about 1.09 for macrogol 23 glycerol cocoate.

IDENTIFICATION

A. Dissolve 1.0 g of macrogol 7 glycerol cocoate in 99 g of a mixture of 10 volumes of *2-propanol R* and 90 volumes of *water R*. Heat the solution to about 65 °C. A turbidity is produced. Allow to cool until the turbidity disappears. The cloud point is between 35 °C and 54 °C.

Heat a 10 g/L solution of macrogol 23 glycerol cocoate in a 100 g/L solution of *sodium chloride R* to about 90 °C. A turbidity is produced. Allow to cool until the turbidity disappears. The cloud point is between 65 °C and 85 °C.

B. Iodine value (see Tests).

C. Saponification value (see Tests).

TESTS

Appearance
The substance to be examined is clear (*2.2.1*) and not more intensely coloured than reference solution Y_2 (*2.2.2, Method I*).

Alkalinity
Dissolve 2.0 g in a hot mixture of 10 mL of *ethanol (96 per cent) R* and 10 mL of *water R*. Add 0.1 mL of *bromothymol blue solution R1*. Not more than 0.5 mL of *0.1 M hydrochloric acid* is required to change the colour of the indicator to yellow.

Acid value (*2.5.1*)
Maximum 5.0, determined on 5.0 g.

Hydroxyl value (*2.5.3, Method A*)
See Table 1122.-1.

Saponification value (*2.5.6*)
See Table 1122.-1.

Table 1122.-1

Number of moles of ethylene oxide reacted per mole (nominal value)	Hydroxyl value	Saponification value (determined on 2.0 g)
7	170 - 210	85 - 105
23	80 - 100	40 - 50

Iodine value (*2.5.4, Method A*)
Maximum 5.0.

Composition of fatty acids
Gas chromatography (*2.4.22, Method A*).
Composition of the fatty-acid fraction of the substance:
— *caproic acid*: maximum 1.0 per cent;
— *caprylic acid*: 5.0 per cent to 10.0 per cent;
— *capric acid*: 4.0 per cent to 10.0 per cent;
— *lauric acid*: 40.0 per cent to 55.0 per cent;
— *myristic acid*: 14.0 per cent to 23.0 per cent;
— *palmitic acid*: 8.0 per cent to 12.0 per cent;
— *stearic acid*: 1.0 per cent to 5.0 per cent;
— *oleic acid*: 5.0 per cent to 10.0 per cent;
— *linoleic acid*: maximum 3.0 per cent.

Ethylene oxide and dioxan (*2.4.25*)
Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Water (*2.5.12*)
Maximum 1.0 per cent, determined on 1.0 g.

Total ash (*2.4.16*)
Maximum 0.3 per cent.

LABELLING
The label states the number of moles of ethylene oxide reacted per mole of substance (nominal value).

Ph Eur

Macrogol 20 Glycerol Monostearate

(Ph. Eur. monograph 2044)

Action and use
Non-ionic surfactant.

Ph Eur

DEFINITION
Macrogol 20 glycerol monostearate is obtained by ethoxylation with ethylene oxide of different types of glycerol stearates, mainly *Glycerol monostearate 40-55 (0495)*.
The number of moles of ethylene oxide reacted per mole of glycerol stearate is 20 (nominal value).

CHARACTERS
Appearance
Pale yellow, oily liquid or gel.

Solubility
Soluble in water at 40 °C and above and in ethanol (96 per cent), practically insoluble in light liquid paraffin and in fatty oils.

Relative density
About 1.07.

IDENTIFICATION
A. Hydroxyl value (see Tests).
B. Saponification value (see Tests).
C. Composition of fatty acids (see Tests).
D. Place 1 g in a test tube and add 0.1 mL of *sulfuric acid R*. Heat the tube until white fumes appear. The fumes turn filter paper impregnated with *alkaline potassium tetraiodomercurate solution R* black.

TESTS
Acid value (*2.5.1*)
Maximum 2.0, determined on 5.0 g.

Hydroxyl value (*2.5.3, Method A*)
65 to 85, determined on 0.350 g.

Iodine value (*2.5.4, Method A*)
Maximum 2.0.

Peroxide value (*2.5.5, Method A*)
Maximum 6.0.

Saponification value (*2.5.6*)
40 to 60.

Composition of fatty acids
Gas chromatography (*2.4.22, Method C*).
Composition of the fatty-acid fraction of the substance:

Type of macrogol 20 glycerol monostearate	Type of glycerol stearate used	Composition of fatty acids
Type I	Type I (obtained using stearic acid 50)	*Stearic acid*: 40.0 per cent to 60.0 per cent, *Sum of the contents of palmitic and stearic acids*: minimum 90.0 per cent.
Type II	Type II (obtained using stearic acid 70)	*Stearic acid*: 60.0 per cent to 80.0 per cent, *Sum of the contents of palmitic and stearic acids*: minimum 90.0 per cent.
Type III	Type III (obtained using stearic acid 95)	*Stearic acid*: 90.0 per cent to 99.0 per cent, *Sum of the contents of palmitic and stearic acids*: minimum 96.0 per cent.

Ethylene oxide and dioxan (*2.4.25, Method A*)
Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Water (*2.5.12*)
Maximum 3.0 per cent, determined on 1.00 g.

Total ash (*2.4.16*)
Maximum 0.2 per cent.

STORAGE
Protected from light.

LABELLING
The label states the type of macrogol 20 glycerol monostearate.

Ph Eur

Macrogol 15 Hydroxystearate

(Ph. Eur. monograph 2052)

Action and use
Non-ionic surfactant.

Ph Eur

DEFINITION

Mixture of mainly monoesters and diesters of 12-hydroxystearic ((12*S*)-hydroxyoctadecanoic) acid and macrogols obtained by ethoxylation of 12-hydroxystearic acid. The number of moles of ethylene oxide reacted per mole of 12-hydroxystearic acid is 15 (nominal value).
It contains free macrogols.

CHARACTERS

Appearance
Yellowish, waxy mass.

Solubility
Very soluble in water, soluble in ethanol (96 per cent), insoluble in liquid paraffin.

It solidifies at about 25 °C.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution To 1.0 g add 100 mL of a 100 g/L solution of *potassium hydroxide R* and boil under a reflux condenser for 30 min. Acidify the warm solution with 20 mL of *hydrochloric acid R* and cool to room temperature. Shake the mixture with 50 mL of *ether R* and allow to stand until a separation of the layers is visible. Separate the clear upper layer, add 5 g of *anhydrous sodium sulfate R*, wait for 30 min, filter and evaporate to dryness on a water-bath. Dissolve 50 mg of the residue in 25 mL of *ether R*.

Reference solution Dissolve 50 mg of *12-hydroxystearic acid R* in 25 mL of *methylene chloride R*.

Plate *TLC octadecylsilyl silica gel plate R.*

Mobile phase *methylene chloride R, glacial acetic acid R, acetone R* (10:40:50 *V/V/V*).

Application 2 µL.

Development Over 2/3 of the plate.

Drying In a current of cold air.

Detection Spray with a 80 g/L solution of *phosphomolybdic acid R* in *2-propanol R* and heat at 120 °C for 1-2 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and colour to the principal spot in the chromatogram obtained with the reference solution.

B. Dissolve 15.0 g in 50 mL of *water R*. The viscosity (*2.2.9*) has a maximum of 20 mPa·s.

C. Free macrogols (see Tests).

TESTS

Appearance of solution
The solution is not more opalescent than reference suspension III (*2.2.1*) and not more intensely coloured than reference solution B_6 or BY_6 (*2.2.2, Method II*).

Dissolve 2.0 g in *water R* and dilute to 20 mL with the same solvent.

Acid value (*2.5.1*)
Maximum 1.0, determined on 2.0 g.

Hydroxyl value (*2.5.3, Method A*)
90 to 110.

Iodine value (*2.5.4, Method A*)
Maximum 2.0.

Peroxide value (*2.5.5, Method A*)
Maximum 5.0.

Saponification value (*2.5.6*)
53 to 63.

Free macrogols
Size-exclusion chromatography (*2.2.30*).

Test solution Dissolve 1.20 g of the substance to be examined in the mobile phase and dilute to 250.0 mL with the mobile phase.

Reference solution (a) Dissolve about 0.4 g of *macrogol 1000 R* in the mobile phase and dilute to 250.0 mL with the mobile phase.

Reference solution (b) Dilute 50.0 mL of reference solution (a) to 100.0 mL with the mobile phase.

Precolumns (2):
— *size*: l = 0.125 m, Ø = 4 mm;
— *stationary phase*: spherical *octadecylsilyl silica gel for chromatography R* (5 µm) with a pore size of 10 nm.

Column:
— *size*: l = 0.30 m, Ø = 7.8 mm;
— *stationary phase*: *hydroxylated polymethacrylate gel R* (6 µm) with a pore size of 12 nm.

Connect both precolumns to the column using a 3-way valve and switch the mobile phase flow according to the following programme:
— 0-114 s: precolumn 1 and column;
— 115 s to the end: precolumn 2 and column;
— 115 s to 7 min: flow back of precolumn 1.

Mobile phase *water for chromatography R, methanol R* (20:80 *V/V*).

Flow rate 1.1 mL/min.

Detection Refractometer.

Injection 50 µL.

Calculate the percentage content of free macrogols using the following expression:

$$\frac{A_1 \times m_2 \times 200}{m_1 \times (A_2 + 2A_3)}$$

m_1 = mass of the substance to be examined in the test solution, in grams;
m_2 = mass of *macrogol 1000 R* in reference solution (a), in grams;
A_1 = area of the peak due to free macrogols in the substance to be examined in the chromatogram obtained with the test solution;
A_2 = area of the peak due to macrogol 1000 in the chromatogram obtained with reference solution (a);
A_3 = area of the peak due to macrogol 1000 in the chromatogram obtained with reference solution (b).

Limit:
— *free macrogols*: 27.0 per cent to 39.0 per cent.

Ethylene oxide and dioxan (*2.4.25*)
Maximum 1 ppm of ethylene oxide and maximum 50 ppm of dioxan.

Water (*2.5.12*)
Maximum 1.0 per cent, determined on 2.00 g.

Total ash (*2.4.16*)
Maximum 0.3 per cent, determined on 1.0 g.

STORAGE

In an airtight container.

Ph Eur

Macrogol Isotridecyl Ether

(Ph. Eur. monograph 2730)

Ph Eur

DEFINITION

Mixture of ethers of mixed macrogols with linear and branched fatty alcohols, mainly $C_{13}H_{28}O$. It contains a variable quantity of free $C_{13}H_{28}O$ and it may contain free macrogols. The number of moles of ethylene oxide reacted per mole of branched $C_{13}H_{28}O$ is 3 or 4 (nominal value).

CHARACTERS

Appearance

Colourless liquid.

Solubility

Practically insoluble in water, soluble or dispersible in ethanol (96 per cent), practically insoluble in light petroleum.

IDENTIFICATION

A. Gas chromatography (*2.2.28*).

Test solution Dissolve 0.200 g of the substance to be examined in *acetone R* and dilute to 10.0 mL with the same solvent.

Reference solution Dissolve 0.35 g of *lauryl alcohol R*, 0.35 g of *tridecyl alcohol R*, 0.15 g of *myristyl alcohol R* and 0.15 g of *ethylene glycol monododecyl ether R* in *acetone R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with *acetone R*.

Column:

- *material*: fused silica;
- *size*: *l* = 30 m, Ø = 0.25 mm;
- *stationary phase*: *methylpolysiloxane R* (film thickness 0.25 µm).

Carrier gas: *hydrogen for chromatography R* or *helium for chromatography R*.

Flow rate: 1 mL/min.

Split ratio 1:50.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 1	120
	1 - 24	120 → 350
	24 - 34	350
Injection port		300
Detector		350

Detection Flame ionisation.

Injection 1 µL.

Retention time Lauryl alcohol = about 6.7 min; tridecyl alcohol = about 7.8 min; myristyl alcohol = about 9 min; ethylene glycol monododecyl ether = about 9.3 min.

System suitability Reference solution:

- *resolution*: minimum 5 between the peaks due to myristyl alcohol and ethylene glycol monododecyl ether.

Results The sum of the areas of the peaks eluting between the peak due to lauryl alcohol and the peak due to tridecyl alcohol is greater than the area of the peak due to tridecyl alcohol and the area of the peak due to lauryl alcohol.

B. Hydroxyl value (see Tests).

C. Iodine value (see Tests).

D. Saponification value (see Tests).

E. Dissolve or disperse 0.1 g in 5 mL of *ethanol (96 per cent) R*. Add 10 mL of *dilute hydrochloric acid R*, 10 mL of *barium chloride solution R1* and 10 mL of a 100 g/L solution of *phosphomolybdic acid R*. A precipitate is formed.

TESTS

Appearance of solution

The solution is not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

Dissolve 5.0 g in *ethanol (96 per cent) R* and dilute to 50 mL with the same solvent.

Alkalinity

Dissolve 2.0 g in a hot mixture of 10 mL of *ethanol (96 per cent) R* and 10 mL of *water R*. Add 0.1 mL of *bromothymol blue solution R1*. Not more than 0.5 mL of *0.1 M hydrochloric acid* is required to change the colour of the indicator to yellow.

Acid value (*2.5.1*)

Maximum 1.0, determined on 5.0 g.

Hydroxyl value (*2.5.3, Method A*)

See Table 2730.-1.

Table 2730.-1

Ethylene oxide units per molecule (nominal value)	Hydroxyl value
3	165 - 185
4	145 - 160

Iodine value (*2.5.4*)

Maximum 2.0.

Saponification value (*2.5.6*)

Maximum 3.0, determined on 10.0 g.

Ethylene oxide and dioxan (*2.4.25*)

Maximum 1 ppm of ethylene oxide and 10 ppm of dioxan.

Water (*2.5.12*)

Maximum 3.0 per cent, determined on 2.00 g.

Sulfated ash (*2.4.14*)

Maximum 0.2 per cent.

Ignite a silica crucible at 600 ± 50 °C for 30 min, allow to cool in a desiccator over a suitable desiccant and weigh. Evenly distribute 1.0 g in the crucible and weigh. Dry at 100-105 °C for 1 h and ignite in a muffle furnace at 600 ± 25 °C until the substance is thoroughly charred. Carry out the test for sulfated ash (*2.4.14*) on the residue obtained, starting from "Moisten the substance to be examined...".

STORAGE

In an airtight container.

LABELLING

The label states the number of moles of ethylene oxide reacted per mole of $C_{13}H_{28}O$ (nominal value).

Ph Eur

Macrogol Lauryl Ether

(Ph. Eur. monograph 1124)

Action and use
Non-ionic surfactant.

Ph Eur

DEFINITION

Mixture of ethers of mixed macrogols with linear fatty alcohols, mainly $C_{12}H_{26}O$. It contains a variable quantity of free $C_{12}H_{26}O$ and may contain free macrogols. The number of moles of ethylene oxide reacted per mole of $C_{12}H_{26}O$ is 3 to 23 (nominal value).

CHARACTERS

— Macrogol lauryl ethers with 3 to 5 units of ethylene oxide per molecule.

Appearance
Colourless liquid.

Solubility
Practically insoluble in water, soluble or dispersible in ethanol (96 per cent), practically insoluble in light petroleum.

— Macrogol lauryl ethers with 9 units of ethylene oxide per molecule.

Appearance
White or almost white, unctuous, hygroscopic mass, melting at 24 °C into a colourless or yellowish, viscous liquid.

Solubility
Freely soluble in water, soluble or dispersible in ethanol (96 per cent), practically insoluble in light petroleum.

— Macrogol lauryl ethers with 10 to 23 units of ethylene oxide per molecule.

Appearance
White or almost white, waxy mass.

Solubility
Soluble or dispersible in water, soluble in ethanol (96 per cent), practically insoluble in light petroleum.

IDENTIFICATION

Carry out tests A, B, C, D, E for macrogol lauryl ethers with 3 to 5 units of ethylene oxide per molecule.

Carry out tests A, B, C, D, F for macrogol lauryl ethers with 9 to 23 units of ethylene oxide per molecule.

A. Hydroxyl value (see Tests).

B. Iodine value (see Tests).

C. Saponification value (see Tests).

D. Dissolve or disperse 0.1 g in 5 mL of *ethanol (96 per cent) R*, add 10 mL of *dilute hydrochloric acid R*, 10 mL of *barium chloride solution R1* and 10 mL of a 100 g/L solution of *phosphomolybdic acid R*. A precipitate is formed.

E. Gas chromatography (*2.2.28*).

Test solution Dissolve 0.200 g of the substance to be examined in *acetone R* and dilute to 10.0 mL with the same solvent.

Reference solution Dissolve 0.15 g of *ethylene glycol monododecyl ether R*, 0.15 g of *lauryl alcohol R* and 0.15 g of *myristyl alcohol R* in *acetone R* and dilute to 100.0 mL with the same solvent.

Column:
— *material*: fused silica;
— *size*: *l* = 30 m, Ø = 0.25 mm;
— *stationary phase*: *phenyl(5)methyl(95)polysiloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R* or *hydrogen for chromatography R*.

Flow rate 1 mL/min.

Split ratio 1:50.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 1	120
	1 - 24	120 → 350
	24 - 34	350
Injection port		300
Detector		350

Detection Flame ionisation.

Injection 1.0 µL.

Identification of peaks Use the chromatogram obtained with the reference solution to identify the peaks due to ethylene glycol monododecyl ether, lauryl alcohol and myristyl alcohol.

Retention time Lauryl alcohol = about 6.7 min; myristyl alcohol = about 8.9 min; ethylene glycol monododecyl ether = about 9.3 min.

System suitability Reference solution:
— *resolution*: minimum 5.0 between the peaks due to myristyl alcohol and ethylene glycol monododecyl ether.

Results The sum of the areas of the peaks eluted before the peak due to lauryl alcohol is less than 10 per cent of the area of the peak due to lauryl alcohol; disregard the peak due to the solvent.

F. Gas chromatography (*2.2.28*).

Test solution Into a 50 mL round-bottomed flask fitted with a reflux condenser introduce 0.300 g of the substance to be examined, 15 mL of *hydriodic acid R*, 2.5 mL of a 603 g/L solution of *phosphorous acid R* and 2 or 3 anti-bumping granules. Heat to 140 °C using an oil bath or an electric heating mantle, then boil for 2 h. Allow to cool to room temperature and rinse the condenser with 5 mL of *ethanol (96 per cent) R*. Add the rinsings to the flask and transfer to a separating funnel, rinsing the flask with 2 quantities, each of 5 mL, of a mixture of equal volumes of *ethanol (96 per cent) R* and *water R*.

Extract with 15 mL of *heptane R* and wash the upper layer with 5 mL portions of a mixture of equal volumes of *ethanol (96 per cent) R* and *water R* until the pH of the lower layer is greater than 3 using a *pH indicator strip R*.

Transfer the upper layer into a 20 mL glass vial with a screw cap and shake with 5 g of *anhydrous sodium sulfate R*. Allow to settle and transfer about 1 mL of the clear supernatant to an autosampler vial.

Reference solution Prepare as described for the test solution using 0.150 g of *2-butyloctanol R*, 0.150 g of *lauryl alcohol R* and 0.150 g of *myristyl alcohol R* instead of the substance to be examined.

Column:
— *material*: fused silica;
— *size*: *l* = 30 m, Ø = 0.32 mm;
— *stationary phase*: *phenyl(5)methyl(95)polysiloxane R* (film thickness 1.0 µm).

Carrier gas *helium for chromatography R* or *hydrogen for chromatography R*.

Flow rate 3 mL/min.

Split ratio 1:30.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 5	100
	5 - 55	100 → 300
	55 - 70	300
Injection port		250
Detector		310

Detection Flame ionisation.

Injection 1.0 µL.

Identification of peaks Use the chromatogram obtained with the reference solution to identify the peaks due to 2-butyloctyl iodide, lauryl iodide and myristyl iodide.

Retention time 2-butyloctyl iodide = about 22.7 min; lauryl iodide = about 25.6 min; myristyl iodide = about 31.1 min.

System suitability Reference solution:

— *resolution*: minimum 5.0 between the peaks due to 2-butyloctyl iodide and lauryl iodide.

Results The sum of the areas of the peaks eluted before the peak due to lauryl iodide is less than 10 per cent of the area of the peak due to lauryl iodide; disregard the peak due to the solvent.

TESTS

Appearance of solution

The solution is not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

Dissolve 5.0 g in *ethanol (96 per cent) R* and dilute to 50 mL with the same solvent.

Alkalinity

Dissolve 2.0 g in a hot mixture of 10 mL of *ethanol (96 per cent) R* and 10 mL of *water R*. Add 0.1 mL of *bromothymol blue solution R1*. Not more than 0.5 mL of *0.1 M hydrochloric acid* is required to change the colour of the indicator to yellow.

Acid value (*2.5.1*)

Maximum 1.0, determined on 5.0 g.

Hydroxyl value (*2.5.3, Method A*)

Ethylene oxide units per molecule (nominal value)	Hydroxyl value
3	165 - 180
4	145 - 165
5	130 - 140
9	90 - 100
10	85 - 95
12	73 - 83
15	64 - 74
20 - 23	40 - 60

Iodine value (*2.5.4*)

Maximum 2.0.

Saponification value (*2.5.6*)

Maximum 3.0, determined on 10.0 g.

Ethylene oxide and dioxan (*2.4.25*)

Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Water (*2.5.12*)

Maximum 3.0 per cent, determined on 2.00 g.

Sulfated ash (*2.4.14*)

Maximum 0.2 per cent.

Ignite a silica crucible at 600 ± 50 °C for 30 min, allow to cool in a desiccator and weigh. Evenly distribute 1.0 g in the crucible and weigh again. Dry at 100-105 °C for 1 h and ignite in a muffle furnace at 600 ± 25 °C until the substance is thoroughly charred. Carry out the test for sulfated ash on the residue obtained, starting from "Moisten the substance to be examined...".

STORAGE

In an airtight container.

LABELLING

The label states the number of moles of ethylene oxide reacted per mole of $C_{12}H_{26}O$ (nominal value).

Ph Eur

Macrogol Oleate

(Ph. Eur. monograph 1618)

Action and use

Non-ionic surfactant.

Ph Eur

DEFINITION

A mixture of monoesters and diesters of mainly oleic (*cis*-9-octadecenoic) acid and macrogols. It may be obtained by ethoxylation of *Oleic acid (0799)* or by esterification of macrogols with oleic acid of animal or vegetable origin. It may contain free macrogols. The average polymer length is equivalent to 5-6 or 10 moles of ethylene oxide per mole (nominal value). A suitable antioxidant may be added.

CHARACTERS

Appearance

Slightly yellowish, viscous liquid.

Solubility

Dispersible in water, soluble in ethanol (96 per cent) and in 2-propanol, dispersible in oils, miscible with fatty oils and with waxes.

Refractive index

About 1.466.

IDENTIFICATION

First identification: A, C.

Second identification: A, B.

A. Saponification value (see Tests).

B. Thin-layer chromatography (*2.2.27*).

Test solution To 20 mg add 10 mL of *methylene chloride R* and mix.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase 25 per cent *V/V* solution of *concentrated ammonia R, 2-propanol R* (20:80 *V/V*).

Application 10 µL.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *potassium iodobismuthate solution R4*; examine the plate about 10 min later.

Results The chromatogram obtained shows 3 principal spots, corresponding, in order of increasing R_F value, to free macrogol, macrogol mono-oleate and macrogol dioleate.

C. Composition of fatty acids (see Tests).

TESTS

Alkalinity
Dissolve 2.0 g in *ethanol (96 per cent) R* and dilute to 20 mL with the same solvent. To 2 mL of this solution add 0.05 mL of *phenol red solution R*. The solution is not red.

Acid value (*2.5.1*)
Maximum 2.0.

Hydroxyl value (*2.5.3, Method A*)
See Table 1618.-1.

Iodine value (*2.5.4, Method A*)
See Table 1618.-1.

Peroxide value (*2.5.5, Method A*)
Maximum 12.0.

Saponification value (*2.5.6*)
See Table 1618.-1.

Table 1618.-1

	5-6 moles of ethylene oxide	10 moles of ethylene oxide
Hydroxyl value	50 - 70	65 - 90
Iodine value	50 - 60	27 - 34
Saponification value	105 - 120	68 - 85

Composition of fatty acids
Gas chromatography (*2.4.22, Method A*).

Composition of the fatty-acid fraction of the substance:
— *myristic acid*: maximum 5.0 per cent;
— *stearic acid*: maximum 6.0 per cent;
— *palmitic acid*: maximum 16.0 per cent;
— *palmitoleic acid*: maximum 8.0 per cent;
— *oleic acid*: 65.0 per cent to 88.0 per cent;
— *linoleic acid*: maximum 18.0 per cent;
— *linolenic acid*: maximum 4.0 per cent;
— *fatty acids with a chain length greater than C_{18}*: maximum 4.0 per cent.

Residual ethylene oxide and dioxan (*2.4.25*)
Maximum 1 ppm of residual ethylene oxide and 10 ppm of residual dioxan.

Water (*2.5.12*)
Maximum 2.0 per cent, determined on 1.00 g using *anhydrous methanol R* as the solvent.

Total ash (*2.4.16*)
Maximum 0.3 per cent, determined on 1.0 g.

STORAGE
In an airtight container.

LABELLING
The label states the number of moles of ethylene oxide per mole (nominal value).

Ph Eur

Macrogol Oleyl Ether

(Ph. Eur. monograph 1125)

Action and use
Non-ionic surfactant.

Ph Eur

DEFINITION
Mixture of ethers of mixed macrogols with linear fatty alcohols, mainly oleyl alcohol. It contains a variable quantity of free oleyl alcohol and it may contain free macrogols. The number of moles of ethylene oxide reacted per mole of oleyl alcohol is 2 to 20 (nominal value). A suitable antioxidant may be added.

CHARACTERS
— Macrogol oleyl ether with 2 to 5 units of ethylene oxide per molecule.

Appearance
Yellow liquid.

Solubility
Practically insoluble in water, soluble in alcohol, practically insoluble in light petroleum.
— Macrogol oleyl ether with 10 to 20 units of ethylene oxide per molecule.

Appearance
Yellowish-white waxy mass.

Solubility
Dispersible or soluble in water, soluble in alcohol, practically insoluble in light petroleum.

IDENTIFICATION
A. Hydroxyl value (see Tests).

B. Iodine value (see Tests).

C. Saponification value (see Tests).

D. Dissolve or disperse 0.1 g in 5 mL of *alcohol R*, add 2 mL of *water R*, 10 mL of *dilute hydrochloric acid R*, 10 mL of *barium chloride solution R1* and 10 mL of a 100 g/L solution of *phosphomolybdic acid R*. A precipitate is formed.

TESTS

Appearance of solution
The solution is not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

Dissolve 5.0 g in *alcohol R* and dilute to 50 mL with the same solvent.

Alkalinity
Dissolve 2.0 g in a hot mixture of 10 mL of *water R* and 10 mL of *alcohol R*. Add 0.1 mL of *bromothymol blue solution R1*. Not more than 0.5 mL of *0.1 M hydrochloric acid* is required to change the colour of the indicator to yellow.

Acid value (*2.5.1*)
Maximum 1.0, determined on 5.0 g.

Hydroxyl value (*2.5.3, Method A*)
See Table 1125.-1.

Iodine value (*2.5.4*)
See Table 1125.-1.

Table 1125.-1

Ethylene oxide units per molecule (nominal value)	Hydroxyl value	Iodine value
2	158 - 178	48 - 74*
5	110 - 125	48 - 56
10	75 - 95	24 - 38
20	40 - 65	14 - 24

* This broad range is needed since 2 different grades of oleyl alcohol may be used for the synthesis. The iodine value does not differ by more than 5 units from the nominal iodine value and is within the limits stated in the table.

Peroxide value (*2.5.5*)
Maximum 10.0.

Saponification value (*2.5.6*)
Maximum 3.0.

Ethylene oxide and dioxan (*2.4.25*)
Maximum 1 ppm of ethylene oxide and 10 ppm of dioxan.

Water (*2.5.12*)
Maximum 3.0 per cent, determined on 2.00 g.

Total ash (*2.4.16*)
Maximum 0.2 per cent, determined on 2.0 g.

STORAGE
In an airtight container, protected from light.

LABELLING
The label states:
— the number of moles of ethylene oxide reacted per mole of oleyl alcohol (nominal value),
— the nominal iodine value for the type with 2 units of ethylene oxide per molecule.

Ph Eur

Macrogol Poly(vinyl alcohol) Grafted Copolymer

(Ph. Eur. monograph 2523)

Ph Eur

DEFINITION
Grafted copolymer of macrogol and poly(vinyl alcohol), having a mean relative molecular mass of about 45 000.

It consists of about 75 per cent of poly(vinyl alcohol) units and 25 per cent of macrogol units. It may contain *Anhydrous colloidal silica (0434)* to improve flowability.

CHARACTERS
Appearance
White or slightly yellowish powder; opalescent solutions may be obtained during testing due to the presence of anhydrous colloidal silica.

Solubility
Very soluble in water, practically insoluble in anhydrous ethanol and in acetone. It dissolves in dilute acids and in dilute solutions of alkali hydroxides.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *macrogol poly(vinyl alcohol) grafted copolymer CRS.*

Preparation Dissolve 0.2 g in 20 mL of *water R*, spread a few drops of the solution on a thallium bromoiodide plate and evaporate the solvent at 110 °C for 30 min.

B. Dissolve 0.4 g in 2 mL of *water R*. Place 1 mL of the solution on a glass plate and allow to dry. A transparent film is formed.

TESTS
pH (*2.2.3*)
5.0 to 8.0.

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 25.0 mL with the same solvent.

Ester value
10 to 75.

Determine the acid value (I_A) as follows. Dissolve 5.00 g in 100 mL of *distilled water R* while stirring with a magnetic stirrer. Titrate with *0.01 M alcoholic potassium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank test under the same conditions.

$$I_A = \frac{0.561(n_1 - n_2)}{m}$$

n_1 = volume of titrant used in the test, in millilitres;
n_2 = volume of titrant used in the blank test, in millilitres;
m = mass of the sample, in grams.

Determine the saponification value (I_S) (*2.5.6*) on 5.00 g, using 50.0 mL of *0.5 M alcoholic potassium hydroxide* and stirring vigorously with a magnetic stirrer.

The ester value (I_E) is calculated from the saponification value (I_S) and the acid value (I_A):

$$I_E = I_S - I_A$$

Ethylene oxide and dioxan (*2.4.25*)
Maximum 1 ppm of ethylene oxide and 10 ppm of dioxan.

Impurity A
Liquid chromatography (*2.2.29*).

Test solution Introduce 0.250 g of the substance to be examined into a 10 mL volumetric flask and add about 1 mL of *methanol R*. Sonicate. Add about 8 mL of *water R* and dilute to 10.0 mL with the same solvent. Filter.

Reference solution (a) Dissolve 5.0 mg of *vinyl acetate CRS* (impurity A) in *methanol R* and dilute to 10.0 mL with the same solvent. Dilute 1.0 mL of the solution to 20.0 mL with *water R*. Dilute 1.0 mL of this solution to 10.0 mL with *water R*.

Reference solution (b) Dissolve 5 mg of *vinyl acetate R* (impurity A) and 5 mg of *1-vinylpyrrolidin-2-one R* in 10 mL of *methanol R* and dilute to 50 mL with *water R*. Dilute 1 mL of the solution to 20 mL with *water R*.

A precolumn containing *end-capped octadecylsilyl silica gel for chromatography R* (5 µm) may be used if a matrix effect is observed.

Column:
— *size*: l = 0.25 m, Ø = 4.0 mm;
— *stationary phase*: *polar end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 30 °C.

Mobile phase:
— *mobile phase A*: *acetonitrile R1, methanol R2, water for chromatography R* (5:5:90 *V/V/V*);
— *mobile phase B*: *methanol R2, acetonitrile R1, water for chromatography R* (5:45:50 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	100	0
2 - 40	100 → 85	0 → 15
40 - 42	85 → 0	15 → 100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 205 nm.

Injection 10 µL.

Retention time Impurity A = about 19 min; 1-vinylpyrrolidin-2-one = about 25 min.

System suitability Reference solution (b):
— *resolution*: minimum 5.0 between the peaks due to impurity A and 1-vinylpyrrolidin-2-one.

Limit:
— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (100 ppm).

Impurity B

Liquid chromatography (*2.2.29*).

Test solution Mix 0.200 g of the substance to be examined with *water R* and dilute to 10.0 mL with the same solvent.

Reference solution Dissolve 30 mg of *citric acid monohydrate R* and 0.100 g of *acetic acid R* (impurity B) in the mobile phase. Shake gently to dissolve and dilute to 100.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *polar end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase 0.50 g/L solution of *sulfuric acid R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 205 nm.

Injection 20 µL. After each injection, rinse the column with a mixture of equal volumes of *acetonitrile R1* and a 0.50 g/L solution of *sulfuric acid R*.

Retention time Impurity B = about 5 min; citric acid = about 7 min.

System suitability Reference solution:
— *resolution*: minimum 2.0 between the peaks due to impurity B and citric acid.

Limit:
— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (1.5 per cent).

Sulfated ash (*2.4.14*)

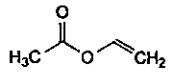
Maximum 3.0 per cent, determined on 5.0 g.

Loss on drying (*2.2.32*)

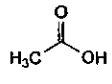
Maximum 5.0 per cent, determined on 1.000 g by drying *in vacuo* at 105 °C.

IMPURITIES

Specified impurities *A, B.*



A. ethenyl acetate,



B. acetic acid.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for macrogol poly(vinyl alcohol) grafted copolymer used as film former in film-coated tablets.

Viscosity (*2.2.10*)

Typically less than 250 mPa·s, determined on a 20 per cent *m/m* solution, using a rotating viscometer at 25 °C and rotation speed of 100 r/min.

Ph Eur

Macrogol 40 Sorbitol Heptaoleate

(Ph. Eur. monograph 2396)

Action and use

Non-ionic surfactant.

Ph Eur

DEFINITION

Mixture of esters of fatty acids, mainly *Oleic acid (0799)*, and sorbitol ethoxylated with approximately 40 moles of ethylene oxide for each mole of sorbitol. 7 moles of oleic acid are used for each mole of sorbitol. It also contains macrogol fatty acid esters.

CHARACTERS

Appearance

Clear or slightly opalescent, yellowish, viscous, hygroscopic liquid.

Solubility

Dispersible in water, soluble in isopropyl myristate, in isopropyl palmitate, in mineral oils and in vegetable fatty oils.

Relative density

About 1.0.

Viscosity (*2.2.9*): about 175 mPa·s at 25 °C.

IDENTIFICATION

First identification: A, D.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *macrogol 40 sorbitol heptaoleate CRS.*

B. Hydroxyl value (see Tests).

C. Saponification value (see Tests).

D. Composition of fatty acids (see Tests).

TESTS

Acid value (*2.5.1*)

Maximum 12.0, determined on 3.0 g.

Hydroxyl value (*2.5.3, Method A*)
22 to 55.

Peroxide value
Maximum 10.0.

Introduce 10.0 g into a 100 mL beaker and dissolve with 20 mL of *glacial acetic acid R*. Add 1 mL of *saturated potassium iodide solution R*, mix and allow to stand for 1 min. Add 50 mL of *carbon dioxide-free water R* and a magnetic stirring bar. Titrate with *0.01 M sodium thiosulfate*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

Determine the peroxide value using the following expression:

$$\frac{(n_1 - n_2) \times M \times 1000}{m}$$

n_1 = volume of *0.01 M sodium thiosulfate* required for the titration of the substance to be examined, in millilitres;
n_2 = volume of *0.01 M sodium thiosulfate* required for the blank titration, in millilitres;
M = molarity of the sodium thiosulfate solution;
m = mass of the substance to be examined, in grams.

Saponification value (*2.5.6*)
90 to 110, determined on 4.0 g.

Use 30.0 mL of 0.5 M alcoholic potassium hydroxide, heat under reflux for 60 min and add 50 mL of *anhydrous ethanol R* before carrying out the titration.

Composition of fatty acids (*2.4.22, Method C*)
Use the mixture of calibrating substances in Table 2.4.22.-3.

Composition of the fatty-acid fraction of the substance:
— *myristic acid*: maximum 5.0 per cent;
— *palmitic acid*: maximum 16.0 per cent;
— *palmitoleic acid*: maximum 8.0 per cent;
— *stearic acid*: maximum 6.0 per cent;
— *oleic acid*: minimum 58.0 per cent;
— *linoleic acid*: maximum 18.0 per cent;
— *linolenic acid*: maximum 4.0 per cent.

Ethylene oxide and dioxan (*2.4.25, Method A*)
Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Water (*2.5.12*)
Maximum 0.5 per cent, determined on 0.50 g.

Sulfated ash
Maximum 0.25 per cent.

Heat a silica crucible to redness for 30 min, allow to cool in a desiccator and weigh. Evenly distribute 1.0 g of the substance to be examined in the crucible and weigh. Dry at 100-105 °C for 1 h and ignite in a muffle furnace at 600 ± 25 °C, until the substance is thoroughly charred. Carry out the test for sulfated ash (*2.4.14*) on the residue obtained, starting from "Moisten the substance to be examined...".

STORAGE
In an airtight container, protected from light.

Ph Eur

Macrogol Stearate

(Ph. Eur. monograph 1234)

Action and use
Non-ionic surfactant.

Ph Eur

DEFINITION
Mixture of monoesters and diesters of mainly stearic (octadecanoic) acid and/or palmitic (hexadecanoic) acid and macrogols. It may be obtained by ethoxylation or by esterification of macrogols with stearic acid 50 (type I) or stearic acid 95 (type II) (see *Stearic acid (1474)*). It may contain free macrogols. The average polymer length is equivalent to 6 to 100 ethylene oxide units per molecule (nominal value).

CHARACTERS

Appearance
White or slightly yellowish, waxy mass.

Solubility
Soluble in ethanol (96 per cent) and in 2-propanol. Macrogol stearate corresponding to a product with 6-9 units of ethylene oxide per molecule is practically insoluble, but freely dispersible in water and miscible with fatty oils and with waxes. Macrogol stearate corresponding to a product with 20-100 units of ethylene oxide per molecule is soluble in water and practically insoluble in fatty oils and in waxes.

IDENTIFICATION
A. Saponification value (see Tests).
B. Composition of fatty acids (see Tests).

TESTS

Alkalinity
Dissolve 2.0 g in *ethanol (96 per cent) R* and dilute to 20 mL with the same solvent. To 2 mL of this solution add 0.05 mL of *phenol red solution R*. The solution is not red.

Melting point (*2.2.15*)
See Table 1234.-1.

Melt about 10 g at 80-90 °C. Introduce a sufficient amount of the substance into the tube by capillary action to form a column of the prescribed height. Allow to stand at 0 °C for 2 h.

Acid value (*2.5.1*)
Maximum 4.0, determined on 2.0 g.

Hydroxyl value (*2.5.3, Method A*)
See Table 1234.-1.

Iodine value (*2.5.4*)
Maximum 2.0.

Saponification value (*2.5.6*)
See Table 1234.-1.

Table 1234.-1

Ethylene oxide units per molecule (nominal value)	Melting point (°C)	Hydroxyl value	Saponification value
6	26 - 37	80 - 110	85 - 115
8 - 9	26 - 35	80 - 105	88 - 100
20	33 - 40	50 - 62	46 - 56
32	46 - 50	20 - 40	30 - 45
40 - 50	38 - 52	23 - 40	20 - 35
100	48 - 60	15 - 30	5 - 20

Composition of fatty acids
Gas chromatography (*2.4.22, Method C*).
Composition of the fatty acid fraction of the substance:

	Type of fatty acid used	Composition of fatty acids
Macrogol stearate type I	Stearic acid 50	*Stearic acid*: 40.0 per cent to 60.0 per cent, *Sum of the contents of palmitic and stearic acids*: not less than 90.0 per cent.
Macrogol stearate type II	Stearic acid 95	*Stearic acid*: 90.0 per cent to 99.0 per cent, *Sum of the contents of palmitic and stearic acids*: not less than 96.0 per cent.

Ethylene oxide and dioxan (*2.4.25*)
Maximum 1 ppm of ethylene oxide and 10 ppm of dioxan.

Water (*2.5.12*)
Maximum 3.0 per cent, determined on 0.50 g. Use as the solvent a mixture of equal volumes of *anhydrous methanol R* and *methylene chloride R*.

Total ash (*2.4.16*)
Maximum 0.3 per cent, determined on 1.0 g.

STORAGE
In an airtight container.

LABELLING
The label states:
— the number of ethylene oxide units per molecule (nominal value);
— the type of macrogol stearate.

Ph Eur

Macrogol Stearyl Ether

(Ph. Eur. monograph 1340)

Action and use
Non-ionic surfactant.

Ph Eur

DEFINITION
Mixture of ethers obtained by ethoxylation of stearyl alcohol. It may contain some free macrogols and various amounts of free stearyl alcohol. The number of moles of ethylene oxide reacted per mole of stearyl alcohol is 2 to 20 (nominal value).

CHARACTERS
Appearance
White or yellowish-white, waxy, unctuous mass, pellets, microbeads or flakes.

Solubility
— macrogol stearyl ether with 2 moles of ethylene oxide reacted per mole: practically insoluble in water, soluble in ethanol (96 per cent) with heating and in methylene chloride;
— macrogol stearyl ether with 10 moles of ethylene oxide reacted per mole: soluble in water and in ethanol (96 per cent);
— macrogol stearyl ether with 20 moles of ethylene oxide reacted per mole: soluble in water, in ethanol (96 per cent) and in methylene chloride.

After melting, it solidifies at about 45 °C.

IDENTIFICATION
A. Hydroxyl value (see Tests).
B. Iodine value (see Tests).
C. Saponification value (see Tests).
D. Thin-layer chromatography (*2.2.27*).
Test solution Dissolve 10.0 g in a mixture of 1 volume of *water R* and 9 volumes of *methanol R* and dilute to 75 mL with the same mixture of solvents. Add 60 mL of *heptane R* and shake for 3 min. The formation of foam can be reduced by the addition of a few drops of *ethanol (96 per cent) R*. Filter the upper layer through *anhydrous sodium sulfate R*, wash the filter with 3 quantities, each of 10 mL, of *heptane R* and evaporate the combined filtrates to dryness. Dissolve 50 mg of the residue in 10 mL of *methanol R* (the solution may be opalescent).
Reference solution Dissolve 25 mg of *stearyl alcohol CRS* in *methanol R* and dilute to 25 mL with the same solvent.
Plate *TLC silica gel plate R.*
Mobile phase *ethyl acetate R.*
Application 20 µL.
Development Over a path of 15 cm.
Drying In air.
Detection Spray with vanillin-sulfuric acid reagent prepared as follows: dissolve 0.5 g of *vanillin R* in 50 mL of *ethanol (96 per cent) R* and dilute to 100 mL with *sulfuric acid R*; allow to dry in air; heat at about 130 °C for 15 min and allow to cool in air.
Results The chromatogram obtained with the test solution shows several spots; one of these spots corresponds to the principal spot in the chromatogram obtained with the reference solution.
E. Dissolve or disperse 0.1 g in 5 mL of *ethanol (96 per cent) R*, add 2 mL of *water R*, 10 mL of *dilute hydrochloric acid R*, 10 mL of *barium chloride solution R1* and 10 mL of a 100 g/L solution of *phosphomolybdic acid R*. A precipitate is formed.

TESTS
Appearance of solution
The solution is not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).
Dissolve 5.0 g in *ethanol (96 per cent) R* and dilute to 50 mL with the same solvent.

Alkalinity
Dissolve 2.0 g in a hot mixture of 10 mL of *ethanol (96 per cent) R* and 10 mL of *water R*. Add 0.1 mL of *bromothymol blue solution R1*. Not more than 0.5 mL of *0.1 M hydrochloric acid* is required to change the colour of the indicator to yellow.

Acid value (*2.5.1*)
Maximum 1.0, determined on 5.0 g.

Hydroxyl value (*2.5.3, Method A*)

Number of moles of ethylene oxide reacted per mole (nominal value)	Hydroxyl value
2	150 - 180
10	75 - 90
20	40 - 60

Iodine value (*2.5.4, Method A*)
Maximum 2.0.

Saponification value (*2.5.6*)
Maximum 3.0, determined on 10.0 g.

Ethylene oxide and dioxan (*2.4.25*)
Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Water (*2.5.12*)
Maximum 3.0 per cent, determined on 1.00 g.

STORAGE
In an airtight container.

LABELLING
The label states the number of moles of ethylene oxide reacted per mole of stearyl alcohol (nominal value).

Ph Eur

Magaldrate

(Ph. Eur. monograph 1539)

$Al_5Mg_{10}(OH)_{31}(SO_4)_2,xH_2O$ 1097 *74978-16-8*
(anhydrous substance)

Action and use
Antacid.

Preparation
Magaldrate Oral Suspension

Ph Eur

DEFINITION
Magaldrate is composed of aluminium and magnesium hydroxides and sulfates. Its composition corresponds approximately to the formula $Al_5Mg_{10}(OH)_{31}(SO_4)_2,xH_2O$.

Content
90.0 per cent to 105.0 per cent (dried substance).

It contains a variable quantity of water.

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water and in ethanol (96 per cent). It is soluble in dilute mineral acids.

IDENTIFICATION
A. Dissolve 0.6 g in 20 mL of *3 M hydrochloric acid R*, add about 30 mL of *water R* and heat to boiling. Adjust to pH 6.2 with *dilute ammonia R1*, continue boiling for a further 2 min, filter and retain the precipitate and the filtrate. To 2 mL of the filtrate add 2 mL of *ammonium chloride solution R* and neutralise with a solution prepared by dissolving 2 g of *ammonium carbonate R* and 2 mL of *dilute ammonia R1* in 20 mL of *water R*; no precipitate is produced. Add *disodium hydrogen phosphate solution R*; a white, crystalline precipitate is produced which does not dissolve in *dilute ammonia R1*.

B. The precipitate retained in identification test A gives the reaction of aluminium (*2.3.1*).

C. The filtrate retained in identification test A gives reaction (a) of sulfates (*2.3.1*).

TESTS

Soluble chlorides
Maximum 3.5 per cent.

To 0.5 g add 25 mL of *dilute nitric acid R* and shake until completely dissolved. Add 10.0 mL of *0.1 M silver nitrate* and 2 mL of *ferric ammonium sulfate solution R2* as indicator. Titrate with *0.1 M ammonium thiocyanate*, shaking vigorously until a persistent brownish-red colour is obtained.

1 mL of *0.1 M silver nitrate* is equivalent to 3.545 mg of Cl.

Soluble sulfates
Maximum 1.9 per cent.

Disperse 0.5 g in 25 mL of *water R*, boil for 5 min, cool, dilute to 25.0 mL with *water R*, mix and filter. To 2.5 mL of the filtrate, add 30 mL of *water R*, neutralise to *blue litmus paper R* with *hydrochloric acid R*, add 3 mL of *1 M hydrochloric acid*, 3 mL of a 120 g/L solution of *barium chloride R* and dilute to 50 mL with *water R*. Mix and allow to stand for 10 min. Any opalescence in the solution is not more intense than that in a standard prepared at the same time in the same manner using 1 mL of *0.01 M sulfuric acid* instead of 2.5 mL of filtrate.

Sulfates
16.0 per cent to 21.0 per cent (dried substance).

Dissolve 0.875 g in a mixture of 5 mL of *glacial acetic acid R* and 10 mL of *water R* and dilute to 25.0 mL with *water R*. Prepare a chromatographic column of 1 cm in internal diameter containing 15 mL of *cation-exchange resin R* (150-300 µm), previously washed with 30 mL of *water R*. Transfer 5.0 mL of the solution to be examined to the column and elute with 15 mL of *water R*. To the eluate add 5 mL of a 53.6 g/L solution of *magnesium acetate R*, 32 mL of *methanol R* and 0.2 mL of *alizarin S solution R*. Add from a burette about 4.0 mL of *0.05 M barium chloride*, add a further 0.2 mL of *alizarin S solution R* and slowly complete the titration until the yellow colour disappears and a violet-red tinge is visible.

1 mL of *0.05 M barium chloride* is equivalent to 4.803 mg of SO_4.

Aluminium hydroxide
32.1 per cent to 45.9 per cent (dried substance).

Dissolve 0.800 g in 10 mL of *dilute hydrochloric acid R*, heating on a water-bath. Cool and dilute to 50.0 mL with *water R*. To 10.0 mL of this solution, add *dilute ammonia R1* until a precipitate begins to appear. Add the smallest quantity of *dilute hydrochloric acid R* needed to dissolve the precipitate and dilute to 20 mL with *water R*. Carry out the complexometric titration of aluminium (*2.5.11*).

1 mL of *0.1 M sodium edetate* is equivalent to 7.80 mg of $Al(OH)_3$.

Magnesium hydroxide
49.2 per cent to 66.6 per cent (dried substance).

Dissolve 0.100 g in 2 mL of *dilute hydrochloric acid R* and transfer to a 500 mL conical flask with the aid of *water R*. Dilute to 200 mL with *water R*, add 20 mL of *triethanolamine R* with shaking, 10 mL of *ammonium chloride buffer solution pH 10.0 R* and about 50 mg of *mordant black 11 triturate R*. Titrate with *0.1 M sodium edetate* until the colour changes from violet to pure blue.

1 mL of *0.1 M sodium edetate* is equivalent to 5.832 mg of $Mg(OH)_2$.

Sodium
Maximum 0.10 per cent.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Weigh 2.00 g into a 100 mL volumetric flask, place in an ice-bath, add 5 mL of *nitric acid R* and swirl to mix. Allow to warm to room temperature and dilute to

100 mL with *water R*. Filter, if necessary, to obtain a clear solution. Dilute 10.0 mL of the filtrate to 100.0 mL with *water R*.

Reference solutions Prepare the reference solutions using *sodium standard solution (200 ppm Na) R*, diluted as necessary with *dilute nitric acid R*.

Source Sodium hollow-cathode lamp.

Wavelength 589 nm.

Atomisation device Air-acetylene flame.

Loss on drying (*2.2.32*)
10.0 per cent to 20.0 per cent, determined on 1.000 g by drying in an oven at 200 °C for 4 h.

ASSAY
To 1.500 g add 50.0 mL of *1 M hydrochloric acid*. Titrate the excess hydrochloric acid with *1 M sodium hydroxide* to pH 3.0, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 mL of *1 M hydrochloric acid* is equivalent to 35.40 mg of $Al_5Mg_{10}(OH)_{31}(SO_4)_2$.

Ph Eur

Magnesium Acetate Tetrahydrate

(Ph. Eur. monograph 2035)

$Mg(CH_3COO)_2,4H_2O$ 214.5 *16674-78-5*

Action and use
Used in dialysis solutions.

Ph Eur

DEFINITION

Content
98.0 per cent to 101.0 per cent of magnesium acetate (anhydrous substance).

CHARACTERS

Appearance
Colourless crystals or white or almost white, crystalline powder.

Solubility
Freely soluble in water and in ethanol (96 per cent).

IDENTIFICATION
A. Dissolve about 100 mg in 2 mL of *water R*. Add 1 mL of *dilute ammonia R1* and heat. A white precipitate is formed that dissolves slowly on addition of 5 mL of *ammonium chloride solution R*. Add 1 mL of *disodium hydrogen phosphate solution R*. A white crystalline precipitate is formed.

B. It gives reaction (b) of acetates (*2.3.1*).

TESTS

pH (*2.2.3*)
7.5 to 8.5.

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Chlorides (*2.4.4*)
Maximum 330 ppm.

Dissolve 1.0 g in *water R* and dilute to 100 mL with the same solvent.

Nitrates
Maximum 3 ppm.

Dissolve 1.0 g in *distilled water R* and dilute to 10 mL with the same solvent, add 5 mg of *sodium chloride R*, 0.05 mL of *indigo carmine solution R* and while stirring, 10 mL of *nitrogen-free sulfuric acid R*. A blue colour is produced which persists for at least 10 min.

Sulfates (*2.4.13*)
Maximum 600 ppm.

Dissolve 0.25 g in *distilled water R* and dilute to 15 mL with the same solvent.

Aluminium (*2.4.17*)
Maximum 1 ppm.

Prescribed solution Dissolve 4.0 g in *water R* and dilute to 100 mL with the same solvent. Add 10 mL of *acetate buffer solution pH 6.0 R*.

Reference solution Mix 2 mL of *aluminium standard solution (2 ppm Al) R*, 10 mL of *acetate buffer solution pH 6.0 R* and 98 mL of *water R*.

Blank solution Mix 10 mL of *acetate buffer solution pH 6.0 R* and 100 mL of *water R*.

Calcium (*2.4.3*)
Maximum 100 ppm.

Dissolve 1.0 g in *distilled water R* and dilute to 15 mL with the same solvent.

Potassium
Maximum 0.1 per cent.

Atomic emission spectrometry (*2.2.22, Method II*).

Test solution Dissolve 0.5 g in *water R* and dilute to 100 mL with the same solvent.

Reference solutions Prepare the reference solutions using *potassium standard solution (600 ppm K) R*, diluted as necessary with *water R*.

Wavelength 766.5 nm.

Sodium
Maximum 0.5 per cent.

Atomic emission spectrometry (*2.2.22, Method II*).

Test solution Dissolve 1.0 g in *water R* and dilute to 100 mL with the same solvent.

Reference solutions Prepare the reference solutions using *sodium standard solution (200 ppm Na) R*, diluted as necessary with *water R*.

Wavelength 589.0 nm.

Readily oxidisable substances
Dissolve 2.0 g in 100 mL of boiling *water R*, add 6 mL of a 150 g/L solution of *sulfuric acid R* and 0.3 mL of *0.02 M potassium permanganate*. Mix and boil gently for 5 min. The pink colour is not completely discharged.

Water (*2.5.12*)
33.0 per cent to 35.0 per cent, determined on 0.100 g.

ASSAY
Dissolve 0.150 g in 300 mL of *water R*. Carry out the complexometric titration of magnesium (*2.5.11*).

1 mL of *0.1 M sodium edetate* is equivalent to 14.24 mg of $C_4H_6MgO_4$.

Ph Eur

Magnesium Aluminometasilicate

(Ph. Eur. monograph 2854)

Action and use
Excipient.

Ph Eur

DEFINITION

Magnesium aluminometasilicate of synthetic origin exists in 2 forms (type A and type B), which differ in their apparent pH. It contains a variable quantity of water.

Content (type A and type B):
— *silicon dioxide* (SiO_2; M_r 60.1): 29.2 per cent to 35.6 per cent (dried substance);
— *aluminium oxide* (Al_2O_3; M_r 102.0): 29.1 per cent to 35.5 per cent (dried substance);
— *magnesium oxide* (MgO; M_r 40.30): 11.4 per cent to 14.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, hygroscopic powder or granules.

Solubility
Practically insoluble in water and in ethanol (96 per cent).

IDENTIFICATION

A. To 0.5 g add 5 mL of a mixture of 1 volume of *sulfuric acid R* and 2 volumes of *water R* and heat until white fumes evolve. Cool, add 20 mL of *water R* and filter. Use the filtrate for identification tests B and C. Wash the residue with *water R*. The residue gives the reaction of silicates (*2.3.1*).

B. Neutralise the filtrate obtained in identification test A with *ammonia R*. A white, gelatinous precipitate is formed. Centrifuge and keep the supernatant for identification test C. Dissolve the precipitate in *dilute hydrochloric acid R*. Add dropwise *dilute sodium hydroxide solution R*. A white, gelatinous precipitate is formed. Filter and add a few drops of *phenolphthalein solution R* to the residue. The residue turns pink. Wash the residue with *water R* until the pink colour is completely discharged and the residue remains white upon addition of a drop of *phenolphthalein solution R*. Sprinkle a few crystals of *sodium fluoride R* on the residue. The residue, in contact with the crystals, turns pink again in a short time.

C. To 2 mL of the supernatant obtained after centrifugation in identification test B, add 1 mL of *dilute ammonia R1* and 1 mL of *ammonium chloride solution R*. Upon the addition of *dilute ammonia R1* a white precipitate may form, which dissolves after addition of the *ammonium chloride solution R*. Add 1 mL of *disodium hydrogen phosphate solution R*. A white precipitate is formed.

TESTS

Solution S
Disperse 10.0 g in 100.0 mL of *water R* and boil gently for 15 min with shaking. After cooling, dilute to 100.0 mL with *water R* and centrifuge. Dilute 50.0 mL of the clear supernatant to 100.0 mL with *water R*.

pH (*2.2.3*)
6.0 to 8.5 for type A; 8.5 to 10.5 for type B.

Disperse 2.0 g in 50 mL of *carbon dioxide-free water R*. Read the pH after the electrode has been immersed in the suspension for 2 min.

Water-soluble salts
Maximum 1.5 per cent.

Evaporate 25 mL of solution S to dryness on a water-bath and heat at 700 °C for 2 h. The residue weighs a maximum of 19 mg.

Chlorides (*2.4.4*)
Maximum 500 ppm.

Dilute 2 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 0.5 per cent.

Dilute 10 mL of solution S to 100 mL with *distilled water R*. Dilute 6 mL of the solution to 15 mL with *distilled water R*.

Iron (*2.4.9*)
Maximum 300 ppm.

Disperse 0.10 g in 8 mL of *dilute nitric acid R*, boil for 1 min, cool and dilute to 30.0 mL with *water R*. Centrifuge and use 10 mL of the clear supernatant.

Loss on drying (*2.2.32*)
Maximum 20.0 per cent, determined on 1.000 g by drying in an oven at 110 °C for 7 h.

Neutralising capacity
Minimum 210 mL of *0.1 M hydrochloric acid* per gram of dried substance.

To 0.200 g in a glass-stoppered flask add 100.0 mL of *0.1 M hydrochloric acid*. Stopper the flask tightly, shake at 37 ± 2 °C for 1 h and filter. Titrate 50.0 mL of the filtrate with *0.1 M sodium hydroxide* to pH 3.5 while stirring thoroughly. Carry out a blank determination.

ASSAY

Silicon dioxide
In a suitable container, disperse 1.000 g (*m*) in 30 mL of *dilute hydrochloric acid R* and evaporate to dryness on a water-bath. Moisten the residue with *hydrochloric acid R* and evaporate to dryness on a water-bath. To the residue add 8 mL of *hydrochloric acid R*, stir, add 25 mL of boiling *water R* and stir again. Allow to stand, then filter the supernatant through filter paper. To the residue in the container add 10 mL of boiling *water R*, stir, allow to stand, then filter the supernatant through the filter paper. Wash the residue in the container with 3 quantities, each of 10 mL, of boiling *water R*, each time filtering the washings through the filter paper. Add 50 mL of *water R* to the residue in the container, heat on a water-bath for 15 min and filter through the filter paper. Wash the residue on the filter paper with boiling *water R* until no further precipitate is formed when 1 mL of *silver nitrate solution R1* is added to 5 mL of the washings. Transfer the residue and the filter paper to a platinum crucible that has been weighed prior to the transfer (*a*). Heat strongly to incinerate and continue to heat at 775-825 °C for 1 h. Cool and weigh (*b*).

Calculate the percentage content of silicon dioxide (SiO_2) using the following expression:

$$\frac{b-a}{m} \times 100$$

Aluminium oxide
In a conical flask disperse 1.250 g in 15 mL of *dilute hydrochloric acid R* and 50 mL of *water R* and heat on a water-bath for 15 min. To this solution add 8 mL of *hydrochloric acid R* and heat on a water-bath for 10 min. After cooling, transfer the solution to a 250 mL volumetric flask, rinse the conical flask with *water R* and add the washings to the volumetric flask. Dilute to volume with *water R*.

Centrifuge and use the supernatant as the test solution. Retain a portion for use in the assay for magnesium oxide.

Carry out the complexometric titration of aluminium (*2.5.11*). Carry out a blank determination.

1 mL of *0.1 M sodium edetate* is equivalent to 5.098 mg of Al_2O_3.

Magnesium oxide

Transfer 100.0 mL of the test solution obtained in the assay for aluminium oxide to a conical flask, add 25 mL of a 50 per cent *V/V* solution of *triethanolamine R* and shake. Add 25 mL of *ammonium chloride buffer solution pH 10.7 R* and 40 mg of *mordant black 11 triturate R*. Titrate with *0.1 M sodium edetate* until the colour changes from violet to pure blue.

1 mL of *0.1 M sodium edetate* is equivalent to 4.030 mg of MgO.

STORAGE

In an airtight container.

LABELLING

The label states the type of magnesium aluminometasilicate.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for magnesium aluminometasilicate used as glidant in tablets and capsules.

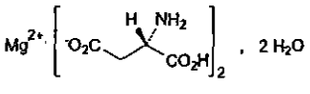
Particle-size distribution (*2.9.31*)

Specific surface area (*2.9.26, Method I*)

Ph Eur

Magnesium Aspartate

(Magnesium Aspartate Dihydrate, Ph. Eur. monograph 1445)



$C_8H_{12}MgN_2O_8,2H_2O$ 324.5 *215533-00-9*

Ph Eur

DEFINITION

Magnesium bis[(3*S*)-3-amino-3-carboxypropanoate] dihydrate.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water.

IDENTIFICATION

Carry out either tests A, C, D, E or tests B, C, D, E.

A. Specific optical rotation (*2.2.7*): + 22.0 to + 24.0 (anhydrous substance).

Dissolve 0.50 g in a 515 g/L solution of *hydrochloric acid R* and dilute to 25.0 mL with the same acid.

B. Enantiomeric purity (see Tests).

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *water R* and dilute to 50 mL with the same solvent.

Reference solution Dissolve 10 mg of *magnesium aspartate dihydrate CRS* in *water R* and dilute to 50 mL with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *glacial acetic acid R, water R, butanol R* (20:20:60 *V/V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *ninhydrin solution R* and heat at 105 °C for 15 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Ignite about 15 mg until a white residue is obtained. Dissolve the residue in 1 mL of *dilute hydrochloric acid R*, neutralise to *red litmus paper R* by adding *dilute sodium hydroxide solution R* and filter if necessary. The solution gives the reaction of magnesium (*2.3.1*).

E. Water (see Tests).

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)

6.0 to 8.0 for solution S.

Enantiomeric purity

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.120 g of the substance to be examined in *water R* and dilute to 100.0 mL with the same solvent.

Reference solution (a) Dissolve 0.100 g of *D-aspartic acid R* (impurity A) in *water R* and dilute to 100.0 mL with the same solvent.

Reference solution (b) Dissolve 0.120 g of the substance to be examined in 90 mL of *water R*, add 0.3 mL of reference solution (a) and dilute to 100 mL with *water R*.

Reference solution (c) Dilute 0.3 mL of reference solution (a) to 100.0 mL with *water R*.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *L-penicillamine coated silica gel for chiral separations R* (5 µm);
— *temperature*: 30 °C.

Mobile phase *2-propanol R*, 0.5 g/L solution of *copper sulfate pentahydrate R* (5:95 *V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µL.

Relative retention With reference to magnesium aspartate (retention time = about 12 min): impurity A = about 0.85.

System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the peaks due to impurity A and magnesium aspartate.

Calculation of percentage content:
— for impurity A, use the concentration of impurity A in reference solution (c).

Limit:
— *impurity A*: maximum 0.3 per cent.

Other dicarboxylic acids

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.600 g of the substance to be examined in 2.0 mL of a 618 g/L solution of *hydrochloric acid R* and dilute to 10.0 mL with *water R*.

Reference solution (a) Dissolve 20.0 mg of *malic acid R* (impurity B) in *water R* and dilute to 20.0 mL with the same solvent.

Reference solution (b) Dissolve 10.0 mg of *maleic acid R* (impurity I) in *water R* and dilute to 10.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with *water R*.

Reference solution (c) Dilute 1 mL of reference solution (b) to 10 mL with reference solution (a).

Reference solution (d) Dilute 1.0 mL of reference solution (a) to 10.0 mL with *water R*.

Reference solution (e) Dissolve 10 mg of *fumaric acid R* (impurity C) in *water R* and dilute to 10 mL with the same solvent. Dilute 1 mL of the solution to 100 mL with *water R*.

Column:
— *size*: *l* = 0.30 m, Ø = 7.8 mm;
— *stationary phase*: *cation-exchange resin R* (9 µm);
— *temperature*: 30 °C.

Mobile phase 0.39 g/L solution of *sulfuric acid R*.

Flow rate 0.6 mL/min.

Detection Spectrophotometer at 214 nm.

Injection 10 µL.

Run time 4 times the retention time of impurity I.

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peaks due to impurities B and I; use the chromatogram obtained with reference solution (e) to identify the peak due to impurity C.

Relative retention With reference to impurity I (retention time = about 7.5 min): impurity B = about 1.2; impurity C = about 2.0.

System suitability Reference solution (c):
— *resolution*: minimum 1.5 between the peaks due to impurities I and B.

Calculation of percentage contents:
— for impurities C and I, use the concentration of impurity I in reference solution (b).
— for impurities other than C and I, use the concentration of impurity B in reference solution (d).

Limits:
— *impurity C*: maximum 0.10 per cent;
— *impurity I*: maximum 0.10 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.3 per cent;
— *reporting threshold*: 0.05 per cent.

Ninhydrin-positive substances

Amino acid analysis (*2.2.56*). For analysis, use Method 1.

The concentrations of the test and reference solutions may be adapted according to the sensitivity of the equipment used. The concentrations of all solutions are adjusted so that the system suitability requirements described in general chapter *2.2.46* are fulfilled, keeping the ratios of concentrations between all solutions as described.

Solution A *dilute hydrochloric acid R1* or a sample preparation buffer suitable for the apparatus used.

Test solution Dissolve 35.0 mg of the substance to be examined in solution A and dilute to 50.0 mL with solution A.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with solution A. Dilute 2.0 mL of this solution to 10.0 mL with solution A.

Reference solution (b) Dissolve 30.0 mg of *proline R* in solution A and dilute to 100.0 mL with solution A. Dilute 1.0 mL of this solution to 250.0 mL with solution A.

Reference solution (c) Dilute 6.0 mL of *ammonium standard solution (100 ppm NH_4) R* to 50.0 mL with solution A. Dilute 1.0 mL of this solution to 100.0 mL with solution A.

Reference solution (d) Dissolve 30 mg of *isoleucine R* and 30 mg of *leucine R* in solution A and dilute to 50 mL with solution A. Dilute 1 mL of the solution to 200 mL with solution A.

Blank solution Solution A.

Inject suitable, equal amounts of the test solution, blank solution and reference solutions (a), (b) and (d) into the amino acid analyser. Run a program suitable for the determination of physiological amino acids.

System suitability Reference solution (d):
— *resolution*: minimum 1.5 between the peaks due to isoleucine and leucine.

Calculation of percentage contents:
— for any ninhydrin-positive substance detected at 570 nm, use the concentration of magnesium aspartate dihydrate in reference solution (a);
— for any ninhydrin-positive substance detected at 440 nm, use the concentration of proline in reference solution (b); if a peak is above the reporting threshold at both wavelengths, use the result obtained at 570 nm for quantification.

Limits:
— *any ninhydrin-positive substance*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.5 per cent;
— *reporting threshold*: 0.05 per cent.

Chlorides (*2.4.4*)

Maximum 200 ppm.

Dilute 10 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)

Maximum 500 ppm.

Dilute 12 mL of solution S to 15 mL with *distilled water R*. Carry out the evaluation of the test after 30 min.

Ammonium
Amino acid analysis (*2.2.56*) as described in the test for ninhydrin-positive substances with the following modification.

Injection Test solution, reference solution (c) and blank solution.

Limit:
— *ammonium at 570 nm*: not more than twice the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.04 per cent), taking into account the peak due to ammonium in the chromatogram obtained with the blank solution.

Iron (*2.4.9*)
Maximum 50 ppm.

In a separating funnel, dissolve 0.20 g in 10 mL of *dilute hydrochloric acid R*. Shake with 3 quantities, each of 10 mL, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 mL of *water R* and shake for 3 min. Use the aqueous layer.

Water (*2.5.12*)
10.0 per cent to 16.0 per cent, determined on 0.100 g.

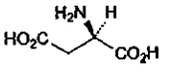
Dissolve the substance to be examined in a mixture of 2 mL of *anhydrous methanol R* and 10 mL of *formamide R1* at 50 °C protected from moisture. Allow to cool. Carry out a blank determination.

ASSAY
Dissolve 0.260 g in 10 mL of *water R* and carry out the complexometric titration of magnesium (*2.5.11*).

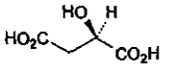
1 mL of *0.1 M sodium edetate* is equivalent to 28.85 mg of $C_8H_{12}MgN_2O_8$.

IMPURITIES
Specified impurities *A, C, I.*

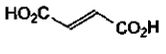
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, D, E, F, G, H.*



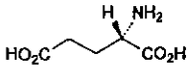
A. (2*R*)-2-aminobutanedioic acid (D-aspartic acid),



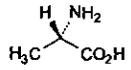
B. (2*RS*)-2-hydroxybutanedioic acid (malic acid),



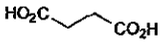
C. (2*E*)-but-2-enedioic acid (fumaric acid),



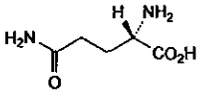
D. (2*S*)-2-aminopentanedioic acid (glutamic acid),



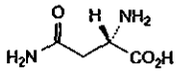
E. (2*S*)-2-aminopropanoic acid (alanine),



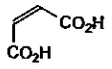
F. butanedioic acid (succinic acid),



G. (2*S*)-2,5-diamino-5-oxopentanoic acid (L-glutamine),



H. (2*S*)-2,4-diamino-4-oxobutanoic acid (asparagine),



I. (2*Z*)-but-2-enedioic acid (maleic acid).

Ph Eur

Heavy Magnesium Carbonate

(Ph. Eur. monograph 0043)

Action and use
Antacid; osmotic laxative.

Preparations
Calcium Carbonate and Heavy Magnesium Carbonate Chewable Tablets
Compound Magnesium Trisilicate Oral Powder

Ph Eur

DEFINITION
Hydrated basic magnesium carbonate.

Content
40.0 per cent to 45.0 per cent, calculated as MgO (M_r 40.30).

CHARACTERS
Appearance
White or almost white powder.

Solubility
Practically insoluble in water. It dissolves in dilute acids with effervescence.

IDENTIFICATION
A. Bulk density (*2.9.34*): minimum 0.25 g/mL.

B. It gives the reaction of carbonates (*2.3.1*).

C. Dissolve about 15 mg in 2 mL of *dilute nitric acid R* and neutralise with *dilute sodium hydroxide solution R*. The solution gives the reaction of magnesium (*2.3.1*).

TESTS
Solution S
Dissolve 5.0 g in 100 mL of *dilute acetic acid R*. When the effervescence has ceased, boil for 2 min, allow to cool and dilute to 100 mL with *dilute acetic acid R*. Filter, if necessary,

through a previously ignited and tared porcelain or silica filter crucible of suitable porosity to give a clear filtrate.

Appearance of solution
Solution S is not more intensely coloured than reference solution B_4 (*2.2.2, Method II*).

Soluble substances
Maximum 1.0 per cent.

Mix 2.00 g with 100 mL of *water R* and boil for 5 min. Filter whilst hot through a sintered-glass filter (40) (*2.1.2*), allow to cool and dilute to 100 mL with *water R*. Evaporate 50 mL of the filtrate to dryness and dry at 100-105 °C. The residue weighs not more than 10 mg.

Substances insoluble in acetic acid
Maximum 0.05 per cent.

Any residue obtained during the preparation of solution S, washed, dried, and ignited at 600 ± 50 °C, weighs not more than 2.5 mg.

Chlorides (*2.4.4*)
Maximum 700 ppm.

Dilute 1.5 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 0.6 per cent.

Dilute 0.5 mL of solution S to 15 mL with *distilled water R*.

Calcium (*2.4.3*)
Maximum 0.75 per cent.

Dilute 2.6 mL of solution S to 150 mL with *distilled water R*. 15 mL of the solution complies with the test.

Iron (*2.4.9*)
Maximum 400 ppm.

Dissolve 0.1 g in 3 mL of *dilute hydrochloric acid R* and dilute to 10 mL with *water R*. Dilute 2.5 mL of the solution to 10 mL with *water R*.

ASSAY
Dissolve 0.150 g in a mixture of 2 mL of *dilute hydrochloric acid R* and 20 mL of *water R*. Carry out the complexometric titration of magnesium (*2.5.11*).

1 mL of *0.1 M sodium edetate* is equivalent to 4.030 mg of MgO.

FUNCTIONALITY-RELATED CHARACTERISTICS
This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for heavy magnesium carbonate used as filler in tablets.

Particle-size distribution (*2.9.31* or *2.9.38*)

Bulk and tapped density (*2.9.34*)

Ph Eur

Light Magnesium Carbonate

(Ph. Eur. monograph 0042)

Action and use
Antacid; osmotic laxative.

Preparations
Aromatic Magnesium Carbonate Mixture
Kaolin Mixture
Magnesium Sulfate Mixture
Magnesium Trisilicate Mixture

Ph Eur

DEFINITION
Hydrated basic magnesium carbonate.

Content
40.0 per cent to 45.0 per cent, calculated as MgO (M_r 40.30).

CHARACTERS
Appearance
White or almost white powder.

Solubility
Practically insoluble in water. It dissolves in dilute acids with effervescence.

IDENTIFICATION
A. Bulk density (*2.9.34*): maximum 0.15 g/mL.

B. It gives the reaction of carbonates (*2.3.1*).

C. Dissolve about 15 mg in 2 mL of *dilute nitric acid R* and neutralise with *dilute sodium hydroxide solution R*. The solution gives the reaction of magnesium (*2.3.1*).

TESTS
Solution S
Dissolve 5.0 g in 100 mL of *dilute acetic acid R*. When the effervescence has ceased, boil for 2 min, allow to cool and dilute to 100 mL with *dilute acetic acid R*. Filter, if necessary, through a previously ignited and tared porcelain or silica filter crucible of suitable porosity to give a clear filtrate.

Appearance of solution
Solution S is not more intensely coloured than reference solution B_4 (*2.2.2, Method II*).

Soluble substances
Maximum 1.0 per cent.

Mix 2.00 g with 100 mL of *water R* and boil for 5 min. Filter whilst hot through a sintered-glass filter (40) (*2.1.2*), allow to cool and dilute to 100 mL with *water R*. Evaporate 50 mL of the filtrate to dryness and dry at 100-105 °C. The residue weighs a maximum of 10 mg.

Substances insoluble in acetic acid
Maximum 0.05 per cent.

Any residue obtained during the preparation of solution S, washed, dried and ignited at 600 ± 50 °C, weighs a maximum of 2.5 mg.

Chlorides (*2.4.4*)
Maximum 700 ppm.

Dilute 1.5 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 0.3 per cent.

Dilute 1 mL of solution S to 15 mL with *distilled water R*.

Arsenic (*2.4.2, Method A*)
Maximum 2 ppm, determined on 10 mL of solution S.

Calcium (*2.4.3*)
Maximum 0.75 per cent.

Dilute 2.6 mL of solution S to 150 mL with *distilled water R*. 15 mL of the solution complies with the test.

Iron (*2.4.9*)
Maximum 400 ppm.

Dissolve 0.1 g in 3 mL of *dilute hydrochloric acid R* and dilute to 10 mL with *water R*. Dilute 2.5 mL of this solution to 10 mL with *water R*.

ASSAY

Dissolve 0.150 g in a mixture of 2 mL of *dilute hydrochloric acid R* and 20 mL of *water R*. Carry out the complexometric titration of magnesium (*2.5.11*).

1 mL of *0.1 M sodium edetate* is equivalent to 4.030 mg of MgO.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for light magnesium carbonate used as filler in oral solid dosage forms.

Particle-size distribution (*2.9.31* or *2.9.38*)

Bulk and tapped density (*2.9.34*)

Ph Eur

Magnesium Chloride Hexahydrate

(Ph. Eur. monograph 0402)

$MgCl_2,6H_2O$ 203.3 *7791-18-6*

Action and use
Used in the treatment of electrolyte deficiencies and in dialysis solutions.

Preparation
Magnesium Chloride Injection

Ph Eur

DEFINITION

Content
98.0 per cent to 101.0 per cent of $MgCl_2,6H_2O$.

CHARACTERS

Appearance
Colourless crystals, hygroscopic.

Solubility
Very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

A. Water (see Tests).

B. It gives reaction (a) of chlorides (*2.3.1*).

C. It gives the reaction of magnesium (*2.3.1*).

TESTS

Solution S
Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity
To 5 mL of solution S add 0.05 mL of *phenol red solution R*. Not more than 0.3 mL of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Bromides
Maximum 500 ppm.

Dilute 2.0 mL of solution S to 10.0 mL with *water R*. To 1.0 mL of this solution add 4.0 mL of *water R*, 2.0 mL of *phenol red solution R3* and 1.0 mL of *chloramine solution R2* and mix immediately. After exactly 2 min, add 0.30 mL of *0.1 M sodium thiosulfate*, mix and dilute to 10.0 mL with *water R*. The absorbance (*2.2.25*) of the solution measured at 590 nm, using *water R* as the compensation liquid, is not greater than that of a standard prepared at the same time and in the same manner using 5.0 mL of a 3 mg/L solution of *potassium bromide R*.

Sulfates (*2.4.13*)
Maximum 100 ppm, determined on solution S.

Aluminium (*2.4.17*)
Maximum 1 ppm, if intended for use in the manufacture of peritoneal dialysis solutions, haemodialysis solutions, or haemofiltration solutions.

Prescribed solution Dissolve 4 g in 100 mL of *water R* and add 10 mL of *acetate buffer solution pH 6.0 R*.

Reference solution Mix 2 mL of *aluminium standard solution (2 ppm Al) R*, 10 mL of *acetate buffer solution pH 6.0 R* and 98 mL of *water R*.

Blank solution Mix 10 mL of *acetate buffer solution pH 6.0 R* and 100 mL of *water R*.

Calcium (*2.4.3*)
Maximum 0.1 per cent.

Dilute 1 mL of solution S to 15 mL with *distilled water R*.

Iron (*2.4.9*)
Maximum 10 ppm, determined on solution S.

Potassium
Maximum 500 ppm, if intended for use in the manufacture of parenteral preparations.

Atomic emission spectrometry (*2.2.22, Method I*).

Test solution Dissolve 1.00 g in *water R* and dilute to 100.0 mL with the same solvent.

Reference solutions Prepare the reference solutions using the following solution, diluted as necessary with *water R*: dissolve 1.144 g of *potassium chloride R*, previously dried at 100-105 °C for 3 h in *water R* and dilute to 1000.0 mL with the same solvent (600 µg of K per millilitre).

Wavelength 766.5 nm.

Water (*2.5.12*)
51.0 per cent to 55.0 per cent, determined on 50.0 mg.

ASSAY

Dissolve 0.300 g in 50 mL of *water R*. Carry out the complexometric titration of magnesium (*2.5.11*).

1 mL of *0.1 M sodium edetate* is equivalent to 20.33 mg of $MgCl_2,6H_2O$.

STORAGE

In an airtight container.

LABELLING

The label states:

— where applicable, that the substance is suitable for use in the manufacture of peritoneal dialysis solutions, haemodialysis solutions or haemofiltration solutions,

— where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

Ph Eur

Partially Hydrated Magnesium Chloride

(Magnesium Chloride 4.5-Hydrate, Ph. Eur. monograph 1341)

$MgCl_2,xH_2O$ with $x \approx 4.5$ 95.21 (anhydrous substance)

Ph Eur

DEFINITION

Content

52.5 per cent to 55.5 per cent (calculated on an as-is basis, without allowing for the results of the test for water).

CHARACTERS

Appearance

White or almost white, hygroscopic, granular powder.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

A. Water (see Tests).

B. It gives reaction (a) of chlorides (*2.3.1*).

C. It gives the reaction of magnesium (*2.3.1*).

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100.0 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 5 mL of solution S add 0.05 mL of *phenol red solution R*. Not more than 0.3 mL of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Bromides

Maximum 500 ppm.

Dilute 2.0 mL of solution S to 10.0 mL with *water R*. To 1.0 mL of the solution add 4.0 mL of *water R*, 2.0 mL of *phenol red solution R3* and 1.0 mL of *chloramine solution R2* and mix immediately. After exactly 2 min, add 0.30 mL of *0.1 M sodium thiosulfate*, mix and dilute to 10.0 mL with *water R*. The absorbance (*2.2.25*) of the solution measured at 590 nm, using *water R* as the compensation liquid, is not greater than that of a standard prepared at the same time and in the same manner using 5.0 mL of a 3 mg/L solution of *potassium bromide R*.

Sulfates (*2.4.13*)

Maximum 100 ppm, determined on solution S.

Aluminium (*2.4.17*)

Maximum 1 ppm, if intended for use in the manufacture of peritoneal dialysis solutions, haemodialysis solutions, or haemofiltration solutions.

Prescribed solution Dissolve 4 g in 100 mL of *water R* and add 10 mL of *acetate buffer solution pH 6.0 R*.

Reference solution Mix 2 mL of *aluminium standard solution (2 ppm Al) R*, 10 mL of *acetate buffer solution pH 6.0 R* and 98 mL of *water R*.

Blank solution Mix 10 mL of *acetate buffer solution pH 6.0 R* and 100 mL of *water R*.

Arsenic (*2.4.2, Method A*)

Maximum 2 ppm, determined on 0.5 g.

Calcium (*2.4.3*)

Maximum 0.1 per cent.

Dilute 1 mL of solution S to 15 mL with *distilled water R*.

Iron (*2.4.9*)

Maximum 10 ppm, determined on solution S.

Potassium

Maximum 500 ppm, if intended for use in the manufacture of parenteral preparations.

Atomic emission spectrometry (*2.2.22, Method I*).

Test solution Dissolve 1.00 g in *water R* and dilute to 100.0 mL with the same solvent.

Reference solutions Prepare the reference solutions using the following solution, diluted as necessary with *water R*: dissolve 1.144 g of *potassium chloride R*, previously dried at 100-105 °C for 3 h, in *water R* and dilute to 1000.0 mL with the same solvent (600 µg of K per millilitre).

Wavelength 766.5 nm.

Water (*2.5.12*)

44.0 per cent to 48.0 per cent, determined on 50.0 mg.

ASSAY

Dissolve 0.250 g in 50 mL of *water R*. Carry out the complexometric titration of magnesium (*2.5.11*).

1 mL of *0.1 M sodium edetate* is equivalent to 9.521 mg of $MgCl_2$.

STORAGE

In an airtight container.

LABELLING

The label states:

— where applicable, that the substance is suitable for use in the manufacture of peritoneal dialysis solutions, haemodialysis solutions or haemofiltration solutions;

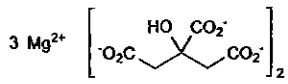
— where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

Ph Eur

Magnesium Citrate

Anhydrous Magnesium Citrate

(Ph. Eur. monograph 2339)



$Mg_3(C_6H_5O_7)_2$ 451.1 *3344-18-1*

Ph Eur

DEFINITION

Trimagnesium bis(2-hydroxypropane-1,2,3-tricarboxylate).

Content

15.0 per cent to 16.5 per cent of Mg (dried substance).

CHARACTERS

Appearance

White or almost white, fine, slightly hygroscopic powder.

Solubility

Soluble in water, practically insoluble in ethanol (96 per cent). It dissolves in dilute hydrochloric acid.

IDENTIFICATION

A. It gives the reaction of citrates (*2.3.1*).

B. It gives the reaction of magnesium (*2.3.1*).

C. pH (see Tests).

D. Loss on drying (see Tests).

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R*, heating at 60 °C, cool and dilute to 100 mL with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension III (*2.2.1*) and not more intensely coloured than reference solutions Y_7 or BY_6 (*2.2.2, Method II*).

pH (*2.2.3*)

6.0 to 8.5 for solution S.

Oxalates

Maximum 280 ppm.

Dissolve 0.50 g in 4 mL of *water R*. Add 3 mL of *hydrochloric acid R* and 1 g of *activated zinc R*. Allow to stand for 5 min. Transfer the liquid to a tube containing 0.25 mL of a 10 g/L solution of *phenylhydrazine hydrochloride R*. Heat to boiling. Cool rapidly, transfer to a graduated cylinder and add an equal volume of *hydrochloric acid R* and 0.25 mL of *potassium ferricyanide solution R*. Shake and allow to stand for 30 min. Any pink colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using 4 mL of a 50 mg/L solution of *oxalic acid R*.

Sulfates (*2.4.13*)

Maximum 0.2 per cent.

Dilute 1.5 mL of solution S to 15 mL with *distilled water R*.

Calcium (*2.4.3*)

Maximum 0.2 per cent.

Dilute 1.0 mL of solution S to 15 mL with *distilled water R*.

Iron (*2.4.9*)

Maximum 100 ppm.

Dilute 2.0 mL of solution S to 10 mL with *distilled water R*.

Loss on drying (*2.2.32*)

Maximum 3.5 per cent, determined on 1.000 g by drying in an oven at 180 ± 10 °C for 5 h.

ASSAY

Dissolve 0.150 g in 50 mL of *water R*. Carry out the complexometric titration of magnesium (*2.5.11*).

1 mL of *0.1 M sodium edetate* is equivalent to 2.431 mg of Mg.

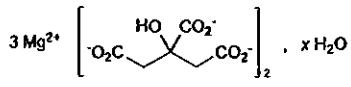
STORAGE

In a non-metallic, airtight container.

Ph Eur

Magnesium Citrate Dodecahydrate

(Ph. Eur. monograph 2401)



$Mg_3(C_6H_5O_7)_2,xH_2O$ with $x \approx 12$ 451.1 (anhydrous substance)

Ph Eur

DEFINITION

Trimagnesium bis(2-hydroxypropane-1,2,3-tricarboxylate) dodecahydrate.

Content

15.0 per cent to 16.5 per cent of Mg (dried substance).

CHARACTERS

Appearance

White or almost white, fine powder.

Solubility

Sparingly soluble in water, practically insoluble in ethanol (96 per cent). It dissolves in dilute hydrochloric acid.

IDENTIFICATION

A. It gives the reaction of citrates (*2.3.1*).

B. It gives the reaction of magnesium (*2.3.1*).

C. Loss on drying (see Tests).

TESTS

Solution S

Dissolve 2.5 g in 15 mL of *dilute hydrochloric acid R* with heating. Cool and dilute to 100 mL with *distilled water R*.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

pH (*2.2.3*)

6.0 to 8.5.

Disperse 5.0 g in *carbon dioxide-free water R* and dilute to 100 mL with the same solvent. Centrifuge and measure the pH of the clear supernatant.

Oxalates

Maximum 280 ppm.

Dissolve 0.50 g in a mixture of 3 mL of *hydrochloric acid R* and 4 mL of *water R* and add 1 g of *activated zinc R*. Allow to stand for 5 min. Transfer the liquid to a tube containing 0.25 mL of a 10 g/L solution of *phenylhydrazine hydrochloride R*. Heat to boiling. Cool rapidly, transfer to a graduated cylinder and add an equal volume of *hydrochloric acid R* and 0.25 mL of *potassium ferricyanide solution R*. Shake and allow to stand for 30 min. Any pink colour in the

solution is not more intense than that of a standard prepared at the same time and in the same manner using 4 mL of a 50 mg/L solution of *oxalic acid R*.

Sulfates (*2.4.13*)
Maximum 0.2 per cent.

Dilute 3.0 mL of solution S to 15 mL with *distilled water R*.

Calcium (*2.4.3*)
Maximum 0.2 per cent.

To a mixture of 2 mL of solution S and 8 mL of *distilled water R*, add about 0.2 mL of *ammonia R* and dilute to 15 mL with *distilled water R*.

Iron (*2.4.9*)
Maximum 100 ppm.

Dilute 4.0 mL of solution S to 10 mL with *distilled water R*.

Loss on drying (*2.2.32*)
29.0 per cent to 36.0 per cent, determined on 1.000 g by drying in an oven at 180 ± 10 °C for 5 h.

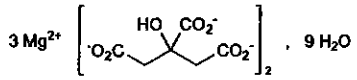
ASSAY
Dissolve 0.200 g in 5 mL of *dilute hydrochloric acid R* with heating. Cool and add 50 mL of *water R*. Adjust to pH 7.0 with *ammonia R*. Carry out the complexometric titration of magnesium (*2.5.11*).

1 mL of *0.1 M sodium edetate* is equivalent to 2.431 mg of Mg.

Ph Eur

Magnesium Citrate Nonahydrate

(Ph. Eur. monograph 2402)



$Mg_3(C_6H_5O_7)_2,9H_2O$ 613 *153531-96-5*

Ph Eur

DEFINITION
Trimagnesium bis(2-hydroxypropane-1,2,3-tricarboxylate) nonahydrate.

Content
15.0 per cent to 16.5 per cent of Mg (dried substance).

CHARACTERS
Appearance
White or almost white, fine powder.

Solubility
Sparingly soluble in water, practically insoluble in ethanol (96 per cent). It dissolves in dilute hydrochloric acid.

IDENTIFICATION
A. It gives the reaction of citrates (*2.3.1*).

B. It gives the reaction of magnesium (*2.3.1*).

C. Loss on drying (see Tests).

TESTS
Solution S
Dissolve 2.5 g in 15 mL of *dilute hydrochloric acid R* with heating. Cool and dilute to 100 mL with *distilled water R*.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

pH (*2.2.3*)
6.0 to 8.5.

Disperse 5.0 g in *carbon dioxide-free water R* and dilute to 100 mL with the same solvent. Centrifuge and measure the pH of the clear supernatant.

Oxalates
Maximum 280 ppm.

Dissolve 0.50 g in a mixture of 3 mL of *hydrochloric acid R* and 4 mL of *water R* and add 1 g of *activated zinc R*. Allow to stand for 5 min. Transfer the liquid to a tube containing 0.25 mL of a 10 g/L solution of *phenylhydrazine hydrochloride R*. Heat to boiling. Cool rapidly, transfer to a graduated cylinder and add an equal volume of *hydrochloric acid R* and 0.25 mL of *potassium ferricyanide solution R*. Shake and allow to stand for 30 min. Any pink colour in the solution is not more intense than that of a standard prepared at the same time and in the same manner using 4 mL of a 50 mg/L solution of *oxalic acid R*.

Sulfates (*2.4.13*)
Maximum 0.2 per cent.

Dilute 3.0 mL of solution S to 15 mL with *distilled water R*.

Calcium (*2.4.3*)
Maximum 0.2 per cent.

To a mixture of 2 mL of solution S and 8 mL of *distilled water R*, add about 0.2 mL of *ammonia R* and dilute to 15 mL with *distilled water R*.

Iron (*2.4.9*)
Maximum 100 ppm.

Dilute 4.0 mL of solution S to 10 mL with *distilled water R*.

Loss on drying (*2.2.32*)
24.0 to 28.0 per cent, determined on 1.000 g by drying in an oven at 180 ± 10 °C for 5 h.

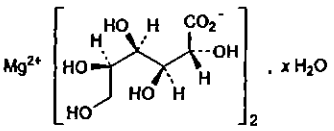
ASSAY
Dissolve 0.200 g in 5 mL of *dilute hydrochloric acid R* with heating. Cool and add 50 mL of *water R*. Adjust to pH 7.0 with *ammonia R*. Carry out the complexometric titration of magnesium (*2.5.11*).

1 mL of *0.1 M sodium edetate* is equivalent to 2.431 mg of Mg.

Ph Eur

Magnesium Gluconate

(Ph. Eur. monograph 2161)



$C_{12}H_{22}MgO_{14},xH_2O$ 414.6
(anhydrous substance)

Ph Eur

DEFINITION
Anhydrous or hydrated magnesium bis[(2*R*,3*S*,4*R*,5*R*)-2,3,4,5,6-pentahydroxyhexanoate] (anhydrous or hydrated magnesium di(D-gluconate)).

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, amorphous, hygroscopic, crystalline or granular powder.

Solubility
Freely soluble in water, slightly soluble in ethanol (96 per cent), very slightly soluble in methylene chloride.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 20 mg of the substance to be examined in 1 mL of *water R*.

Reference solution Dissolve 20 mg of *calcium gluconate CRS* in 1 mL of *water R*, heating if necessary in a water-bath at 60 °C.

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *concentrated ammonia R, ethyl acetate R, water R, ethanol (96 per cent) R* (10:10:30:50 *V/V/V/V*).

Application 1 µL.

Development Over 3/4 of the plate.

Drying At 105 °C for 20 min, then allow to cool to room temperature.

Detection Spray with a solution containing 25 g/L of *ammonium molybdate R* and 10 g/L of *cerium sulfate R* in *dilute sulfuric acid R*, then heat at 105 °C for about 10 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

B. To 10 mL of solution S (see Tests) add 3 mL of *ammonium chloride solution R*. A slight opalescence may be observed. Add 10 mL of *disodium hydrogen phosphate solution R*. A white precipitate is formed that does not dissolve upon the addition of 2 mL of *dilute ammonia R1*.

TESTS

Solution S
Dissolve 1.0 g in *water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

Sucrose and reducing sugars
Dissolve 0.5 g in a mixture of 2 mL of *hydrochloric acid R1* and 10 mL of *water R*. Boil for 5 min, allow to cool, add 10 mL of *sodium carbonate solution R* and allow to stand for 10 min. Dilute to 25 mL with *water R* and filter. To 5 mL of the filtrate add 2 mL of *cupri-tartaric solution R* and boil for 1 min. Allow to stand for 2 min. No red precipitate is formed.

Chlorides (*2.4.4*)
Maximum 500 ppm.

Dilute 5 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 500 ppm.

Dissolve 2.0 g in a mixture of 10 mL of *acetic acid R* and 90 mL of *distilled water R*.

Water (*2.5.32*)
Maximum 12.0 per cent, determined on 80 mg.

Microbial contamination
TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*).

TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

ASSAY

Dissolve 0.350 g in 100 mL of *water R* and carry out the complexometric titration of magnesium (*2.5.11*).

1 mL of *0.1 M sodium edetate* is equivalent to 41.46 mg of $C_{12}H_{22}MgO_{14}$.

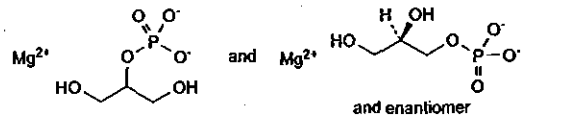
STORAGE

In an airtight container.

Ph Eur

Magnesium Glycerophosphate

(Ph. Eur. monograph 1446)



$C_3H_7MgO_6P$ 194.4

Action and use
Excipient.

Preparations
Magnesium Glycerophosphate Chewable Tablets
Magnesium Glycerophosphate Oral Solution

Ph Eur

DEFINITION

Mixture, in variable proportions, of magnesium salts of (*RS*)-2,3-dihydroxypropyl phosphate and 2-hydroxy-1-(hydroxymethyl)ethyl phosphate, which may be hydrated.

Content
11.0 per cent to 12.5 per cent of Mg (dried substance).

CHARACTERS

Appearance
White or almost white powder, hygroscopic.

Solubility
Practically insoluble in ethanol (96 per cent). It dissolves in dilute solutions of acids.

IDENTIFICATION

A. Mix 1 g with 1 g of *potassium hydrogen sulfate R* in a test tube fitted with a glass tube. Heat strongly and direct the white vapour towards a piece of filter paper impregnated with a freshly prepared 10 g/L solution of *sodium nitroprusside R*. The filter paper develops a blue colour in contact with *piperidine R*.

B. Ignite 0.1 g in a crucible. Take up the residue with 5 mL of *nitric acid R* and heat on a water-bath for 1 min. Filter. The filtrate gives reaction (b) of phosphates (*2.3.1*).

C. It gives the reaction of magnesium (*2.3.1*).

TESTS

Solution S
Dissolve 2.5 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is not more opalescent than reference suspension III (*2.2.1*).

Acidity
Dissolve 1.0 g in 100 mL of *carbon dioxide-free water R*. Add 0.1 mL of *phenolphthalein solution R*. Not more than

1.5 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Glycerol and ethanol (96 per cent)-soluble substances
Maximum 1.5 per cent.

Shake 1.0 g with 25 mL of *ethanol (96 per cent) R* for 2 min. Filter and wash the residue with 5 mL of *ethanol (96 per cent) R*. Combine the filtrate and the washings, evaporate to dryness on a water-bath and dry the residue at 70 °C for 1 h. The residue weighs a maximum of 15 mg.

Chlorides (*2.4.4*)
Maximum 0.15 per cent.

Dissolve 1.0 g in *water R* and dilute to 100 mL with the same solvent. Dilute 3.5 mL of this solution to 15 mL with *water R*.

Phosphates (*2.4.11*)
Maximum 0.5 per cent.

Dilute 4 mL of solution S to 100 mL with *water R*. Dilute 1 mL of this solution to 100 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 0.1 per cent.

Dilute 3 mL of solution S to 15 mL with *distilled water R*.

Iron (*2.4.9*)
Maximum 150 ppm.

Dissolve 67 mg in *water R* and dilute to 10 mL with the same solvent.

Loss on drying (*2.2.32*)
Maximum 12.0 per cent, determined on 1.000 g by drying in an oven at 150 °C for 4 h.

ASSAY
Dissolve 0.200 g in 40 mL of *water R*. Carry out the complexometric titration of magnesium (*2.5.11*).

1 mL of *0.1 M sodium edetate* is equivalent to 2.431 mg of Mg.

STORAGE
In an airtight container.

Ph Eur

Magnesium Hydroxide

(Ph. Eur. monograph 0039)

$Mg(OH)_2$ 58.32 *1309-42-8*

Action and use
Antacid; osmotic laxative.

Preparations
Co-magaldrox Oral Suspension

Co-magaldrox Tablets

Magnesium Hydroxide Mixture

Ph Eur

DEFINITION
Content
95.0 per cent to 100.5 per cent of $Mg(OH)_2$.

CHARACTERS
Appearance
White or almost white, fine, amorphous powder.

Solubility
Practically insoluble in water. It dissolves in dilute acids.

IDENTIFICATION
A. Dissolve about 15 mg in 2 mL of *dilute nitric acid R* and neutralise with *dilute sodium hydroxide solution R*. The solution gives the reaction of magnesium (*2.3.1*).

B. Loss on ignition (see Tests).

TESTS
Solution S
Dissolve 5.0 g in a mixture of 50 mL of *acetic acid R* and 50 mL of *distilled water R*. Not more than slight effervescence is produced. Boil for 2 min, cool and dilute to 100 mL with *dilute acetic acid R*. Filter, if necessary, through a previously ignited and tared porcelain or silica filter crucible of suitable porosity to give a clear filtrate. Keep the residue for the test for substances insoluble in acetic acid.

Appearance of solution
Solution S is not more intensely coloured than reference solution B_3 (*2.2.2, Method II*).

Soluble substances
Maximum 2.0 per cent.

Mix 2.00 g with 100 mL of *water R* and boil for 5 min. Filter whilst hot through a sintered-glass filter (40) (*2.1.2*), allow to cool and dilute to 100 mL with *water R*. Evaporate 50 mL of the filtrate to dryness and dry at 100-105 °C. The residue weighs a maximum of 20 mg.

Substances insoluble in acetic acid
Maximum 0.1 per cent.

Any residue obtained during the preparation of solution S, washed, dried, and ignited at 600 ± 50 °C, weighs not more than 5 mg.

Chlorides (*2.4.4*)
Maximum 0.1 per cent.

Dilute 1 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 1.0 per cent.

Dilute 0.3 mL of solution S to 15 mL with *distilled water R*.

Calcium (*2.4.3*)
Maximum 1.5 per cent.

Dilute 1.3 mL of solution S to 150 mL with *distilled water R*.

Iron (*2.4.9*)
Maximum 0.07 per cent.

Dissolve 0.15 g in 5 mL of *dilute hydrochloric acid R* and dilute to 10 mL with *water R*. Dilute 1 mL of the solution to 10 mL with *water R*.

Loss on ignition
29.0 per cent to 32.5 per cent.

Heat 0.5 g gradually to 900 ± 50 °C and ignite to constant mass.

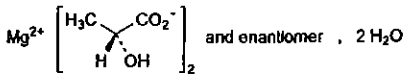
ASSAY
Dissolve 0.100 g in a mixture of 2 mL of *dilute hydrochloric acid R* and 20 mL of *water R*. Carry out the complexometric titration of magnesium (*2.5.11*).

1 mL of *0.1 M sodium edetate* is equivalent to 5.832 mg of $Mg(OH)_2$.

Ph Eur

Magnesium Lactate Dihydrate

(Ph. Eur. monograph 2160)



$C_6H_{10}MgO_6,2H_2O$ 238.5

Ph Eur

DEFINITION

Magnesium bis(2-hydroxypropanoate) or mixture of magnesium (2*R*)-, (2*S*)- and (2*RS*)-2-hydroxypropanoate dihydrate.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline or granular powder.

Solubility
Slightly soluble in water, soluble in boiling water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. It gives the reaction of lactates (*2.3.1*).

B. It gives the reaction of magnesium (*2.3.1*).

TESTS

Solution S
Dissolve 5.0 g with heating in *carbon dioxide-free water R* prepared from *distilled water R*, allow to cool and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

pH (*2.2.3*)
6.5 to 8.5 for solution S.

Chlorides (*2.4.4*)
Maximum 200 ppm.

Dilute 5 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 400 ppm.

Dilute 7.5 mL of solution S to 15 mL with *distilled water R*.

Iron (*2.4.9*)
Maximum 50 ppm.

Dilute 4 mL of solution S to 10 mL with *water R*.

Loss on drying (*2.2.32*)
14.0 per cent to 17.0 per cent, determined on 0.500 g by drying in an oven at 125 °C.

ASSAY

Dissolve 0.180 g in *water R* and dilute to 300 mL with the same solvent. Carry out the complexometric titration of magnesium (*2.5.11*).

1 mL of *0.1 M sodium edetate* is equivalent to 20.25 mg of $C_6H_{10}MgO_6$.

Ph Eur

Heavy Magnesium Oxide

(Ph. Eur. monograph 0041)

MgO 40.30 *1309-48-4*

Action and use
Antacid; osmotic laxative.

Ph Eur

DEFINITION

Content
98.0 per cent to 100.5 per cent of MgO (ignited substance).

CHARACTERS

Appearance
Fine, white or almost white powder.

Solubility
Practically insoluble in water. It dissolves in dilute acids with at most slight effervescence.

IDENTIFICATION

A. Bulk density (*2.9.34*): minimum 0.25 g/mL.

B. Dissolve about 15 mg in 2 mL of *dilute nitric acid R* and neutralise with *dilute sodium hydroxide solution R*. The solution gives the reaction of magnesium (*2.3.1*).

C. Loss on ignition (see Tests).

TESTS

Solution S
Dissolve 5.0 g in a mixture of 30 mL of *distilled water R* and 70 mL of *acetic acid R*, boil for 2 min, allow to cool and dilute to 100 mL with *dilute acetic acid R*. Filter, if necessary, through a previously ignited and tared porcelain or silica filter crucible of suitable porosity to give a clear filtrate. Keep the residue for the test for substances insoluble in acetic acid.

Appearance of solution
Solution S is not more intensely coloured than reference solution B_3 (*2.2.2, Method II*).

Soluble substances
Maximum 2.0 per cent.

To 2.00 g add 100 mL of *water R* and boil for 5 min. Filter whilst hot through a sintered-glass filter (40) (*2.1.2*), allow to cool and dilute to 100 mL with *water R*. Evaporate 50 mL of the filtrate to dryness and dry at 100-105 °C. The residue weighs a maximum of 20 mg.

Substances insoluble in acetic acid
Maximum 0.1 per cent.

Any residue obtained during the preparation of solution S, washed, dried and ignited at 600 ± 50 °C, weighs a maximum of 5 mg.

Chlorides (*2.4.4*)
Maximum 0.1 per cent.

Dilute 1 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 1.0 per cent.

Dilute 0.3 mL of solution S to 15 mL with *distilled water R*.

Calcium (*2.4.3*)
Maximum 1.5 per cent.

Dilute 1.3 mL of solution S to 150 mL with *distilled water R*. 15 mL of the solution complies with the test.

Iron (*2.4.9*)
Maximum 0.07 per cent.

Dissolve 0.15 g in 5 mL of *dilute hydrochloric acid R* and dilute to 10 mL with *water R*. Dilute 1 mL of the solution to 10 mL with *water R*.

Loss on ignition
Maximum 8.0 per cent, determined on 1.00 g at 900 ± 25 °C.

ASSAY
Dissolve 0.320 g in 20 mL of *dilute hydrochloric acid R* and dilute to 100.0 mL with *water R*. Using 20.0 mL of the solution, carry out the complexometric titration of magnesium (*2.5.11*).

1 mL of *0.1 M sodium edetate* is equivalent to 4.030 mg of MgO.

FUNCTIONALITY-RELATED CHARACTERISTICS
This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for heavy magnesium oxide used as filler in oral solid dosage forms.

Particle-size distribution (*2.9.31* or *2.9.38*)

Bulk and tapped density (*2.9.34*)

Ph Eur

Light Magnesium Oxide

Light Magnesia

(Ph. Eur. monograph 0040)

MgO	40.30	*1309-48-4*

Action and use
Antacid; osmotic laxative.

Preparation
Compound Sodium Picosulfate Powder for Oral Solution

Ph Eur

DEFINITION
Content
98.0 per cent to 100.5 per cent of MgO (ignited substance).

CHARACTERS
Appearance
Fine, white or almost white, amorphous powder.

Solubility
Practically insoluble in water. It dissolves in dilute acids with at most slight effervescence.

IDENTIFICATION
A. Bulk density (*2.9.34*): maximum 0.15 g/mL.

B. Dissolve about 15 mg in 2 mL of *dilute nitric acid R* and neutralise with *dilute sodium hydroxide solution R*. The solution gives the reaction of magnesium (*2.3.1*).

C. Loss on ignition (see Tests).

TESTS
Solution S
Dissolve 5.0 g in a mixture of 30 mL of *distilled water R* and 70 mL of *acetic acid R*, boil for 2 min, allow to cool and dilute to 100 mL with *dilute acetic acid R*. Filter, if necessary, through a previously ignited and tared porcelain or silica filter crucible of a suitable porosity to give a clear filtrate. Keep the residue for the test for substances insoluble in acetic acid.

Appearance of solution
Solution S is not more intensely coloured than reference solution B_2 (*2.2.2, Method II*).

Soluble substances
Maximum 2.0 per cent.

To 2.00 g add 100 mL of *water R* and boil for 5 min. Filter whilst hot through a sintered-glass filter (40) (*2.1.2*), allow to cool and dilute to 100 mL with *water R*. Evaporate 50 mL of the filtrate to dryness and dry at 100-105 °C. The residue weighs a maximum of 20 mg.

Substances insoluble in acetic acid
Maximum 0.1 per cent.

Any residue obtained during the preparation of solution S, washed, dried, and ignited at 600 ± 50 °C, weighs a maximum of 5 mg.

Chlorides (*2.4.4*)
Maximum 0.15 per cent.

Dilute 0.7 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 1.0 per cent.

Dilute 0.3 mL of solution S to 15 mL with *distilled water R*.

Calcium (*2.4.3*)
Maximum 1.5 per cent.

Dilute 1.3 mL of solution S to 150 mL with *distilled water R*. 15 mL of this solution complies with the test.

Iron (*2.4.9*)
Maximum 0.1 per cent.

Dissolve 50 mg in 5 mL of *dilute hydrochloric acid R* and dilute to 10 mL with *water R*. Dilute 2 mL of this solution to 10 mL with *water R*.

Loss on ignition
Maximum 8.0 per cent, determined on 1.00 g at 900 ± 25 °C.

ASSAY
Dissolve 0.320 g in 20 mL of *dilute hydrochloric acid R* and dilute to 100.0 mL with *water R*. Using 20.0 mL of this solution, carry out the complexometric titration of magnesium (*2.5.11*).

1 mL of *0.1 M sodium edetate* is equivalent to 4.030 mg of MgO.

FUNCTIONALITY-RELATED CHARACTERISTICS
This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related

characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for light magnesium oxide used as filler in oral solid dosage forms.

Particle-size distribution (*2.9.31* or *2.9.38*)

Bulk and tapped density (*2.9.34*)

Ph Eur

Magnesium Peroxide

(Ph. Eur. monograph 1540)

Ph Eur

DEFINITION

Mixture of magnesium peroxide and magnesium oxide.

Content

22.0 per cent to 28.0 per cent of MgO_2 (M_r 56.30).

CHARACTERS

Appearance

White or slightly yellow, amorphous, light powder.

Solubility

Practically insoluble in water and in ethanol (96 per cent). It dissolves in dilute mineral acids.

IDENTIFICATION

A. Dissolve about 15 mg in 2 mL of *dilute nitric acid R* and neutralise with *dilute sodium hydroxide solution R*. The solution gives the reaction of magnesium (*2.3.1*).

B. Dissolve 50 mg in 2 mL of *dilute sulfuric acid R*. Add 2 mL of a 5 g/L solution of *potassium permanganate R* and shake. The solution becomes colourless with evolution of gas.

TESTS

Solution S1

Dissolve cautiously 5.0 g in 40 mL of *hydrochloric acid R1*. Cautiously evaporate the solution to 10 mL and dilute to 100 mL with a mixture of equal volumes of *acetic acid R* and *distilled water R*. Filter, if necessary, through a previously ignited and tared porcelain or silica filter crucible of suitable porosity to give a clear filtrate. Keep the residue for the test for acid insoluble substances.

Solution S2

Dilute 5 mL of solution S1 to 25 mL with *distilled water R*.

Appearance of solution

Solution S1 is not more intensely coloured than reference solution B_4 (*2.2.2, Method II*).

Acidity or alkalinity

To 2.0 g add 100 mL of *carbon dioxide-free water R* and heat to boiling for 5 min. Filter whilst hot through a sintered-glass filter (40) (*2.1.2*), allow to cool and dilute to 100 mL with *carbon dioxide-free water R*. To 15 mL of the filtrate, add 0.1 mL of *phenolphthalein solution R*. The solution is red. Not more than 0.2 mL of *0.1 M hydrochloric acid* is necessary to change the colour of the indicator. Keep the filtrate for the test for soluble substances.

Acid insoluble substances

Maximum 0.1 per cent.

Any residue obtained during the preparation of solution S1, washed, dried and ignited at 600 ± 50 °C, weighs a maximum of 5 mg.

Soluble substances

Maximum 1.5 per cent.

Take 50 mL of the filtrate obtained in the test for acidity or alkalinity, evaporate to dryness and dry at 100-105 °C. The residue weighs a maximum of 15 mg.

Chlorides (*2.4.4*)

Maximum 0.1 per cent.

Dissolve 50 mg in 5 mL of *dilute nitric acid R* and dilute to 15 mL with *water R*.

Sulfates (*2.4.13*)

Maximum 0.5 per cent.

Dilute 3 mL of solution S2 to 15 mL with *distilled water R*.

Arsenic (*2.4.2, Method A*)

Maximum 4 ppm, determined on 5 mL of solution S1.

Calcium (*2.4.3*)

Maximum 1.0 per cent.

Dilute 1 mL of solution S2 to 15 mL with *distilled water R*.

Iron (*2.4.9*)

Maximum 500 ppm.

Dilute 2 mL of solution S2 to 10 mL with *water R*.

ASSAY

Dissolve 80.0 mg, shaking cautiously, in a mixture, previously cooled to 20 °C, of 10 mL of *sulfuric acid R* and 90 mL of *water R*. Titrate with *0.02 M potassium permanganate* until a pink colour is obtained.

1 mL of *0.02 M potassium permanganate* is equivalent to 2.815 mg of MgO_2.

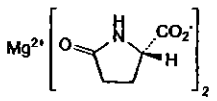
STORAGE

Protected from light.

Ph Eur

Magnesium Pidolate

(Ph. Eur. monograph 1619)



$C_{10}H_{12}MgN_2O_6$ 280.5 *62003-27-4*

Ph Eur

DEFINITION

Magnesium bis[(2*S*)-5-oxopyrrolidine-2-carboxylate].

Content

8.49 per cent to 8.84 per cent of Mg (A_r = 24.31) (anhydrous substance).

CHARACTERS

Appearance

Amorphous, white or almost white powder, hygroscopic.

Solubility

Very soluble in water, soluble in methanol, practically insoluble in methylene chloride.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 60 mg of the substance to be examined in 2 mL of *water R* and dilute to 10 mL with *methanol R*.

Reference solution Dissolve 55 mg of *pidolic acid CRS* in 2 mL of *water R* and dilute to 10 mL with *methanol R*.

Plate *TLC silica gel plate R*.

Mobile phase *methanol R, glacial acetic acid R, methylene chloride R* (15:20:65 *V/V/V*).

Application 1 µL.

Development Over 2/3 of the plate.

Drying At 100-105 °C for 15 min.

Detection Spray with *strong sodium hypochlorite solution R*. Allow to stand for 10 min and spray abundantly with *glacial acetic acid R*. Allow to stand again for 10 min and dry at 100-105 °C for 2 min. Spray with *potassium iodide and starch solution R* until spots appear.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution. The chromatogram obtained with the test solution may show 2 faint secondary spots.

B. To 0.15 mL of solution S (see Tests) add 1.8 mL of *water R*. The solution gives the reaction of magnesium (*2.3.1*).

TESTS

Solution S

Dissolve 5.00 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50.0 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_8 (*2.2.2, Method I*).

pH (*2.2.3*)

5.5 to 7.0 for solution S.

Specific optical rotation (*2.2.7*)

−26.5 to −23.3 (anhydrous substance), determined on solution S.

Impurity A

Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.250 g of the substance to be examined in 4 mL of *water R* and dilute to 50.0 mL with *methanol R*.

Reference solution (a) Dissolve 60.0 mg of *glutamic acid R* in 50 mL of *water R* and dilute to 100.0 mL with *methanol R*. Dilute 1.0 mL of the solution to 20.0 mL with *methanol R*.

Reference solution (b) Dissolve 10 mg of *aspartic acid R* and 10 mg of *glutamic acid R* in *water R* and dilute to 25 mL with the same solvent. Dilute 1 mL of the solution to 10 mL with *water R*.

Plate *TLC silica gel plate R*.

Mobile phase *glacial acetic acid R, water R, butanol R* (20:20:60 *V/V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *ninhydrin solution R* and heat at 100-105 °C for 15 min.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Limit:

— *impurity A*: any spot due to impurity A is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.6 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.500 g of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve 50.0 mg of *pidolate impurity B CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 5.0 mL of the solution to 50.0 mL with the mobile phase.

Reference solution (c) Dilute 10.0 mL of reference solution (b) to 100.0 mL with the mobile phase.

Reference solution (d) Dilute 1.0 mL of *nitrate standard solution (100 ppm NO_3) R* to 100.0 mL with the mobile phase.

Reference solution (e) Dilute 6.0 mL of reference solution (a) to 10.0 mL with reference solution (b).

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 1.56 g of *sodium dihydrogen phosphate R* in 1000 mL of *water for chromatography R* and adjust to pH 2.5 with a 10 per cent *V/V* solution of *phosphoric acid R*.

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µL of the test solution and reference solutions (b), (c), (d) and (e).

Run time 4 times the retention time of pidolic acid.

Retention times Pidolic acid = about 4.5 min; impurity B = about 7.5 min.

System suitability Reference solution (e):

— *resolution*: minimum 10 between the peaks due to pidolic acid and impurity B.

Limits:

— *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);

— *total of other impurities*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent); disregard any peak due to the nitrate ion (NO_3^-).

Chlorides (*2.4.4*)

Maximum 500 ppm.

Dilute 1.0 mL of solution S to 15.0 mL with *water R*.

Nitrates

Examine the chromatogram obtained with the test solution in the test for related substances.

Limit:
— *nitrates*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (200 ppm).

Sulfates (*2.4.13*)
Maximum 0.1 per cent.

Dilute 1.5 mL of solution S to 15.0 mL with *distilled water R*.

Iron (*2.4.9*)
Maximum 200 ppm.

Dilute 0.5 mL of solution S to 10 mL with *water R*.

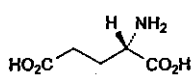
Water (*2.5.12*)
Maximum 8.0 per cent, determined on 0.200 g.

ASSAY
Dissolve 0.300 g in 50 mL of *water R*. Carry out the complexometric titration of magnesium (*2.5.11*).

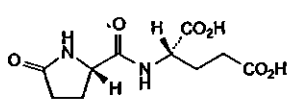
1 mL of *0.1 M sodium edetate* is equivalent to 2.431 mg of Mg.

STORAGE
In an airtight container.

IMPURITIES
Specified impurities *A, B.*



A. (2*S*)-2-aminopentanedioic acid (glutamic acid),



B. (2*S*)-2-[(2*S*)-5-oxopyrrolidine-2-carboxamido] pentanedioic acid.

Ph Eur

Magnesium Stearate[1]

(Ph. Eur. monograph 0229)

Action and use
Excipient.

Ph Eur

DEFINITION
Compound of magnesium with a mixture of solid organic acids and consisting mainly of variable proportions of magnesium stearate and magnesium palmitate obtained from sources of vegetable or animal origin.

Content
— *magnesium* (Mg; A_r 24.305): 4.0 per cent to 5.0 per cent (dried substance);
— *stearic acid in the fatty acid fraction*: minimum 40.0 per cent;
— *sum of stearic acid and palmitic acid in the fatty acid fraction*: minimum 90.0 per cent.

♦CHARACTERS
Appearance
White or almost white, very fine, light powder, greasy to the touch.

Solubility
Practically insoluble in water and in anhydrous ethanol.♦

IDENTIFICATION
First identification: C, D.

◊Second identification: A, B, D.

A. Freezing point (*2.2.18*): minimum 53 °C, determined on the residue obtained in the preparation of solution S (see Tests).

B. Acid value (*2.5.1*): 195 to 210.

Dissolve 0.200 g of the residue obtained in the preparation of solution S in 25 mL of the prescribed mixture of solvents.◊

C. Examine the chromatograms obtained in the assay of stearic acid and palmitic acid.

Results The 2 principal peaks in the chromatogram obtained with the test solution are similar in retention time to the 2 principal peaks in the chromatogram obtained with the reference solution.

D. To 1 mL of solution S add 1 mL of *dilute ammonia R1*; a white precipitate is formed that dissolves on addition of 1 mL of *ammonium chloride solution R*. Add 1 mL of a 120 g/L solution of *disodium hydrogen phosphate dodecahydrate R*; a white crystalline precipitate is formed.

TESTS
Solution S
To 5.0 g add 50 mL of *peroxide-free ether R*, 20 mL of *dilute nitric acid R* and 20 mL of *water R* and heat under a reflux condenser until dissolution is complete. Allow to cool. In a separating funnel, separate the aqueous layer and shake the ether layer with 2 quantities, each of 4 mL, of *water R*. Combine the aqueous layers, wash with 15 mL of *peroxide-free ether R* and dilute to 50.0 mL with *water R* (solution S). Evaporate the organic layer to dryness and dry the residue at 100-105 °C. Keep the residue for identification tests A and B.

Acidity or alkalinity
To 1.0 g add 20 mL of *carbon dioxide-free water R* and boil for 1 min with continuous shaking. Cool and filter.
To 10 mL of the filtrate add 0.05 mL of *bromothymol blue solution R4*. Not more than 0.05 mL of *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Chlorides
Maximum 0.1 per cent.

Dilute 10.0 mL of solution S to 40 mL with *water R*. Neutralise if necessary with *nitric acid R* using *litmus R* as indicator. Add 1 mL of *nitric acid R* and 1 mL of *0.1 M silver nitrate* and dilute to 50 mL with *water R*. Mix and allow to stand for 5 min protected from light. The turbidity, if any, is not greater than that produced in a solution containing 1.4 mL of *0.02 M hydrochloric acid*.

Sulfates
Maximum 1.0 per cent.

Dilute 6.0 mL of solution S to 40 mL with *water R*. Neutralise if necessary with *hydrochloric acid R* using *litmus R* as indicator. Add 1 mL of *3 M hydrochloric acid R* and 3 mL of a 120 g/L solution of *barium chloride R* and dilute to 50 mL with *water R*. Mix and allow to stand for 10 min.

[1] *This monograph has undergone pharmacopoeial harmonisation. See chapter 5.8 Pharmacopoeial harmonisation.*

The turbidity, if any, is not greater than that produced in a solution containing 3.0 mL of *0.02 M sulfuric acid.*

Cadmium

Maximum 3 ppm.

Atomic absorption spectrometry (*2.2.23, Method II*).

For the preparation of all aqueous solutions and for the rinsing of glassware before use, employ water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a content of cadmium, lead and nickel as practicable and store all reagent solutions in containers of borosilicate glass. Clean glassware before use by soaking in a warm 773 g/L solution of nitric acid R for 30 min and by rinsing with deionised water.

Blank solution Dilute 25 mL of *cadmium- and lead-free nitric acid R* to 100.0 mL with *water R*.

Modifier solution Dissolve 20 g of *ammonium dihydrogen phosphate R* and 1 g of *magnesium nitrate R* in *water R* and dilute to 100 mL with the same solvent. Alternatively, use an appropriate matrix modifier as recommended by the graphite furnace atomic absorption (GFAA) spectrometer manufacturer.

Test solution Place 0.100 g of the substance to be examined in a polytetrafluoroethylene digestion bomb and add 2.5 mL of *cadmium- and lead-free nitric acid R*. Close and seal the bomb according to the manufacturer's operating instructions *(when using a digestion bomb, be thoroughly familiar with the safety and operating instructions. Carefully follow the bomb manufacturer's instructions regarding care and maintenance of these digestion bombs. Do not use metal jacketed bombs or liners which have been used with hydrochloric acid due to contamination from corrosion of the metal jacket by hydrochloric acid)*. Heat the bomb in an oven at 170 °C for 3 h. Cool the bomb slowly in air to room temperature according to the bomb manufacturer's instructions. Place the bomb in a fume cupboard and open carefully as corrosive gases may be expelled. Dissolve the residue in *water R* and dilute to 10.0 mL with the same solvent.

Reference solution Prepare a solution of 0.0030 µg/mL of Cd by suitable dilutions of a 0.00825 µg/mL solution of *cadmium nitrate tetrahydrate R* in the blank solution.

Dilute 1.0 mL of the test solution to 10.0 mL with the blank solution. Prepare mixtures of this solution, the reference solution and the blank solution in the following proportions: (1.0:0:1.0 *V/V/V*), (1.0:0.5:0.5 *V/V/V*), (1.0:1.0:0 *V/V/V*). To each mixture add 50 µL of modifier solution and mix. These solutions contain respectively 0 µg, 0.00075 µg and 0.0015 µg of cadmium per millilitre from the reference solution (keep the remaining test solution for use in the test for lead and nickel).

Source Cadmium hollow-cathode lamp.

Wavelength 228.8 nm.

Atomisation device Furnace.

Platform Pyrolytically coated with integrated tube.

Operating conditions Use the temperature programme recommended for cadmium by the GFAA spectrometer manufacturer. An example of temperature parameters for GFAA analysis of cadmium is shown below.

Stage	Final temperature (°C)	Ramp time (s)	Hold time (s)
Drying	110	10	20
Ashing	600	10	30
Atomisation	1800	0	5

Lead

Maximum 10 ppm.

Atomic absorption spectrometry (*2.2.23, Method II*).

For the preparation of all aqueous solutions and for the rinsing of glassware before use, employ water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a content of cadmium, lead and nickel as practicable and store all reagent solutions in containers of borosilicate glass. Clean glassware before use by soaking in a warm 773 g/L solution of nitric acid R for 30 min and by rinsing with deionised water.

Blank solution Use the solution described in the test for cadmium.

Modifier solution Use the solution described in the test for cadmium.

Test solution Use the solution described in the test for cadmium.

Reference solution Prepare a solution of 0.100 µg/mL of Pb by suitable dilutions of *lead standard solution (100 ppm Pb) R* with the blank solution.

Prepare mixtures of the test solution, the reference solution and the blank solution in the following proportions: (1.0:0:1.0 *V/V/V*), (1.0:0.5:0.5 *V/V/V*), (1.0:1.0:0 *V/V/V*). To each mixture add 50 µL of modifier solution and mix. These solutions contain respectively 0 µg, 0.025 µg and 0.05 µg of lead per millilitre from the reference solution.

Source Lead hollow-cathode lamp.

Wavelength 283.3 nm.

Atomisation device Furnace.

Platform Pyrolytically coated with integrated tube.

Operating conditions Use the temperature programme recommended for lead by the GFAA spectrometer manufacturer. An example of temperature parameters for GFAA analysis of lead is shown below.

Stage	Final temperature (°C)	Ramp time (s)	Hold time (s)
Drying	110	10	20
Ashing	450	10	30
Atomisation	2000	0	5

Nickel

Maximum 5 ppm.

Atomic absorption spectrometry (*2.2.23, Method II*).

For the preparation of all aqueous solutions and for the rinsing of glassware before use, employ water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a content of cadmium, lead and nickel as practicable and store all reagent solutions in containers of borosilicate glass. Clean glassware before use by soaking in a warm 773 g/L solution of nitric acid R for 30 min and by rinsing with deionised water.

Blank solution Use the solution described in the test for cadmium.

Modifier solution Dissolve 20 g of *ammonium dihydrogen phosphate R* in *water R* and dilute to 100 mL with the same solvent. Alternatively, use an appropriate matrix modifier as recommended by the GFAA spectrometer manufacturer.

Test solution Use the solution described in the test for cadmium.

Reference solution Prepare a solution of 0.050 µg/mL of Ni by suitable dilutions of a 0.2477 µg/mL solution of *nickel nitrate hexahydrate R* in the blank solution.

Prepare mixtures of the test solution, the reference solution and the blank solution in the following proportions: (1.0:0:1.0 *V/V/V*), (1.0:0.5:0.5 *V/V/V*), (1.0:1.0:0 *V/V/V*). To each mixture add 50 µL of matrix modifier solution and mix. These reference solutions contain respectively 0 µg, 0.0125 µg and 0.025 µg of nickel per millilitre from the reference solution.

Source Nickel hollow-cathode lamp.

Wavelength 232.0 nm.

Atomisation device Furnace.

Platform Pyrolytically coated with integrated tube.

Operating conditions Use the temperature programme recommended for nickel by the GFAA spectrometer manufacturer. An example of temperature parameters for GFAA analysis of nickel is shown below.

Stage	Final temperature (°C)	Ramp time (s)	Hold time (s)
Drying	110	10	20
Ashing	1000	20	30
Atomisation	2300	0	5

Loss on drying (*2.2.32*)
Maximum 6.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

♦Microbial contamination
TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*).

TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

Absence of *Escherichia coli* (*2.6.13*).

Absence of *Salmonella* (*2.6.13*).♦

ASSAY

Magnesium
To 0.500 g in a 250 mL conical flask add 50 mL of a mixture of equal volumes of *anhydrous ethanol R* and *butanol R*, 5 mL of *concentrated ammonia R*, 3 mL of *ammonium chloride buffer solution pH 10.0 R*, 30.0 mL of *0.1 M sodium edetate* and 15 mg of *mordant black 11 triturate R*. Heat at 45-50 °C until the solution is clear and titrate with *0.1 M zinc sulfate* until the colour changes from blue to violet. Carry out a blank titration.

1 mL of *0.1 M sodium edetate* is equivalent to 2.431 mg of Mg.

Stearic acid and palmitic acid
Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution In a conical flask fitted with a reflux condenser, dissolve 0.10 g of the substance to be examined in 5 mL of *boron trifluoride-methanol solution R*. Boil under a reflux condenser for 10 min. Add 4 mL of *heptane R* through the condenser and boil again under a reflux condenser for 10 min. Allow to cool. Add 20 mL of *saturated sodium chloride solution R*. Shake and allow the layers to separate. Dry the organic layer over 0.1 g of *anhydrous sodium sulfate R* (previously washed with *heptane R*). Dilute 1.0 mL of the solution to 10.0 mL with *heptane R*.

Reference solution Prepare the reference solution in the same manner as the test solution using 50.0 mg of *palmitic acid CRS* and 50.0 mg of *stearic acid CRS* instead of the substance to be examined.

Column:
— *material*: fused silica;
— *size*: *l* = 30 m, Ø = 0.32 mm;
— *stationary phase*: *macrogol 20 000 R* (film thickness 0.5 µm).

Carrier gas *helium for chromatography R*.

Flow rate 2.4 mL/min.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 2	70
	2 - 36	70 → 240
	36 - 41	240
Injection port		220
Detector		260

Detection Flame ionisation.

Injection 1 µL.

Relative retention With reference to methyl stearate: methyl palmitate = about 0.9.

System suitability Reference solution:
— *resolution*: minimum 5.0 between the peaks due to methyl palmitate and methyl stearate;
— *relative standard deviation*: maximum 3.0 per cent for the areas of the peaks due to methyl palmitate and methyl stearate, determined on 6 injections; maximum 1.0 per cent for the ratio of the areas of the peaks due to methyl palmitate to the areas of the peaks due to methyl stearate, determined on 6 injections.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for magnesium stearate used as lubricant in tablets and capsules.

Particle-size distribution (*2.9.31*)

Specific surface area (*2.9.26, Method I*)
Determine the specific surface area in the P/P_o range of 0.05 to 0.15.

Sample outgassing 2 h at 40 °C.

Thermogravimetry (*2.2.34*)

Magnesium Sulfate Heptahydrate

Epsom Salts

Magnesium Sulphate Heptahydrate

(Ph. Eur. monograph 0044)

$MgSO_4,7H_2O$ 246.5 *10034-99-8*

Action and use
Osmotic laxative; used in treatment of electrolyte deficiency.

Preparations
Magnesium Sulfate Injection

Magnesium Sulfate Mixture

Magnesium Sulfate, Potassium Chloride and Sodium Chloride Infusion

Ph Eur

DEFINITION
Content
99.0 per cent to 100.5 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder or brilliant, colourless crystals.

Solubility
Freely soluble in water, very soluble in boiling water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION
A. It gives the reactions of sulfates (*2.3.1*).

B. It gives the reaction of magnesium (*2.3.1*).

TESTS
Solution S
Dissolve 5.0 g in *water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity
To 10 mL of solution S add 0.05 mL of *phenol red solution R*. Not more than 0.2 mL of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Chlorides (*2.4.4*)
Maximum 300 ppm.

Dilute 1.7 mL of solution S to 15 mL with *water R*.

Iron (*2.4.9*)
Maximum 20 ppm.

Dilute 5 mL of solution S to 10 mL with *water R*.

Loss on drying (*2.2.32*)
48.0 per cent to 52.0 per cent, determined on 0.500 g by drying in an oven at 110-120 °C for 1 h and then at 400 °C to constant mass.

ASSAY
Dissolve 0.450 g in 100 mL of *water R* and carry out the complexometric titration of magnesium (*2.5.11*).

1 mL of *0.1 M sodium edetate* is equivalent to 12.04 mg of $MgSO_4$.

Ph Eur

Dried Magnesium Sulfate

Dried Epsom Salts; Dried Magnesium Sulphate

Preparation
Magnesium Sulfate Paste

DEFINITION
Dried Magnesium Sulfate contains not less than 62.0% and not more than 70.0% of $MgSO_4$. It may be prepared by drying magnesium sulfate at 100° until it has lost approximately 25% of its weight.

CHARACTERISTICS
A white powder.

Freely soluble in *water*; dissolves more rapidly in hot *water*.

IDENTIFICATION
Yields the reactions characteristic of *magnesium salts* and of *sulfates*, Appendix VI.

TESTS
Acidity or alkalinity
To 10 mL of a 7.5% w/v solution in *carbon dioxide-free water* add 0.05 mL of *phenol red solution*. Not more than 0.2 mL of either *0.01M hydrochloric acid VS* or *0.01M sodium hydroxide VS* is required to change the colour of the solution.

Arsenic
0.33 g dissolved in 25 mL of *water* complies with the *limit test for arsenic*, Appendix VII (3 ppm).

Iron
0.33 g dissolved in 10 mL of *water* complies with the *limit test for iron*, Appendix VII (30 ppm).

Chloride
0.13 g dissolved in 15 mL of *water* complies with the *limit test for chlorides*, Appendix VII (400 ppm).

Insoluble matter
7.5 g dissolves in 20 mL of *water*, producing a solution which may be slightly turbid at first but which becomes clear in a few minutes.

ASSAY
Dissolve 0.3 g in 50 mL of *water* and carry out the *complexometric titration of magnesium*, Appendix VIII D. Each mL of 0.1M *disodium edetate VS* is equivalent to 12.04 mg of $MgSO_4$.

Magnesium Trisilicate

(Ph. Eur. monograph 0403)

Action and use
Antacid.

Preparations
Magnesium Trisilicate Mixture

Compound Magnesium Trisilicate Chewable Tablets

Compound Magnesium Trisilicate Oral Powder

Ph Eur

DEFINITION
It has a variable composition corresponding approximately to $Mg_2Si_3O_8,xH_2O$.

Content
— *magnesium oxide* (MgO; M_r 40.30): minimum 29.0 per cent (ignited substance),
— *silicon dioxide* (SiO_2; M_r 60.1): minimum 65.0 per cent (ignited substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Practically insoluble in water and in ethanol (96 per cent).

IDENTIFICATION

A. 0.25 g gives the reaction of silicates (*2.3.1*).

B. 1 mL of solution S (see Tests) neutralised with *dilute sodium hydroxide solution R* gives the reaction of magnesium (*2.3.1*).

TESTS

Solution S
To 2.0 g add a mixture of 4 mL of *nitric acid R* and 4 mL of *distilled water R*. Heat to boiling with frequent shaking. Add 12 mL of *distilled water R* and allow to cool. Filter or centrifuge to obtain a clear solution and dilute to 20 mL with *distilled water R*.

Alkalinity
To 10.0 g in a 200 mL conical flask, add 100.0 g of *water R* and heat on a water-bath for 30 min. Allow to cool and make up to the initial mass with *water R*. Allow to stand and filter or centrifuge until a clear liquid is obtained. To 10 mL of this liquid add 0.1 mL of *phenolphthalein solution R*. Not more than 1.0 mL of *0.1 M hydrochloric acid* is required to change the colour of the indicator.

Water-soluble salts
Maximum 1.5 per cent.

In a platinum dish, evaporate to dryness on a water-bath 20.0 mL of the liquid obtained in the test for alkalinity. The residue, ignited to constant mass at 900 ± 50 °C, weighs a maximum of 30 mg.

Chlorides (*2.4.4*)
Maximum 500 ppm.

Dilute 0.5 mL of solution S to 15 mL with *water R*. Prepare the standard using a mixture of 5 mL of *chloride standard solution (5 ppm Cl) R* and 10 mL of *water R*.

Sulfates (*2.4.13*)
Maximum 0.5 per cent.

Dilute 0.3 mL of solution S to 15 mL with *distilled water R*.

Arsenic (*2.4.2, method A*)
Maximum 4 ppm, determined on 2.5 mL of solution S.

Loss on ignition
17 per cent to 34 per cent, determined on 0.5 g by ignition to constant mass at 900 ± 50 °C in a platinum crucible.

Acid-absorbing capacity
Suspend 0.25 g in *0.1 M hydrochloric acid*, dilute to 100.0 mL with the same acid and allow to stand for 2 h in a water-bath at 37 ± 0.5 °C, with frequent shaking. Allow to cool. To 20.0 mL of the supernatant solution add 0.1 mL of *bromophenol blue solution R* and titrate with *0.1 M sodium hydroxide* until a blue colour is obtained. The acid-absorbing capacity is not less than 100.0 mL of *0.1 M hydrochloric acid* per gram.

ASSAY

Magnesium oxide
To 1.000 g in a 200 mL conical flask, add 35 mL of *hydrochloric acid R* and 60 mL of *water R* and heat in a water-bath for 15 min. Allow to cool, filter, wash the conical flask and the residue with *water R* and dilute the combined filtrate and washings to 250.0 mL with *water R*. Neutralise 50.0 mL of the solution with *strong sodium hydroxide solution R* (about 8 mL). Carry out the complexometric titration of magnesium (*2.5.11*).

1 mL of *0.1 M sodium edetate* is equivalent to 4.030 mg of MgO.

Silicon dioxide
To 0.700 g add 10 mL of *dilute sulfuric acid R* and 10 mL of *water R*. Heat for 90 min on a water-bath with frequent shaking, replacing the evaporated water. Allow to cool and decant onto an ashless filter paper (diameter 7 cm). Wash the precipitate by decantation with 3 quantities, each of 5 mL, of hot *water R*, transfer it to the filter and wash it with hot *water R* until 1 mL of the filtrate remains clear after the addition of 0.05 mL of *dilute hydrochloric acid R* and 2 mL of *barium chloride solution R1*. Incinerate the filter and its contents in a platinum crucible, then ignite the residue (SiO_2) at 900 ± 50 °C to constant mass.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for magnesium trisilicate used as a lubricant in tablets and capsules.

Particle-size distribution (*2.9.31*)

Specific surface area (*2.9.26, Method I*)

Ph Eur

Refined Maize Oil

(Ph. Eur. monograph 1342)

Ph Eur

DEFINITION

Fatty oil obtained from the seeds of *Zea mays* L. by mechanical expression or by extraction. It is then refined.

PRODUCTION

The oil is prepared using materials and methods designed to ensure that the content of brassicasterol (*2.4.23*) in the sterol fraction of the oil is not greater than 0.3 per cent.

CHARACTERS

Appearance
Clear, light yellow or yellow oil.

Solubility
Practically insoluble in water and in ethanol (96 per cent), miscible with light petroleum (bp: 40-60 °C) and with methylene chloride.

Relative density
About 0.920.

Refractive index
About 1.474.

IDENTIFICATION

First identification: B.

Second identification: A.

A. Identification of fatty oils by thin-layer chromatography (*2.3.2*).

Results The chromatogram obtained with the test solution is similar to the chromatogram obtained with the reference solution.

B. Composition of fatty acids (see Tests).

TESTS

Acid value (*2.5.1*)

Maximum 0.5, or maximum 0.3 if intended for use in the manufacture of parenteral preparations, determined on 10.0 g.

Peroxide value (*2.5.5, Method A*)

Maximum 10.0, or maximum 5.0 if intended for use in the manufacture of parenteral preparations.

Unsaponifiable matter (*2.5.7*)

Maximum 2.8 per cent, determined on 5.0 g.

Alkaline impurities (*2.4.19*)

It complies with the test.

Composition of fatty acids (*2.4.22, Method A*)

Use the mixture of calibrating substances in Table 2.4.22.-3.

Composition of the fatty-acid fraction of the oil:

- *sum of fatty acids of chain length less than C_{16}*: maximum 0.6 per cent;
- *palmitic acid*: 8.6 per cent to 16.5 per cent;
- *stearic acid*: maximum 3.3 per cent;
- *oleic acid and isomer*: 20.0 per cent to 42.2 per cent;
- *linoleic acid*: 39.4 per cent to 65.6 per cent;
- *linolenic acid*: 0.5 per cent to 1.5 per cent;
- *arachidic acid*: maximum 0.8 per cent;
- *eicosenoic acid*: maximum 0.5 per cent;
- *behenic acid*: maximum 0.5 per cent;
- *any other fatty acid*: for each fatty acid, maximum 0.5 per cent.

Water (*2.5.32*)

Maximum 0.1 per cent, determined on 1.00 g.

STORAGE

Protected from light, at a temperature not exceeding 25 °C.

LABELLING

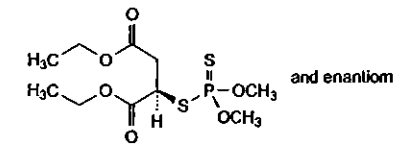
The label states:

- where applicable, that the substance is suitable for use in the manufacture of parenteral preparations;
- whether the oil is obtained by mechanical expression or by extraction.

Ph Eur

Malathion

(Ph. Eur. monograph 1343)



$C_{10}H_{19}O_6PS_2$ 330.4 *121-75-5*

Action and use

Organophosphorus insecticide.

Preparations

Malathion Lotion

Malathion Shampoo

Ph Eur

DEFINITION

Diethyl (2*RS*)-2-(dimethoxyphosphinodithioyl)butanedioate.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Clear, colourless or slightly yellowish liquid.

Solubility

Slightly soluble in water, miscible with acetone, with cyclohexane, with ethanol (96 per cent) and with vegetable oils.

It solidifies at about 3 °C.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison malathion CRS.

TESTS

Relative density (*2.2.5*)

1.220 to 1.240.

Optical rotation (*2.2.7*)

−0.1° to + 0.1°.

Dissolve 2.50 g in *ethanol (96 per cent) R* and dilute to 25.0 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture water R, acetonitrile R (1:3 *V/V*).

Test solution (a) Dissolve 0.10 g of the substance to be examined in the solvent mixture and dilute to 5.0 mL with the solvent mixture.

Test solution (b) Dilute 1.0 mL of test solution (a) to 10.0 mL with the solvent mixture.

Reference solution (a) Dissolve 0.100 g of *malathion CRS* in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (b) Dilute 0.5 mL of test solution (a) to 100.0 mL with the solvent mixture.

Reference solution (c) Dissolve 5.0 mg of *malathion impurity A CRS* and 5.0 mg of *malathion impurity B CRS* in the solvent mixture, then dilute 50.0 mL with the solvent mixture.

Reference solution (d) Dilute 2.0 mL of reference solution (c) to 10.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (10 µm);
— *temperature*: 35 °C.

Mobile phase *acetonitrile R, water R* (45:55 *V/V*).

Flow rate 1 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µL of test solution (a) and reference solutions (b), (c) and (d).

Retention time Impurity B = about 3.5 min; impurity A = about 5 min; malathion = about 16 min.

System suitability Reference solution (c):
— *resolution*: minimum 2.0 between the peaks due to impurities B and A.

Limits:
— *impurity A*: not more than 3 times the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.3 per cent);
— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.1 per cent);
— *sum of impurities other than A and B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);
— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.12*)
Maximum 0.1 per cent, determined on 2.000 g.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution (b) and reference solution (a).

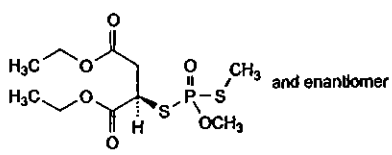
System suitability Reference solution (a):
— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections.

Calculate the percentage content of $C_{10}H_{19}O_6PS_2$ from the declared content of *malathion CRS*.

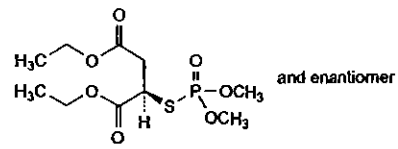
STORAGE
In an airtight container, protected from light.

IMPURITIES
Specified impurities *A, B.*

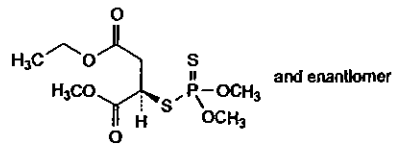
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C.*



A. diethyl (2*RS*)-2-[(methoxy)(methylsulfanyl)-*S*-phosphinothioyl]butanedioate (isomalathion),



B. diethyl (2*RS*)-2-(dimethoxy-*S*-phosphinothioyl)butanedioate (maloxon),



C. ethyl and methyl (2*RS*)-2-(dimethoxyphosphinodithioyl) butanedioate (methyl analogue).

Ph Eur

Maleic Acid

(Ph. Eur. monograph 0365)

$C_4H_4O_4$ 116.1 *110-16-7*

Ph Eur

DEFINITION
Maleic acid contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (*Z*)-butenedioic acid, calculated with reference to the anhydrous substance.

CHARACTERS
A white or almost white, crystalline powder, freely soluble in water and in alcohol.

IDENTIFICATION
A. Dilute 5 mL of solution S (see Tests) to 10 mL with *water R*. The pH of the dilution is less than 2.

B. Examine the chromatograms obtained in the test for fumaric acid. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 0.1 g in 10 mL of *water R* (solution a). To 0.3 mL of solution (a) add a solution of 10 mg of *resorcinol R* in 3 mL of *sulfuric acid R*. Heat on a water-bath for 15 min; no colour develops. To 3 mL of solution (a) add 1 mL of *bromine water R*. Heat on a water-bath to remove the bromine (15 min), heat to boiling and cool. To 0.2 mL of this solution add a solution of 10 mg of *resorcinol R* in 3 mL of *sulfuric acid R*. Heat on a water-bath for 15 min. A violet-pink colour develops.

TESTS
Solution S
Dissolve 5.0 g in *water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

Fumaric acid
Examine by thin-layer chromatography (*2.2.27*), using *silica gel GF_{254} R* as the coating substance.

Test solution (a) Dissolve 0.5 g of the substance to be examined in *acetone R* and dilute to 5 mL with the same solvent.

Test solution (b) Dilute 1 mL of test solution (a) to 50 mL with *acetone R*.

Reference solution (a) Dissolve 20 mg of *maleic acid CRS* in *acetone R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 15 mg of *fumaric acid CRS* in *acetone R* and dilute to 10 mL with the same solvent.

Reference solution (c) Mix 5 mL of reference solution (a) and 5 mL of reference solution (b).

Apply separately to the plate 5 µL of test solutions (a) and (b), 5 µL of reference solutions (a) and (b) and 10 µL of reference solution (c). Develop in an unsaturated tank over a path of 10 cm using a mixture of 12 volumes of *anhydrous formic acid R*, 16 volumes of *chloroform R*, 32 volumes of *butanol R* and 44 volumes of *heptane R*. Dry the plate at 100 °C for 15 min and examine in ultraviolet light at 254 nm. Any spot corresponding to fumaric acid in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Iron
To 10 mL of solution S add 2 mL of *dilute hydrochloric acid R* and 0.05 mL of *bromine water R*. After 5 min, remove the excess of bromine by passing a current of air and add 3 mL of *potassium thiocyanate solution R*. Shake. Prepare a standard at the same time and in the same manner, using a mixture of 5 mL of *iron standard solution (1 ppm Fe) R*, 1 mL of *dilute hydrochloric acid R*, 6 mL of *water R* and 0.05 mL of *bromine water R*. Allow both solutions to stand for 5 min. Any red colour in the test solution is not more intense than that in the standard (5 ppm).

Water (*2.5.12*)
Not more than 2.0 per cent, determined on 1.00 g by the semi-micro determination of water.

Sulfated ash (*2.4.14*)
Not more than 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.500 g in 50 mL of *water R*. Titrate with *1 M sodium hydroxide* using 0.5 mL of *phenolphthalein solution R* as indicator.

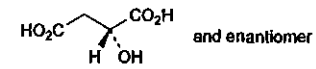
1 mL of *1 M sodium hydroxide* is equivalent to 58.04 mg of $C_4H_4O_4$.

STORAGE
Store in a glass container, protected from light.

Ph Eur

Malic Acid

(Ph. Eur. monograph 2080)



and enantiomer

$C_4H_6O_5$ 134.1 *6915-15-7*

Action and use
Excipient.

Ph Eur

DEFINITION
(2*RS*)-2-Hydroxybutanedioic acid.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water and in alcohol, sparingly soluble in acetone.

IDENTIFICATION
A. Melting point (*2.2.14*): 128 °C to 132 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of malic acid.*

TESTS
Solution S
Dissolve 5.00 g in *water R* and dilute to 25 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Optical rotation (*2.2.7*)
−0.10° to + 0.10°, determined on solution S.

Water-insoluble substances
Maximum 0.1 per cent.

Dissolve 25.0 g in 100 mL of *water R*, filter the solution through a tared sintered-glass filter (16) (*2.1.2*), wash the filter with hot *water R* and dry at 100-105 °C to constant weight. The residue weighs a maximum of 25 mg.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 100.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dissolve 10.0 mg of *fumaric acid R* and 4.0 mg of *maleic acid R* in 25 mL of the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (b) Dilute 2.5 mL of reference solution (a) to 100.0 mL with the mobile phase.

Reference solution (c) Dissolve 20.0 mg of the substance to be examined in the mobile phase, add 1.0 mL of reference solution (a) and dilute to 20.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.30 m, Ø = 7.8 mm,
— *stationary phase*: *ion-exclusion resin for chromatography R* (9 µm),
— *temperature*: 37 °C.

Mobile phase *0.005 M sulfuric acid.*

Flow rate 0.6 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µL.

Run time Twice the retention time of the principal peak in the chromatogram obtained with the test solution.

Relative retention With reference to malic acid (retention time = about 10 min): impurity B = about 0.8; impurity A = about 1.5.

System suitability Reference solution (c):
— *resolution*: minimum 2.5 between the peaks due to impurity B and malic acid.

Limits:
— *impurity A*: not more than twice the area of the corresponding peak in the chromatogram obtained with reference solution (b) (1.0 per cent),
— *impurity B*: not more than 0.25 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.05 per cent),
— *any other impurity*: for each impurity, not more than 0.5 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) (0.1 per cent),
— *total of other impurities*: not more than 2.5 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) (0.5 per cent),
— *disregard limit*: 0.1 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) (0.02 per cent).

Water (*2.5.12*)
Maximum 2.0 per cent, determined on 1.00 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

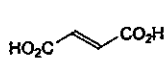
ASSAY

Dissolve 0.500 g in 50 mL of *carbon dioxide-free water R*. Titrate with *1 M sodium hydroxide* determining the end-point potentiometrically (*2.2.20*).

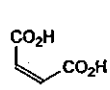
1 mL of *1 M sodium hydroxide* is equivalent to 67.05 mg of $C_4H_6O_5$.

IMPURITIES

Specified impurities *A, B.*



A. (*E*)-butenedioic acid (fumaric acid),

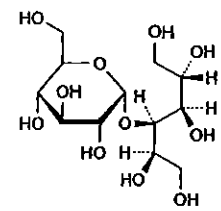


B. (*Z*)-butenedioic acid (maleic acid).

Ph Eur

Maltitol

(Ph. Eur. monograph 1235)



$C_{12}H_{24}O_{11}$ 344.3 *585-88-6*

Action and use
Sweetening agent.

Ph Eur

DEFINITION

4-*O*-α-D-Glucopyranosyl-D-glucitol (D-maltitol).

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Very soluble in water, practically insoluble in anhydrous ethanol.

IDENTIFICATION

First identification: A.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *maltitol CRS.*

B. Melting point (*2.2.14*): 148 °C to 151 °C.

C. Specific optical rotation (*2.2.7*): + 105.5 to + 108.5 (anhydrous substance).

Dissolve 5.00 g in *water R* and dilute to 100.0 mL with the same solvent.

D. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 25 mg of the substance to be examined in *water R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dissolve 25 mg of *maltitol CRS* in *water R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 25 mg of *maltitol CRS* and 25 mg of *sorbitol CRS* in *water R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *water R, ethyl acetate R, propanol R* (10:20:70 *V/V/V*).

Application 2 µL.

Development Over 3/4 of the plate.

Drying In air.

Detection Spray with *4-aminobenzoic acid solution R*. Dry in a current of cold air until the acetone is removed. Heat at 100-105 °C for 15 min. Allow to cool and spray with a 2 g/L solution of *sodium periodate R*. Dry in a current of cold air. Heat at 100 °C for 15 min.

System suitability Reference solution (b):
— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 5.0 g in *water R* and dilute to 50 mL with the same solvent.

Conductivity (*2.2.38*)

Maximum 20 $\mu S \cdot cm^{-1}$.

Dissolve 20.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100.0 mL with the same solvent. Measure the conductivity of the solution, while gently stirring with a magnetic stirrer.

Reducing sugars

Maximum 0.2 per cent, expressed as glucose equivalent.

Dissolve 5.0 g in 6 mL of *water R* with the aid of gentle heat. Cool and add 20 mL of *cupri-citric solution R* and a few glass beads. Heat so that boiling begins after 4 min and maintain boiling for 3 min. Cool rapidly and add 100 mL of a 2.4 per cent *V/V* solution of *glacial acetic acid R* and 20.0 mL of *0.025 M iodine*. With continuous shaking, add 25 mL of a mixture of 6 volumes of *hydrochloric acid R* and 94 volumes of *water R* and, when the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulfate* using 1 mL of *starch solution R*, added towards the end of the titration as indicator. Not less than 12.8 mL of *0.05 M sodium thiosulfate* is required.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 5.0 g of the substance to be examined in 20 mL of *water R* and dilute to 100.0 mL with the same solvent.

Reference solution (a) Dissolve 0.50 g of *maltitol CRS* in 2.0 mL of *water R* and dilute to 10.0 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *water R*.

Reference solution (c) Dilute 10.0 mL of reference solution (b) to 100.0 mL with *water R*.

Reference solution (d) Dissolve 0.5 g of *maltitol R* and 0.5 g of *sorbitol R* in 5 mL of *water R* and dilute to 10 mL with the same solvent.

Column:
- *size*: *l* = 0.3 m, Ø = 7.8 mm;
- *stationary phase*: *strong cation-exchange resin (calcium form) R* (9 µm);
- *temperature*: 85 ± 1 °C.

Mobile phase Degassed *water for chromatography R*.

Flow rate 0.5 mL/min.

Detection Differential refractometer maintained at a constant temperature (e.g. 35 °C).

Injection 20 µL of the test solution and reference solutions (b), (c) and (d).

Run time 3 times the retention time of maltitol.

Relative retention With reference to maltitol (retention time = about 16 min): impurity B = about 0.8; impurity A = about 1.8.

System suitability Reference solution (d):
- *resolution*: minimum 2.0 between the peaks due to maltitol and impurity A.

Limits:
- *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);
- *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Water (*2.5.12*)

Maximum 1.0 per cent, determined on 1.00 g.

Microbial contamination

If intended for use in the manufacture of parenteral preparations:
- TAMC: acceptance criterion: 10^2 CFU/g (*2.6.12*).

If not intended for use in the manufacture of parenteral preparations:
- TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*);
- TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*);
- absence of *Escherichia coli* (*2.6.13*);
- absence of *Salmonella* (*2.6.13*).

Bacterial endotoxins (*2.6.14*)

If intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins:
- less than 4 IU/g for parenteral preparations having a concentration of less than 100 g/L of maltitol;
- less than 2.5 IU/g for parenteral preparations having a concentration of 100 g/L or more of maltitol.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

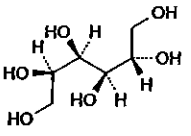
Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{12}H_{24}O_{11}$ taking into account the assigned content of *maltitol CRS*.

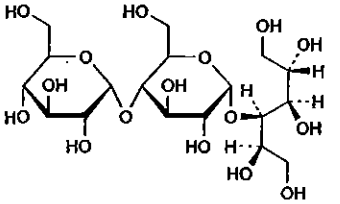
LABELLING

The label states:
- where applicable, the maximum concentration of bacterial endotoxins;
- where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

IMPURITIES



A. D-glucitol (D-sorbitol),



B. α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-D-glucitol (maltotriitol).

Liquid Maltitol

(Ph. Eur. monograph 1236)

Action and use
Excipient.

Ph Eur

DEFINITION
Aqueous solution of a hydrogenated, partly hydrolysed starch, composed of a mixture of mainly 4-*O*-α-D-glucopyranosyl-D-glucitol (D-maltitol) with D-glucitol (D-sorbitol) and hydrogenated oligo- and polysaccharides.

Content
— *D-maltitol* ($C_{12}H_{24}O_{11}$): minimum 50.0 per cent *m/m* (anhydrous substance) and 95.0 per cent to 105.0 per cent of the content stated on the label;
— *D-sorbitol* ($C_6H_{14}O_6$): maximum 8.0 per cent *m/m* (anhydrous substance);
— *anhydrous substance*: 68.0 per cent *m/m* to 85.0 per cent *m/m*.

CHARACTERS
Appearance
Clear, colourless, syrupy liquid.

Solubility
Miscible with water and with glycerol.

IDENTIFICATION
First identification: A.

Second identification: B, C.

A. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

B. Thin-layer chromatography (*2.2.27*).

Test solution Dilute 0.35 g of the substance to be examined to 100 mL with *water R*.

Reference solution (a) Dissolve 20 mg of *maltitol CRS* in *water R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 20 mg of *maltitol CRS* and 20 mg of *sorbitol CRS* in *water R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *water R, ethyl acetate R, propanol R* (10:20:70 *V/V/V*).

Application 2 µL.

Development Over 3/4 of the plate.

Drying In air.

Detection Spray with *4-aminobenzoic acid solution R*. Dry in a current of cold air until the acetone is removed. Heat at 100-105 °C for 15 min. Allow to cool and spray with a 2 g/L solution of *sodium periodate R*. Dry in a current of cold air. Heat at 100 °C for 15 min.

System suitability Reference solution (b):
— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and colour to the principal spot in the chromatogram obtained with reference solution (a).

C. To 3 mL of a freshly prepared 100 g/L solution of *pyrocatechol R*, add 6 mL of *sulfuric acid R* while cooling in iced water. To 3 mL of the cooled mixture, add 0.3 mL of solution S (see Tests). Heat gently over a naked-flame for about 30 s. A pink colour develops.

TESTS
Solution S
Dilute 7.0 g to 50 mL with *water R*.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Conductivity (*2.2.38*)
Maximum 10 µS·cm^{-1}, measured on undiluted liquid maltitol while gently stirring with a magnetic stirrer.

Reducing sugars
Maximum 0.2 per cent, calculated as glucose equivalent.

To 5.0 g add 6 mL of *water R*, 20 mL of *cupri-citric solution R* and a few glass beads. Heat so that boiling begins after 4 min and maintain boiling for 3 min. Cool rapidly and add 100 mL of a 2.4 per cent *V/V* solution of *glacial acetic acid R* and 20.0 mL of *0.025 M iodine*. With continuous shaking, add 25 mL of a mixture of 6 volumes of *hydrochloric acid R* and 94 volumes of *water R* and, when the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulfate* using 1 mL of *starch solution R*, added towards the end of the titration, as indicator. Not less than 12.8 mL of *0.05 M sodium thiosulfate* is required.

Water (*2.5.12*)
15.0 per cent *m/m* to 32.0 per cent *m/m*, determined on 0.100 g. Use as solvent a mixture of equal volumes of *anhydrous methanol R* and *formamide R1*. Carry out the titration at about 50 °C.

ASSAY
Liquid chromatography (*2.2.29*).

Test solution Mix 1.00 g of the solution to be examined with 20 mL of *water R* and dilute to 50.0 mL with the same solvent.

Reference solution (a) Dissolve 50.0 mg of *maltitol CRS* in 2 mL of *water R* and dilute to 5.0 mL with the same solvent.

Reference solution (b) Dissolve 8.0 mg of *sorbitol CRS* in 2 mL of *water R* and dilute to 5.0 mL with the same solvent.

Reference solution (c) Dissolve 50 mg of *maltitol R* and 50 mg of *sorbitol R* in 2 mL of *water R* and dilute to 5 mL with the same solvent.

Column:
— *size*: l = 0.3 m, Ø = 7.8 mm;
— *stationary phase*: *strong cation-exchange resin (calcium form) R* (9 µm);
— *temperature*: 85 ± 2 °C.

Mobile phase Degassed *water for chromatography R*.

Flow rate 0.5 mL/min.

Detection Differential refractometer maintained at a constant temperature (e.g. 35 °C).

Injection 20 µL.

Run time 3 times the retention time of maltitol.

Relative retention With reference to maltitol (retention time = about 16 min): sorbitol = about 1.8.

System suitability Reference solution (c):
— *resolution*: minimum 2.0 between the peaks due to maltitol and sorbitol.

Calculate the percentage contents of D-maltitol ($C_{12}H_{24}O_{11}$) and D-sorbitol ($C_6H_{14}O_6$) taking into account the assigned contents of *maltitol CRS* and *sorbitol CRS*.

LABELLING
The label states the content of D-maltitol.

Ph Eur

Maltodextrin

(Ph. Eur. monograph 1542)

Action and use
Excipient.

Ph Eur

DEFINITION
Mixture of glucose, disaccharides and polysaccharides, obtained by the partial hydrolysis of starch.

The degree of hydrolysis, expressed as dextrose equivalent (DE), is less than 20 (nominal value).

CHARACTERS

Appearance
White or almost white, slightly hygroscopic powder or granules.

Solubility
Freely soluble in water.

IDENTIFICATION
A. Dissolve 0.1 g in 2.5 mL of *water R* and heat with 2.5 mL of *cupri-tartaric solution R*. A red precipitate is formed.

B. Dip, for 1 s, a suitable stick with a reactive pad containing glucose-oxidase, peroxidase and a hydrogen-donating substance, such as tetramethylbenzidine, in a 100 g/L solution of the substance to be examined. Observe the colour of the reactive pad; within 60 s a colour change is observed, characteristic of the hydrogen-donating substance used (from yellow to green or blue if tetramethylbenzidine is used).

C. It is a powder or granules.

D. Dextrose equivalent (see Tests).

TESTS

Solution S
Dissolve 12.5 g in *carbon dioxide-free water R* and dilute to 50.0 mL with the same solvent.

pH *(2.2.3)*
4.0 to 7.0.

Mix 1 mL of a 223.6 g/L solution of *potassium chloride R* and 30 mL of solution S.

Sulfur dioxide *(2.5.29)*
Maximum 20 ppm.

Loss on drying *(2.2.32)*
Maximum 6.0 per cent, determined on 10.00 g by drying in an oven at 105 °C.

Sulfated ash *(2.4.14)*
Maximum 0.5 per cent, determined on 1.0 g.

Dextrose equivalent
(DE): within 2 DE units of the nominal value.

Weigh an amount of the substance to be examined equivalent to 2.85-3.15 g of reducing carbohydrates, calculated as dextrose equivalent, into a 500 mL volumetric flask. Dissolve in *water R* and dilute to 500.0 mL with the same solvent. Transfer the solution to a 50 mL burette.

Pipette 25.0 mL of *cupri-tartaric solution R* into a 250 mL flask and add 18.5 mL of the test solution from the burette, mix and add a few glass beads. Place the flask on a hot plate, previously adjusted so that the solution begins to boil within 2 min ± 15 s. Allow to boil for exactly 120 s, add 1 mL of a 1 g/L solution of *methylene blue R* and titrate with the test solution (V_1) until the blue colour disappears. Maintain the solution at boiling throughout the titration.

Standardise the cupri-tartaric solution using a 6.00 g/L solution of *glucose R* (V_0).

Calculate the dextrose equivalent using the following expression:

$$\frac{300 \times V_0 \times 100}{V_1 \times M \times D}$$

V_0 = total volume of glucose standard solution, in millilitres;
V_1 = total volume of test solution, in millilitres;
M = sample mass, in grams;
D = percentage content of dry matter in the substance.

Microbial contamination
TAMC: acceptance criterion 10^3 CFU/g *(2.6.12)*.

TYMC: acceptance criterion 10^2 CFU/g *(2.6.12)*.

Absence of *Escherichia coli* *(2.6.13)*.

Absence of *Salmonella* *(2.6.13)*.

LABELLING
The label states the dextrose equivalent (DE) (= nominal value).

FUNCTIONALITY-RELATED CHARACTERISTICS
This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for maltodextrin used as filler and binder in tablets and capsules.

Dextrose equivalent
(see Tests).

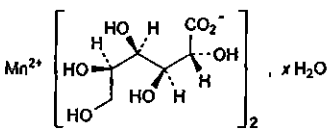
Particle-size distribution *(2.9.31* or *2.9.38)*

Powder flow *(2.9.36)*

Ph Eur

Manganese Gluconate

(Ph. Eur. monograph 2162)



$C_{12}H_{22}MnO_{14},xH_2O$ 445.2
(anhydrous substance)

Ph Eur

DEFINITION
Anhydrous or hydrated manganese(II) bis[(2*R*,3*S*,4*R*,5*R*)-2,3,4,5,6-pentahydroxyhexanoate] (anhydrous or hydrated manganese(II) di(D-gluconate)).

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or pale pink, slightly hygroscopic, crystalline powder.

Solubility
Soluble in water, practically insoluble in anhydrous ethanol, insoluble in methylene chloride.

IDENTIFICATION
A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 20 mg of the substance to be examined in 1 mL of *water R*.

Reference solution Dissolve 20 mg of *calcium gluconate CRS* in 1 mL of *water R*, heating if necessary in a water-bath at 60 °C.

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *concentrated ammonia R, ethyl acetate R, water R, ethanol (96 per cent) R* (10:10:30:50 *V/V/V/V*).

Application 1 µL.

Development Over 3/4 of the plate.

Drying At 105 °C for 20 min, then allow to cool to room temperature.

Detection Spray with a solution containing 25 g/L of *ammonium molybdate R* and 10 g/L of *cerium sulfate R* in *dilute sulfuric acid R*, and heat at 105 °C for about 10 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

B. Dissolve 50 mg in 5 mL of *water R*. Add 0.5 mL of *ammonium sulfide solution R*. A pale pink precipitate is formed that dissolves upon the addition of 1 mL of *glacial acetic acid R*.

TESTS

Solution S
Dissolve 1.0 g in *water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than intensity 6 of the range of reference solutions of the most appropriate colour (*2.2.2, Method II*).

Sucrose and reducing sugars
Dissolve 0.5 g in a mixture of 2 mL of *hydrochloric acid R1* and 10 mL of *water R*. Boil for 5 min, allow to cool, add 10 mL of *sodium carbonate solution R* and allow to stand for 10 min. Dilute to 25 mL with *water R* and filter. To 5 mL of the filtrate add 2 mL of *cupri-tartaric solution R* and boil for 1 min. Allow to stand for 2 min. No red precipitate is formed.

Chlorides (*2.4.4*)
Maximum 500 ppm.

Dilute 5 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 500 ppm.

Dissolve 2.0 g in a mixture of 10 mL of *acetic acid R* and 90 mL of *distilled water R*.

Zinc
Maximum 50 ppm.

To 10 mL of solution S add 1 mL of *sulfuric acid R* and 0.1 mL of *potassium ferrocyanide solution R*. After 30 s, any opalescence in the solution is not more intense than that in a mixture of 1.0 mL of *zinc standard solution (10 ppm Zn) R*, 9 mL of *water R*, 1 mL of *sulfuric acid R* and 0.1 mL of *potassium ferrocyanide solution R*.

Water (*2.5.32*)
Maximum 9.0 per cent, determined on 80 mg.

Microbial contamination
TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*).

TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

ASSAY
Dissolve 0.400 g in 50 mL of *water R*. Add 10 mg of *ascorbic acid R*, 20 mL of *ammonium chloride buffer solution pH 10.0 R* and 0.2 mL of a 2 g/L solution of *mordant black 11 R* in *triethanolamine R*. Titrate with *0.1 M sodium edetate* until the colour changes from violet to pure blue.

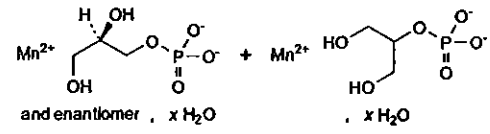
1 mL of *0.1 M sodium edetate* is equivalent to 44.52 mg of $C_{12}H_{22}MnO_{14}$.

STORAGE
In a non-metallic, airtight container.

Ph Eur

Hydrated Manganese Glycerophosphate

(Ph. Eur. monograph 2163)



$C_3H_7MnO_6P_xH_2O$ 225.0
(anhydrous substance)

Ph Eur

DEFINITION
Mixture of variable proportions of hydrated manganese(II) (2*RS*)-2,3-dihydroxypropyl phosphate and hydrated manganese(II) 2-hydroxy-1-(hydroxymethyl)ethyl phosphate.

Content
97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
White or pale pink, hygroscopic powder.

Solubility
Practically insoluble in water and in ethanol (96 per cent). It is freely soluble in dilute mineral acids.

IDENTIFICATION
A. Mix 1 g with 1 g of *potassium hydrogen sulfate R* in a test tube fitted with a delivery tube. Heat strongly and direct the white vapour towards a piece of filter paper impregnated with a freshly prepared 10 g/L solution of *sodium nitroprusside R*. The filter paper develops a blue colour in contact with *piperidine R*.

B. Disperse 50 mg in 5 mL of *water R*. Add 0.5 mL of *ammonium sulfide solution R*. A pale pink precipitate is formed that dissolves on the addition of 1 mL of *acetic acid R*.

C. Ignite 0.1 g in a crucible. Take up the residue with 5 mL of *nitric acid R* and heat on a water-bath for 1 min. Filter. The filtrate gives reaction (b) of phosphates (*2.3.1*).

TESTS

Solution S

Dissolve 5.0 g in 20 mL of *dilute hydrochloric acid R*. Filter if necessary. Add *dilute ammonia R1* until a precipitate is formed. Dissolve the precipitate by adding the minimum quantity needed of *dilute hydrochloric acid R* and dilute to 100 mL with *distilled water R*.

Glycerol and ethanol (96 per cent)-soluble substances

Maximum 1.0 per cent.

Shake 1.00 g with 25 mL of *ethanol (96 per cent) R* for 1 min. Filter. Evaporate the filtrate to dryness on a water-bath and dry the residue at 70 °C for 1 h. The residue weighs a maximum of 10 mg.

Chlorides (*2.4.4*)

Maximum 0.15 per cent.

Dissolve 0.22 g in a mixture of 1 mL of *nitric acid R* and 10 mL of *water R* and dilute to 100 mL with *water R*.

Phosphates (*2.4.11*)

Maximum 0.3 per cent.

Dilute 1.0 mL of solution S to 100.0 mL with *water R*. To 10 mL of this solution add 140 mL of *water R*.

Sulfates (*2.4.13*)

Maximum 0.2 per cent.

Dilute 5 mL of solution S to 50 mL with *distilled water R*.

Iron (*2.4.9*)

Maximum 50 ppm.

Dilute 4 mL of solution S to 10 mL with *water R*.

Loss on drying (*2.2.32*)

Maximum 12.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

ASSAY

To 0.200 g add 1.5 mL of *1 M hydrochloric acid*, 50 mL of *water R*, 10 mg of *ascorbic acid R* and 20 mL of *ammonium chloride buffer solution pH 10.0 R*. Stir until dissolution. Immediately add 0.3 mL of a 2 g/L solution of *mordant black 11 R* in *triethanolamine R* and titrate with *0.1 M sodium edetate* until the colour changes from violet to pure blue.

1 mL of *0.1 M sodium edetate* is equivalent to 22.50 mg of $C_3H_7MnO_6P$.

STORAGE

In an airtight container.

Ph Eur

Manganese Sulfate

Manganese Sulphate

$MnSO_4,4H_2O$ 223.1 *7785-87-7*

DEFINITION

Manganese Sulfate is manganese(II) sulfate tetrahydrate. It contains not less than 98.0% and not more than 100.5% of $MnSO_4$, calculated with reference to the substance ignited at 450° to 500°.

CHARACTERISTICS

Pale pink crystals or crystalline powder.

Freely soluble in *water*; practically insoluble in *ethanol (96%)*.

IDENTIFICATION

A. Dissolve 0.5 g in 10 mL of *water* and add 1 mL of *sodium sulfide solution*. A pink precipitate is produced which is soluble in 6M *acetic acid*.

B. To 0.1 g add 2 g of *lead(IV) oxide* and 5 mL of *nitric acid*, boil gently for a few minutes, add 100 mL of *water* and filter. A purple solution is produced.

C. Yields the reactions characteristic of *sulfates*, Appendix VI.

TESTS

Arsenic

0.25 g complies with the *limit test for arsenic*, Appendix VII (4 ppm).

Heavy metals

Dissolve 0.50 g in 50 mL of *water*, add 1 mL of 1M *acetic acid* and pass *hydrogen sulfide* through the solution for 20 seconds. The colour produced within 2 minutes is not more intense than that obtained by treating 10 mL of *lead standard solution (2 ppm Pb)* diluted to 50 mL in the same manner (40 ppm).

Iron

Dissolve 1.0 g in 10 mL of *water* and add 2 mL of 1M *hydrochloric acid* and, dropwise, 0.05M *potassium permanganate* until a permanent pink colour is produced. Add 5 mL of a 10% w/v solution of *ammonium thiocyanate* and 20 mL of a mixture of equal volumes of *isoamyl alcohol* and *amyl acetate*, shake well and allow to separate. Any colour in the upper layer is not more intense than that obtained by treating 2 mL of *iron standard solution (20 ppm Fe)* diluted to 10 mL in the same manner (40 ppm).

Zinc

Dissolve 2.0 g in 10 mL of *water*, add 3 mL of 1M *hydrochloric acid* and 0.3 mL of a freshly prepared 3% w/v solution of *potassium hexacyanoferrate(II)*, mix and allow to stand for 15 minutes. Any turbidity produced is not more intense than that obtained by treating 10 mL of *zinc standard solution (100 ppm Zn)* in the same manner (500 ppm).

Chloride

15 mL of a 1.0% w/v solution complies with the *limit test for chlorides*, Appendix VII (330 ppm).

Loss on ignition

When ignited to constant weight at 450° to 500°, loses 31.0 to 34.0% of its weight. Use 1 g.

ASSAY

Dissolve 0.15 g in 40 mL of *water*, add 8 mL of freshly boiled and cooled *nitric acid*, cool, add 1.5 g of *sodium bismuthate* and shake for 2 minutes. Add 25 mL of a mixture of 3 volumes of *nitric acid* and 97 volumes of *water*, filter, wash the residue with 40 mL of the mixture, collecting the filtrate and washings in 50 mL of 0.1M *ammonium iron(II) sulfate VS*, and titrate immediately with 0.02M *potassium permanganate VS*. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of ammonium iron(II) sulfate required. Each mL of 0.1M *ammonium iron(II) sulfate VS* is equivalent to 3.020 mg of $MnSO_4$.

Manganese Sulfate Monohydrate

Manganese Sulphate Monohydrate

(Ph. Eur. monograph 1543)

$MnSO_4,H_2O$ 169.0 *10034-96-5*

Ph Eur

DEFINITION

Content

99.0 per cent to 101.0 per cent (ignited substance).

CHARACTERS

Appearance

Pale pink crystalline powder, slightly hygroscopic.

Solubility

Freely soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Solution S (see Tests) gives reaction (a) of sulfates (*2.3.1*).

B. Dissolve 50 mg in 5 mL of *water R*. Add 0.5 mL of *sodium sulfide solution R*. A pale pink precipitate is formed which dissolves on the addition of 1 mL of *anhydrous acetic acid R*.

C. Loss on ignition (see Tests).

TESTS

Solution S

Dissolve 10.0 g in *distilled water R* and dilute to 100 mL with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II (*2.2.1*).

Chlorides (*2.4.4*)

Maximum 100 ppm.

Dilute 5 mL of solution S to 15 mL with *water R*.

Iron (*2.4.9*)

Maximum 10 ppm, determined on solution S.

Zinc

Maximum 50 ppm.

To 10 mL of solution S add 1 mL of *sulfuric acid R* and 0.1 mL of *potassium ferrocyanide solution R*. After 30 s, any opalescence in the solution is not more intense than that in a mixture of 5 mL of *zinc standard solution (10 ppm Zn) R*, 5 mL of *water R*, 1 mL of *sulfuric acid R* and 0.1 mL of *potassium ferrocyanide solution R*.

Loss on ignition

10.0 per cent to 12.0 per cent, determined on 1.00 g at 500 ± 50 °C.

ASSAY

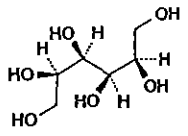
Dissolve 0.150 g in 50 mL of *water R*. Add 10 mg of *ascorbic acid R*, 20 mL of *ammonium chloride buffer solution pH 10.0 R* and 0.2 mL of a 2 g/L solution of *mordant black 11 R* in *triethanolamine R*. Titrate with *0.1 M sodium edetate* until the colour changes from violet to pure blue.

1 mL of *0.1 M sodium edetate* is equivalent to 15.10 mg of $MnSO_4$.

Ph Eur

Mannitol[1]

(Ph. Eur. monograph 0559)



$C_6H_{14}O_6$ 182.2 *69-65-8*

Action and use

Diuretic.

Preparation

Mannitol Infusion

Ph Eur

DEFINITION

D-Mannitol.

Content

97.0 per cent to 102.0 per cent (dried substance).

♦CHARACTERS

Appearance

White or almost white crystals or powder.

Solubility

Freely soluble in water, practically insoluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).♦

IDENTIFICATION

First identification: C.

◊*Second identification: A, B, D.*

A. Specific optical rotation (*2.2.7*): + 23 to + 25 (dried substance).

Dissolve 2.00 g of the substance to be examined and 2.6 g of *disodium tetraborate R* in about 20 mL of *water R* at 30 °C; shake continuously for 15-30 min without further heating. Dilute the resulting clear solution to 25.0 mL with *water R*.

B. Melting point (see Tests).◊

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison mannitol CRS.

If the spectra obtained in the solid state show differences, dissolve separately in 2 glass vials 25 mg of the substance to be examined and 25 mg of the reference substance in 0.25 mL of *distilled water R* without heating. The solutions obtained are clear. Evaporate to dryness by heating in a microwave oven with a power range of 600-700 W for 20 min or by heating in an oven at 100 °C for 1 h then gradually applying vacuum until a dry residue is obtained. Non-sticky, white or slightly yellowish powders are obtained. Record new spectra using the residues.

◊D. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 25 mg of the substance to be examined in *water R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dissolve 25 mg of *mannitol CRS* in *water R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 25 mg of *mannitol R* and 25 mg of *sorbitol R* in *water R* and dilute to 10 mL with the same solvent.

Plate TLC silica gel plate R.

[1] *This monograph has undergone pharmacopoeial harmonisation. See chapter 5.8 Pharmacopoeial harmonisation.*

Mobile phase *water R, ethyl acetate R, propanol R* (10:20:70 *V/V/V*).

Application 2 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *4-aminobenzoic acid solution R* and dry in a current of cold air until the acetone is removed; heat at 100 °C for 15 min, allow to cool then spray with a 2 g/L solution of *sodium periodate R*; dry in a current of cold air and heat at 100 °C for 15 min.

System suitability Reference solution (b):
— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).◊

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 5.0 g in *water R* and dilute to 50 mL with the same solvent.

Conductivity (*2.2.38*)

Maximum 20 µS·cm^{-1}.

Dissolve 20.0 g in *carbon dioxide-free water R* prepared from *distilled water R* by heating at 40-50 °C and dilute to 100.0 mL with the same solvent. After cooling, measure the conductivity of the solution while gently stirring with a magnetic stirrer.

Melting point (*2.2.14*)

165 °C to 170 °C.

Reducing sugars

Maximum 0.1 per cent (calculated as glucose equivalent).

To 7.0 g add 13 mL of *water R*. Boil gently with 40 mL of *cupri-tartaric solution R* for 3 min, and allow to stand for 2 min. A precipitate is formed. Filter through a sintered-glass filter (16) (*2.1.2*) coated with *diatomaceous earth R* or a sintered-glass filter (10) (*2.1.2*). Wash the precipitate with hot *water R* (about 50-60 °C) until the washing is no longer alkaline, and filter the washings through the same sintered-glass filter. Discard the filtrate. Immediately dissolve the precipitate in 20 mL of *ferric sulfate solution R*, filter through the same sintered-glass filter, and wash the filter with 15-20 mL of *water R*. Combine the washings and the filtrate, heat to 80 °C, and titrate with *0.02 M potassium permanganate*. Not more than 3.2 mL is required to change the colour of the solution from green to pink so that the colour persists for at least 10 s.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.50 g of the substance to be examined in 2.5 mL of *water R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dissolve 0.50 g of *mannitol CRS* in 2.5 mL of *water R* and dilute to 10.0 mL with the same solvent.

Reference solution (b) Dilute 2.0 mL of the test solution to 100.0 mL with *water R*.

Reference solution (c) Dilute 0.5 mL of reference solution (b) to 20.0 mL with *water R*.

Reference solution (d) Dissolve 0.25 g of *mannitol R* and 0.25 g of *sorbitol R* (impurity A) in 5 mL of *water R* and dilute to 10 mL with the same solvent.

Reference solution (e) Dissolve 0.5 g of *maltitol R* (impurity B) and 0.5 g of *isomalt R* (impurity C) in 5 mL of *water R* and dilute to 100 mL with the same solvent. Dilute 2 mL of the solution to 10 mL with *water R*.

Column:
— *size*: *l* = 0.3 m, Ø = 7.8 mm;
— *stationary phase*: *strong cation-exchange resin (calcium form) R* (9 µm);
— *temperature*: 85 ± 2 °C.

Mobile phase Degassed *water for chromatography R*.

Flow rate 0.5 mL/min.

Detection Differential refractometer maintained at a constant temperature (e.g. 40 °C).

Injection 20 µL of the test solution and reference solutions (b), (c), (d) and (e).

Run time 1.5 times the retention time of mannitol.

Identification of impurities Use the chromatogram obtained with reference solution (d) to identify the peak due to impurity A and the chromatogram obtained with reference solution (e) to identify the peaks due to impurities B and C.

Relative retention With reference to mannitol (retention time = about 20 min): impurity C (1st peak) = about 0.6; impurity B = about 0.7; impurity C (2nd peak) = about 0.73; impurity A = about 1.2. Impurity C elutes in 2 peaks. Coelution of impurity B and the 2nd peak due to impurity C may be observed.

System suitability Reference solution (d):
— *resolution*: minimum 2.0 between the peaks due to mannitol and impurity A.

Limits:
— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);
— *sum of impurities B and C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);
— *unspecified impurities*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);
— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);
— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Microbial contamination

If intended for use in the manufacture of parenteral preparations:
— TAMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

If not intended for use in the manufacture of parenteral preparations:
— TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*);
— TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*);
— absence of *Escherichia coli* (*2.6.13*);
◊—absence of *Salmonella* (*2.6.13*).◊

♦**Bacterial endotoxins** (*2.6.14*)
If intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins:
— less than 4 IU/g for parenteral preparations having a concentration of 100 g/L or less of mannitol;
— less than 2.5 IU/g for parenteral preparations having a concentration of more than 100 g/L of mannitol.♦

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

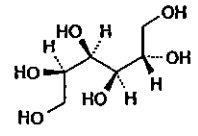
Calculate the percentage content of $C_6H_{14}O_6$ taking into account the assigned content of *mannitol CRS*.

LABELLING

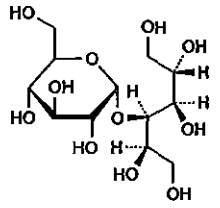
The label states:
— where applicable, the maximum concentration of bacterial endotoxins;
— where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

IMPURITIES

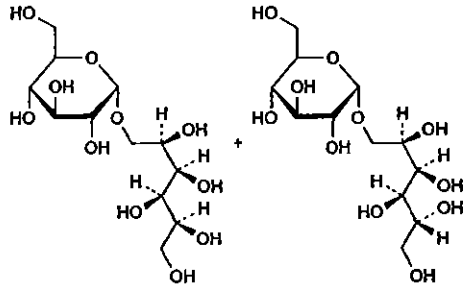
Specified impurities *A, B, C.*



A. D-glucitol (D-sorbitol),



B. 4-*O*-α-D-glucopyranosyl-D-glucitol (D-maltitol),



C. mixture of 6-*O*-α-D-glucopyranosyl-D-glucitol and 1-*O*-α-D-glucopyranosyl-D-mannitol (isomalt).

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for mannitol used as filler in tablets and capsules.

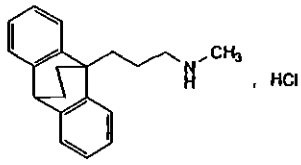
Particle-size distribution (*2.9.31* or *2.9.38*)

Powder flow (*2.9.36*)

Ph Eur

Maprotiline Hydrochloride

(Ph. Eur. monograph 1237)



$C_{20}H_{24}ClN$ 313.9 *10347-81-6*

Action and use
Antidepressant.

Ph Eur

DEFINITION

3-(9,10-Ethanoanthracen-9(10*H*)-yl)-*N*-methylpropan-1-amine hydrochloride.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Slightly soluble in water, freely soluble in methanol, soluble in ethanol (96 per cent), sparingly soluble in methylene chloride, very slightly soluble in acetone.

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 10 mg in *1 M hydrochloric acid* and dilute to 100 mL with the same acid.

Spectral range 250-300 nm.

Absorption maxima At 265 nm and 272 nm.

Absorption minimum At 268 nm.

Absorbance ratio A_{272}/A_{265} = 1.1 to 1.3.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *maprotiline hydrochloride CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 5 mL with the same solvent.

Reference solution (a) Dissolve 25 mg of *maprotiline hydrochloride CRS* in *methanol R* and dilute to 5 mL with the same solvent.

Reference solution (b) Dissolve 10 mg of *maprotiline impurity D CRS* in reference solution (a) and dilute to 2 mL with reference solution (a).

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *ethyl acetate R, dilute ammonia R1, 2-butanol R1* (4:5:14 *V/V/V*).

Application 5 µL.

Development Over half of the plate.

Drying In a current of warm air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):
— the chromatogram shows 2 clearly separated principal spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dilute 0.5 mL of solution S (see Tests) to 2 mL with *methanol R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 1.0 g in *methanol R* and dilute to 20 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.10 g of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 10.0 mL with the mobile phase. Dilute 2.0 mL of this solution to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve 1.0 mg of *maprotiline impurity D CRS* in the test solution and dilute to 10.0 mL with the test solution.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *silica gel for chromatography R* (5 µm).

Mobile phase Dissolve about 0.580 g of *ammonium acetate R* in 200 mL of *water R* and add 2 mL of a 70 g/L solution of *concentrated ammonia R*; add 150 mL of *2-propanol R* and 650 mL of *methanol R*; the resulting apparent pH value is between 8.2 and 8.4.

Flow rate 1 mL/min.

Detection Spectrophotometer at 272 nm.

Injection 20 µL.

Run time 1.5 times the retention time of maprotiline.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity D.

Relative retention With reference to maprotiline (retention time = about 10 min): impurity A = about 0.3; impurity B = about 0.5; impurity C = about 0.7; impurity D = about 0.8; impurity E = about 1.3.

System suitability Reference solution (b):
— *resolution*: 1.8 to 3.2 between the peaks due to impurity D and maprotiline; if necessary, adjust the pH of the mobile phase, in steps of 0.1 pH unit, by adding a 50 per cent *V/V* solution of *acetic acid R* if the resolution is less than 1.8, or by adding a 70 g/L solution of *concentrated ammonia R* if the resolution is greater than 3.2.

Limits:
— *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 80 °C at a pressure not exceeding 2.5 kPa for 6 h.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

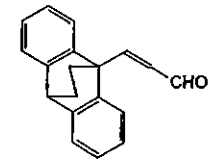
ASSAY

Dissolve 0.250 g in a mixture of 5 mL of *0.1 M hydrochloric acid* and 50 mL of *ethanol (96 per cent) R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

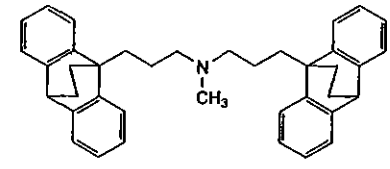
1 mL of *0.1 M sodium hydroxide* is equivalent to 31.39 mg of $C_{20}H_{24}ClN$.

IMPURITIES

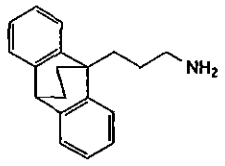
Specified impurities *A, B, C, D, E.*



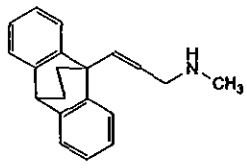
A. 3-(9,10-ethanoanthracen-9(10*H*)-yl)prop-2-enal,



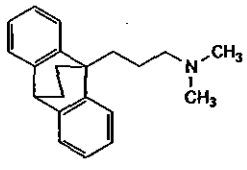
B. 3-(9,10-ethanoanthracen-9(10*H*)-yl)-*N*-[3-(9,10-ethanoanthracen-9(10*H*)-yl)propyl]-*N*-methylpropan-1-amine,



C. 3-(9,10-ethanoanthracen-9(10*H*)-yl)propan-1-amine,



D. 3-(9,10-ethanoanthracen-9(10*H*)-yl)-*N*-methylprop-2-en-1-amine (dehydromaprotiline),

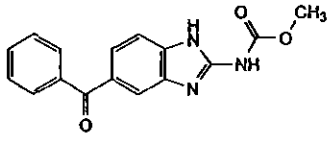


E. 3-(9,10-ethanoanthracen-9(10*H*)-yl)-*N*,*N*-dimethylpropan-1-amine.

Ph Eur

Mebendazole

(Ph. Eur. monograph 0845)



$C_{16}H_{13}N_3O_3$ 295.3 *31431-39-7*

Action and use
Benzimadazole antihelminthic.

Preparations
Mebendazole Chewable Tablets
Mebendazole Oral Suspension

Ph Eur

DEFINITION
Methyl (5-benzoyl-1*H*-benzimidazol-2-yl)carbamate.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white powder.

Solubility
Practically insoluble in water, in ethanol (96 per cent) and in methylene chloride.

It shows polymorphism (*5.9*). The acceptable crystalline form corresponds to *mebendazole CRS*.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Preparation Examine the substances without prior treatment.

Comparison *mebendazole CRS*.

TESTS
Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in *dimethylformamide R* and dilute to 25.0 mL with the same solvent.

Reference solution (a) Dissolve 5.0 mg of *mebendazole for system suitability CRS* (containing impurities A, B, C, D, E, F and G) in *dimethylformamide R* and dilute to 5.0 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *dimethylformamide R*. Dilute 5.0 mL of this solution to 20.0 mL with *dimethylformamide R*.

Column:
— *size*: *l* = 0.10 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm);
— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: 7.5 g/L solution of *ammonium acetate R*;
— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	80 → 70	20 → 30
15 - 20	70 → 10	30 → 90
20 - 25	10	90

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 250 nm.

Injection 10 µL.

Identification of impurities Use the chromatogram supplied with *mebendazole for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D, E, F and G.

Relative retention With reference to mebendazole (retention time = about 12 min): impurity A = about 0.4; impurity B = about 0.5; impurity C = about 0.7; impurity D = about 1.1; impurity E = about 1.3; impurity F = about 1.4; impurity G = about 1.6.

System suitability Reference solution (a):
— *peak-to-valley ratio*: minimum 4, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to mebendazole.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity G by 1.4;
— *impurity G*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
— *impurities A, B, C, D, E, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);
— *unspecified impurities*: for each impurity, not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

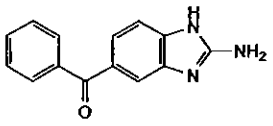
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.250 g in 3 mL of *anhydrous formic acid R* and add 50 mL of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

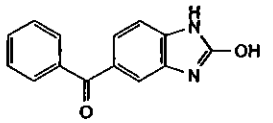
1 mL of *0.1 M perchloric acid* is equivalent to 29.53 mg of $C_{16}H_{13}N_3O_3$.

STORAGE
Protected from light.

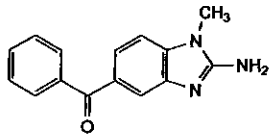
IMPURITIES
Specified impurities *A, B, C, D, E, F, G.*



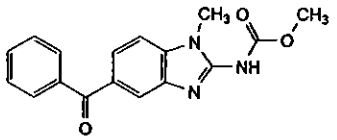
A. (2-amino-1*H*-benzimidazol-5-yl)phenylmethanone,



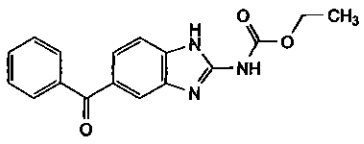
B. (2-hydroxy-1*H*-benzimidazol-5-yl)phenylmethanone,



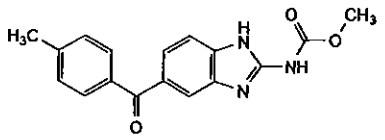
C. (2-amino-1-methyl-1*H*-benzimidazol-5-yl) phenylmethanone,



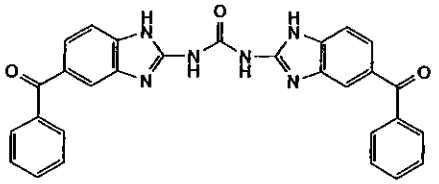
D. methyl (5-benzoyl-1-methyl-1*H*-benzimidazol-2-yl) carbamate,



E. ethyl (5-benzoyl-1*H*-benzimidazol-2-yl)carbamate,



F. methyl [5-(4-methylbenzoyl)-1*H*-benzimidazol-2-yl] carbamate,

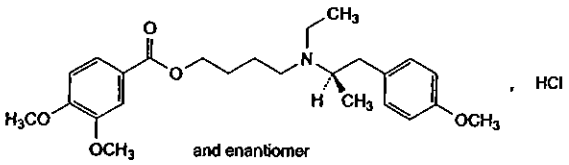


G. *N,N'*-bis(5-benzoyl-1*H*,-benzimidazol-2-yl)urea.

Ph Eur

Mebeverine Hydrochloride

(Ph. Eur. monograph 2097)



$C_{25}H_{36}ClNO_5$ 466.0 *2753-45-9*

Action and use
Smooth muscle relaxant; antispasmodic.

Preparation
Mebeverine Tablets

Ph Eur

DEFINITION
4-[Ethyl[(2*RS*)-1-(4-methoxyphenyl)propan-2-yl]amino]butyl 3,4-dimethoxybenzoate hydrochloride.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Very soluble in water and in methylene chloride, freely soluble in ethanol (96 per cent).

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *mebeverine hydrochloride CRS.*

B. Dissolve 25 mg in 2 mL of *water R*, acidify with *dilute nitric acid R* and centrifuge. The supernatant gives reaction (a) of chlorides (*2.3.1*) starting from 'add 0.4 mL of *silver nitrate solution R1*'.

TESTS
pH (*2.2.3*)
4.5 to 6.5.

Dissolve 0.2 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R, water R* (50:50 *V/V*).

Test solution Dissolve 0.100 g of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve the contents of a vial of *mebeverine for system suitability CRS* (containing impurity J) in 1 mL of the solvent mixture.

Column:
— *size*: *l* = 0.25 m, Ø = 4.0 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: mix 1 volume of *triethylamine R* and 100 volumes of *water for chromatography R* and adjust to pH 5.0 with *phosphoric acid R*;
— *mobile phase B*: *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 6	75	25
6 - 20	75 → 30	25 → 70
20 - 40	30	70

Flow rate 1 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 2 µL.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity J.

Relative retention With reference to mebeverine (retention time = about 15 min): impurity J = about 0.82.

System suitability:
— *resolution*: minimum 3.5 between the peaks due to impurity J and mebeverine in the chromatogram obtained with reference solution (b);
— *signal-to-noise ratio*: minimum 70 for the principal peak in the chromatogram obtained with reference solution (a).

Calculation of percentage contents:
— *correction factor*: multiply the peak area of impurity J by 3.5;
— for each impurity, use the concentration of mebeverine hydrochloride in reference solution (a).

Limits:
— *impurity J*: maximum 0.2 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.3 per cent;
— *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 1 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

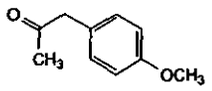
ASSAY
Dissolve 0.400 g in a mixture of 20 mL of *acetic anhydride R* and 30 mL of *glacial acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 46.60 mg of $C_{25}H_{36}ClNO_5$.

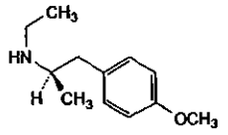
STORAGE
Protected from light.

IMPURITIES
Specified impurities *J*.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, E, F, G, H, I, K, N, O.*

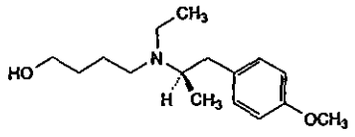


A. 1-(4-methoxyphenyl)propan-2-one,



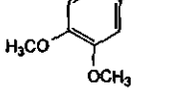
and enantiomer

B. (2*RS*)-*N*-ethyl-1-(4-methoxyphenyl)propan-2-amine,

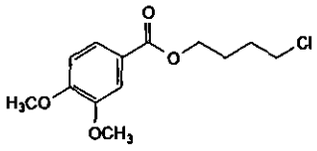


and enantiomer

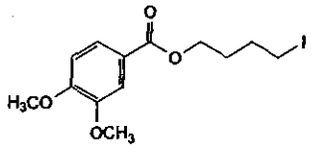
C. 4-[ethyl[(2*RS*)-1-(4-methoxyphenyl)propan-2-yl]amino] butan-1-ol,



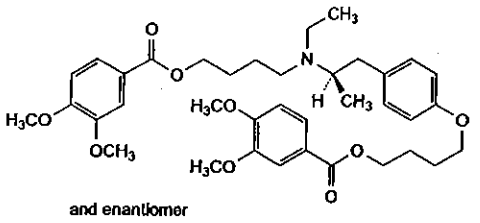
D. 3,4-dimethoxybenzoic acid,



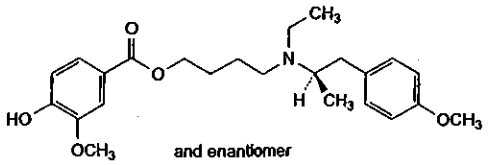
E. 4-chlorobutyl 3,4-dimethoxybenzoate,



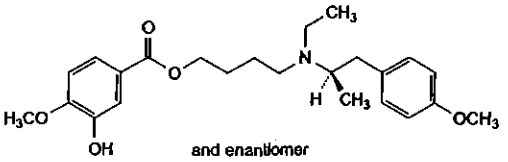
F. 4-iodobutyl 3,4-dimethoxybenzoate,



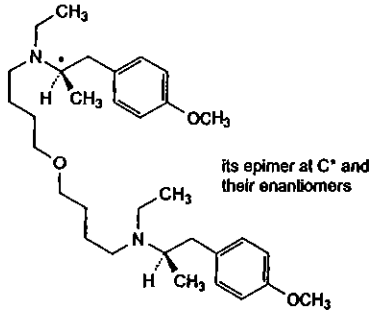
G. 4-[[(2*RS*)-1-[4-[4-[(3,4-dimethoxybenzoyl)oxy]butoxy]phenyl]propan-2-yl](ethyl)amino]butyl 3,4-dimethoxybenzoate,



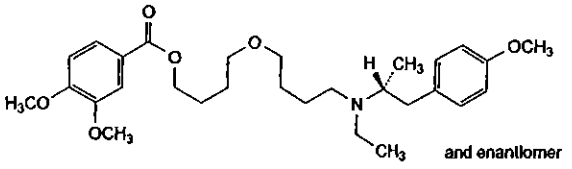
H. 4-[ethyl[(2*RS*)-1-(4-methoxyphenyl)propan-2-yl]amino]butyl 4-hydroxy-3-methoxybenzoate,



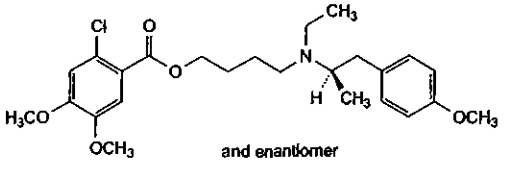
I. 4-[ethyl[(2*RS*)-1-(4-methoxyphenyl)propan-2-yl]amino]butyl 3-hydroxy-4-methoxybenzoate,



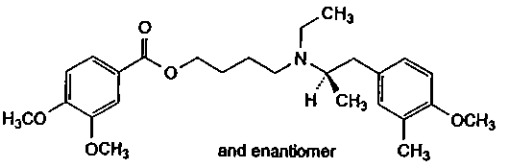
J. 4,4′-oxybis[*N*-ethyl-*N*-[(2*RS*)-1-(4-methoxyphenyl)propan-2-yl]butan-1-amine],



K. 4-[4-[ethyl[(2*RS*)-1-(4-methoxyphenyl)propan-2-yl]amino]butoxy]butyl 3,4-dimethoxybenzoate,



N. 4-[ethyl[(2*RS*)-1-(4-methoxyphenyl)propan-2-yl]amino]butyl 2-chloro-4,5-dimethoxybenzoate,

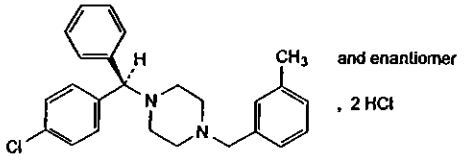


O. 4-[ethyl[(2*RS*)-1-(4-methoxy-3-methylphenyl)propan-2-yl]amino]butyl 3,4-dimethoxybenzoate.

Ph Eur

Meclozine Hydrochloride

(Meclozine Dihydrochloride, Ph. Eur. monograph 0622)



$C_{25}H_{29}Cl_3N_2$ 463.9 *1104-22-9*

Action and use
Histamine H_1 receptor antagonist; antihistamine.

Ph Eur

DEFINITION
1-[(*RS*)-(4-Chlorophenyl)phenylmethyl]-4-[(3-methylphenyl)methyl]piperazine dihydrochloride.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or yellowish-white, slightly hygroscopic, crystalline powder.

Solubility
Slightly soluble in water, soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION
First identification: B, D.
Second identification: A, C, D.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 15.0 mg in *0.1 M hydrochloric acid* and dilute to 100.0 mL with the same acid. Dilute 10.0 mL of this solution to 100.0 mL with *0.1 M hydrochloric acid.*

Spectral range 220-350 nm.

Absorption maximum At 232 nm.

Specific absorbance at the absorption maximum 345 to 380 (anhydrous substance).

The solution also shows a weak absorbance without a defined maximum between 260 nm and 300 nm.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *meclozine dihydrochloride CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 50 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 50 mg of *meclozine dihydrochloride CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel GF_{254} plate R.*

Mobile phase *diethylamine R, toluene R, cyclohexane R* (10:15:75 *V/V/V*).

Application 10 µL.

Development Over 2/3 of the plate.

Drying In a current of warm air for 5 min.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve about 15 mg in 2 mL of *ethanol (96 per cent) R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Acidity or alkalinity

Calculate the acidity or alkalinity from the titration volumes obtained in the assay using the following equation:

$$A = V_2 - 2V_1$$

V_1 = volume of *0.1 M sodium hydroxide* added at the 1st point of inflexion;

V_2 = volume of *0.1 M sodium hydroxide* added at the 2nd point of inflexion.

A is not less than −0.3 mL and not more than 0.3 mL for 0.350 g of the substance to be examined.

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R, water R* (50:50 *V/V*).

Test solution Dissolve 50 mg of the substance to be examined in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 7.5 mg of *meclozine impurity B CRS* and 7.5 mg of *meclozine impurity H CRS* in the solvent mixture and dilute to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 100.0 mL with the solvent mixture.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped ethylene-bridged octadecylsilyl silica gel for chromatography (hybrid material) R* (3.5 µm);
- *temperature*: 35 °C.

Mobile phase:
- *mobile phase A*: 0.1 per cent *V/V* solution of *concentrated ammonia R*;
- *mobile phase B*: *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	60	40
3 - 13	60 → 15	40 → 85
13 - 23	15 → 5	85 → 95
23 - 33	5	95

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 225 nm.

Injection 10 µL.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B and H.

Relative retention With reference to meclozine (retention time = about 18 min): impurity B = about 0.45; impurity H = about 0.49.

System suitability Reference solution (b):
- *resolution*: minimum 1.5 between the peaks due to impurities B and H.

Limits:
- *impurity B*: not more than 1.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)

Maximum 5.0 per cent, determined on 0.200 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.350 g in 50 mL of *ethanol (96 per cent) R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 46.39 mg of $C_{25}H_{29}Cl_3N_2$.

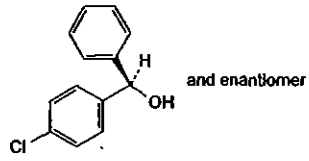
STORAGE

In an airtight container.

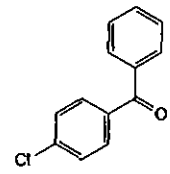
IMPURITIES

Specified impurities *B.*

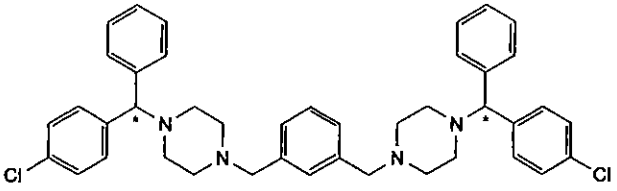
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C, D, E, F, H.*



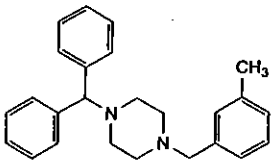
B. (*RS*)-(4-chlorophenyl)phenylmethanol,



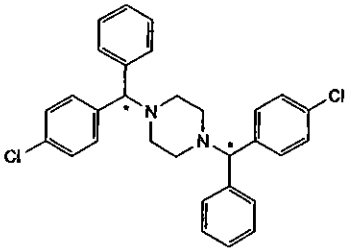
C. (4-chlorophenyl)phenylmethanone (4-chlorobenzophenone),



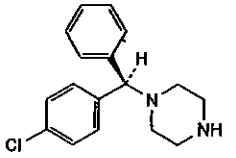
D. 1,1′-(1,3-phenylenebismethylene)bis[4-[(4-chlorophenyl)phenylmethyl]piperazine],



E. 1-(diphenylmethyl)-4-[(3-methylphenyl)methyl]piperazine,



F. 1,4-bis[(4-chlorophenyl)phenylmethyl]piperazine,



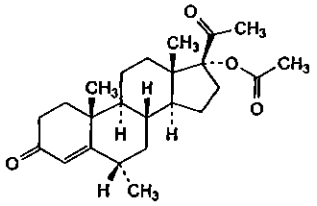
and enantiomer

H. 1-[(*RS*)-(4-chlorophenyl)phenylmethyl]piperazine.

Ph Eur

Medroxyprogesterone Acetate

(Ph. Eur. monograph 0673)



$C_{24}H_{34}O_4$ 386.5 *71-58-9*

Action and use
Progestogen.

Preparations
Medroxyprogesterone Injection
Medroxyprogesterone Tablets

Ph Eur

DEFINITION
6α-Methyl-3,20-dioxopregn-4-en-17-yl acetate.

Content
97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, freely soluble in methylene chloride, soluble in acetone, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *medroxyprogesterone acetate CRS.*

TESTS

Specific optical rotation (*2.2.7*)
+ 47 to + 53 (dried substance).
Dissolve 0.250 g in *acetone R* and dilute to 25.0 mL with the same solvent.

Impurity F
Liquid chromatography (*2.2.29*).
Test solution Dissolve 20 mg of the substance to be examined in 5.0 mL of *acetonitrile R1* and dilute to 10.0 mL with *water for chromatography R.*
Reference solution (a) Dilute 1.0 mL of the test solution to 20.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.
Reference solution (b) Dissolve 10 mg of *medroxyprogesterone acetate for peak identification CRS* (containing impurity F) in 3.0 mL of *acetonitrile R1* and dilute to 5.0 mL with *water for chromatography R.*
Column:
— *size*: *l* = 0.10 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm).
Mobile phase *water for chromatography R, acetonitrile R1* (44:56 *V/V*).
Flow rate 1.0 mL/min.
Detection Spectrophotometer at 200 nm.
Injection 25 µL.
Identification of impurities Use the chromatogram supplied with *medroxyprogesterone acetate for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity F.
Relative retention With reference to medroxyprogesterone acetate (retention time = about 8 min):
impurity F = about 1.8.
Limit:
— *correction factor*: for the calculation of content, multiply the peak area of impurity F by 1.8;
— *impurity F*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent).

Related substances
Liquid chromatography (*2.2.29*).
Solvent mixture *acetonitrile R, water R* (50:50 *V/V*).
Test solution Dissolve 20 mg of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.
Reference solution (a) Dissolve 4 mg of *medroxyprogesterone acetate for system suitability CRS* (containing impurities A, B, C, D, E, G and I) in the solvent mixture and dilute to 2.0 mL with the solvent mixture.
Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture.

Reference solution (c) Dilute 1.0 mL of reference solution (b) to 10.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.25 m, Ø = 3.0 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 60 °C.

Mobile phase *tetrahydrofuran R, acetonitrile R, water R* (12:23:65 *V/V/V*).

Flow rate 0.9 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µL.

Run time Twice the retention time of medroxyprogesterone acetate.

Identification of impurities Use the chromatogram supplied with *medroxyprogesterone acetate for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D, E, G and I.

Relative retention With reference to medroxyprogesterone acetate (retention time = about 20 min):
impurity A = about 0.3; impurity I = about 0.5;
impurity H = about 0.65; impurity B = about 0.7;
impurity C = about 0.8; impurity G = about 0.85;
impurity D = about 0.9; impurity E = about 0.95.

System suitability Reference solution (a):
— *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to medroxyprogesterone acetate.

Limits:
— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.5; impurity G = 2.6;
— *impurity D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
— *impurity B*: not more than 0.7 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.7 per cent);
— *impurity A*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent);
— *impurities C, E, G, I*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);
— *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 0.500 g by drying in an oven at 105 °C for 3 h.

ASSAY

Dissolve 50.0 mg in *ethanol (96 per cent) R* and dilute to 50.0 mL with the same solvent. Dilute 2.0 mL of the solution to 100.0 mL with *ethanol (96 per cent) R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 241 nm.

Calculate the content of $C_{24}H_{34}O_4$ taking the specific absorbance to be 420.

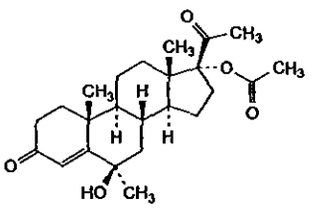
STORAGE

Protected from light.

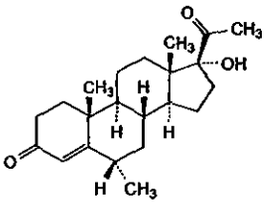
IMPURITIES

Specified impurities *A, B, C, D, E, F, G, I.*

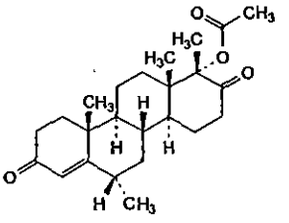
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *H.*



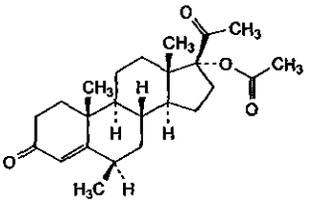
A. 6β-hydroxy-6-methyl-3,20-dioxopregn-4-en-17-yl acetate (6-hydroxymedroxyprogesterone acetate),



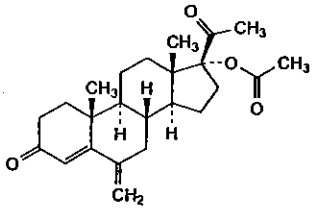
B. 17-hydroxy-6α-methylpregn-4-ene-3,20-dione (medroxyprogesterone),



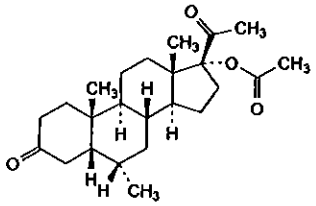
C. 6α,17a-dimethyl-3,17-dioxo-*D*-homoandrost-4-en-17aα-yl acetate,



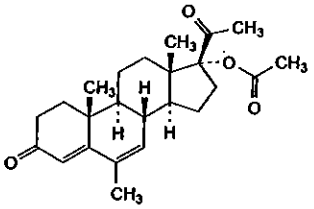
D. 6β-methyl-3,20-dioxopregn-4-en-17-yl acetate (6-epimedroxyprogesterone acetate),



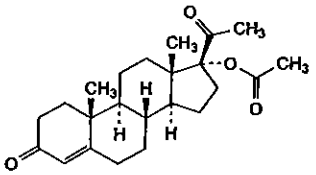
E. 6-methylidene-3,20-dioxopregn-4-en-17-yl acetate (6-methylenehydroxyprogesterone acetate),



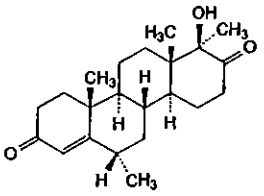
F. 6α-methyl-3,20-dioxo-5β-pregnan-17-yl acetate (4,5-dihydromedroxyprogesterone acetate),



G. 6-methyl-3,20-dioxopregna-4,6-dien-17-yl acetate (megestrol acetate),



H. 3,20-dioxopregn-4-en-17-yl acetate (hydroxyprogesterone acetate),

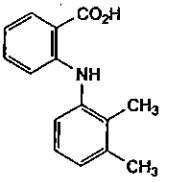


I. 17aβ-hydroxy-6,17a-dimethyl-*D*-homoandrost-4-ene-3,17-dione.

Ph Eur

Mefenamic Acid

(Ph. Eur. monograph 1240)



$C_{15}H_{15}NO_2$ 241.3 *61-68-7*

Action and use
Cyao-oxygenase Inhibitor, anargeric anti-inflammatory.

Preparations
Mefenamic Acid Capsules
Mefenamic Acid Tablets

Ph Eur

DEFINITION
2-(2,3-Dimethylanilino)benzoic acid.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, microcrystalline powder.

Solubility
Practically insoluble in water, slightly soluble in ethanol (96 per cent) and in methylene chloride. It dissolves in dilute solutions of alkali hydroxides.

It shows polymorphism (*5.9*).

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *mefenamic acid CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *ethanol (96 per cent) R*, evaporate to dryness and record new spectra using the residues.

TESTS
Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 50 mg of *2-chlorobenzoic acid R* (impurity C) and 50 mg of *benzoic acid R* (impurity D) in the mobile phase and dilute to 100 mL with the mobile phase. Dilute 1 mL of the solution to 100 mL with the mobile phase.

Reference solution (c) Dissolve 10.0 mg of *mefenamic acid impurity A CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 100.0 mL with the mobile phase.

Reference solution (d) Dissolve 20.0 mg of *benzoic acid R* in the mobile phase and dilute to 1000.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 100.0 mL with the mobile phase.

Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase:* spherical *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 14 volumes of *tetrahydrofuran R*, 40 volumes of a 5.75 g/L solution of *ammonium dihydrogen phosphate R* adjusted to pH 5.0 with *dilute ammonia R2*, and 46 volumes of *acetonitrile R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µL.

Run time 4 times the retention time of mefenamic acid.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities C and D.

Relative retention With reference to mefenamic acid (retention time = about 8 min): impurity C = about 0.3; impurity D = about 0.35; impurity A = about 0.5.

System suitability:
— *resolution:* minimum 3.0 between the peaks due to impurities C and D in the chromatogram obtained with reference solution (b);
— *signal-to-noise ratio:* minimum 10 for the principal peak in the chromatogram obtained with reference solution (d).

Limits:
— *correction factors:* for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity C = 5.9; impurity D = 4.0;
— *impurities C, D:* for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *impurity A:* not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (100 ppm);
— *unspecified impurities:* for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total:* not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *disregard limit:* 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent); disregard the peak due to impurity A.

Loss on drying *(2.2.32)*
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

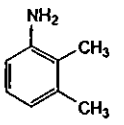
Sulfated ash *(2.4.14)*
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve with the aid of ultrasound 0.200 g in 100 mL of warm *anhydrous ethanol R*, previously neutralised to *phenol red solution R*. Add 0.1 mL of *phenol red solution R* and titrate with *0.1 M sodium hydroxide*.

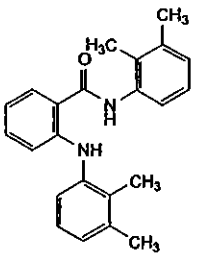
1 mL of *0.1 M sodium hydroxide* is equivalent to 24.13 mg of $C_{15}H_{15}NO_2$.

IMPURITIES
Specified impurities *A, C, D.*

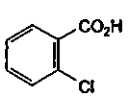
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, E.*



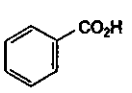
A. 2,3-dimethylaniline,



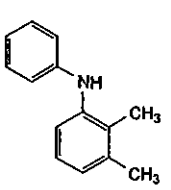
B. 2-(2,3-dimethylanilino)-*N*-(2,3-dimethylphenyl) benzamide,



C. 2-chlorobenzoic acid,



D. benzoic acid,

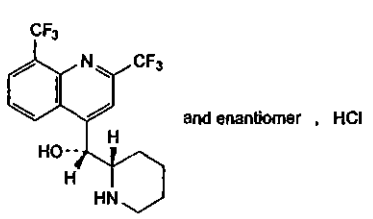


E. 2,3-dimethyl-*N*-phenylaniline.

Ph Eur

Mefloquine Hydrochloride

(Ph. Eur. monograph 1241)



$C_{17}H_{17}ClF_6N_2O$ 414.8 *51773-92-3*

Action and use
Antiprotozoal (malaria).

Ph Eur

DEFINITION

(*RS*)-[2,8-Bis(trifluoromethyl)quinolin-4-yl][(2*SR*)-piperidin-2-yl]methanol hydrochloride.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or slightly yellow, crystalline powder.

Solubility

Very slightly soluble in water, freely soluble in methanol, soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

mp

About 260 °C, with decomposition.

IDENTIFICATION

First identification: A, E.

Second identification: B, C, D, E.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *mefloquine hydrochloride CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 8 mg of the substance to be examined in *methanol R* and dilute to 5 mL with the same solvent.

Reference solution (a) Dissolve 8 mg of *mefloquine hydrochloride CRS* in *methanol R* and dilute to 5 mL with the same solvent.

Reference solution (b) Dilute 2.5 mL of the test solution to 100 mL with *methanol R.*

Reference solution (c) To 1 mL of reference solution (b) add 1 mL of a 0.016 g/L solution of *quinidine sulfate R* in *methanol R.*

Plate *TLC silica gel F_{254} plate R.*

Pretreatment Develop the plate with a mixture of 20 volumes of *methanol R* and 80 volumes of *methylene chloride R*, and dry at 100-105 °C for 15 min before use.

Mobile phase *anhydrous acetic acid R, methanol R, methylene chloride R* (10:10:80 *V/V*).

Application 20 µL.

Development Over a path of 10 cm.

Drying In a current of warm air for 15 min.

Detection Examine in ultraviolet light at 254 nm; lightly spray with a mixture prepared immediately before use of 1 volume of *sulfuric acid R* and 40 volumes of *iodoplatinate reagent R*; spray with *strong hydrogen peroxide solution R.*

System suitability Reference solution (c):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Mix about 10 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until a practically white residue is obtained. Allow to cool, then add 2 mL of *water R*, 0.05 mL of *phenolphthalein solution R1* and about 1 mL of *dilute hydrochloric acid R* to make the solution colourless. Filter. To the filtrate add a freshly prepared mixture of 0.1 mL of *alizarin S solution R* and 0.1 mL of *zirconyl nitrate solution R.* Mix, allow to stand for 5 min and compare the colour of the solution with a blank prepared in the same manner. The test solution is yellow and the blank is red.

D. To about 20 mg add 0.2 mL of *sulfuric acid R.* Blue fluorescence appears in ultraviolet light at 365 nm.

E. It gives reaction (b) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 2.50 g in *methanol R* and dilute to 50.0 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_7 (*2.2.2, Method I*).

Optical rotation (*2.2.7*)

−0.2° to + 0.2°, determined on solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.10 g of the substance to be examined in the mobile phase and dilute to 25.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 50.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 20.0 mL with the mobile phase.

Reference solution (b) Dissolve 8 mg of *mefloquine hydrochloride CRS* and 8 mg of *quinidine sulfate R* in the mobile phase, then dilute to 50.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 100.0 mL with the mobile phase.

Precolumn:

— *size: l* = 0.025 m, Ø = 4 mm;

— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Column:

— *size: l* = 0.25 m, Ø = 4 mm;

— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 1 g of *tetraheptylammonium bromide R* in a mixture of 200 volumes of *methanol R*, 400 volumes of a 1.5 g/L solution of *sodium hydrogen sulfate R* and 400 volumes of *acetonitrile R.*

Flow rate 0.8 mL/min.

Detection Spectrophotometer at 280 nm.

Equilibration With the mobile phase at a flow rate of 2 mL/min for about 30 min.

Injection 20 µL.

Run time 10 times the retention time of mefloquine.

Retention time Quinidine = about 2 min; mefloquine = about 4 min; impurity B = about 15 min; impurity A = about 36 min.

System suitability Reference solution (b):

— *resolution*: minimum 8.5 between the peaks due to quinidine and mefloquine.

Limits:

— *impurity with a relative retention with reference to mefloquine of about 0.7*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *sum of impurities other than the impurity with a relative retention with reference to mefloquine of about 0.7*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

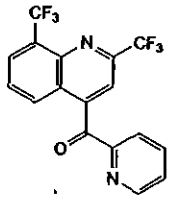
Water (*2.5.12*)
Maximum 3.0 per cent, determined on 1.000 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

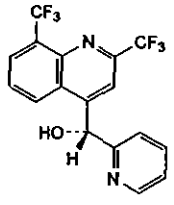
ASSAY
Dissolve 0.350 g in 15 mL of *anhydrous formic acid R* and add 40 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).
1 mL of *0.1 M perchloric acid* is equivalent to 41.48 mg of $C_{17}H_{17}ClF_6N_2O$.

STORAGE
Protected from light.

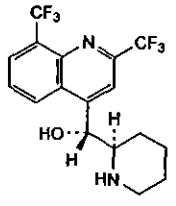
IMPURITIES



A. [2,8-bis(trifluoromethyl)quinolin-4-yl](pyridin-2-yl) methanone,



B. (*RS*)-[2,8-bis(trifluoromethyl)quinolin-4-yl](pyridin-2-yl) methanol,

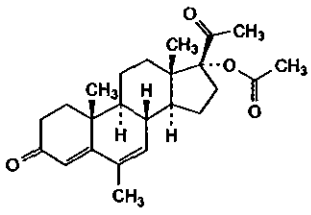


C. (*RS*)-[2,8-bis(trifluoromethyl)quinolin-4-yl][(2*RS*)-piperidin-2-yl]methanol.

Ph Eur

Megestrol Acetate

(Ph. Eur. monograph 1593)



$C_{24}H_{32}O_4$ 384.5 *595-33-5*

Action and use
Progestogen.

Preparation
Megestrol Tablets

Ph Eur

DEFINITION
6-Methyl-3,20-dioxopregna-4,6-dien-17-yl acetate.

Content
97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, soluble in acetone, sparingly soluble in ethanol (96 per cent).

mp
About 217 °C.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *megestrol acetate CRS*.

TESTS

Specific optical rotation (*2.2.7*)
+ 14.0 to + 17.0 (dried substance).
Dissolve 2.50 g in *methylene chloride R* and dilute to 25.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).
Solvent mixture *acetic acid R, water R, acetonitrile R1* (0.1:20:80 *V/V/V*).
Test solution (a) Dissolve 0.100 g of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.
Test solution (b) Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.
Reference solution (a) Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.
Reference solution (b) Dissolve 10 mg of *megestrol acetate for system suitability CRS* (containing impurities A, D, G, H, I, J and L) in 1.0 mL of the solvent mixture.
Reference solution (c) Dissolve 10 mg of *megestrol acetate for peak identification CRS* (containing impurities B, C and E) in 1.0 mL of the solvent mixture.

Reference solution (d) Dissolve 50.0 mg of *megestrol acetate CRS* in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (e) Dissolve the contents of a vial of *megestrol acetate for impurity K identification CRS* in 1.0 mL of the solvent mixture.

Column:
- *size*: *l* = 0.15 m; Ø = 4.6 mm;
- *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (3 µm);
- *temperature*: 40 °C.

Mobile phase:
- *mobile phase A*: *tetrahydrofuran R, acetonitrile R1, water R* (7.5:12.5:80 *V/V/V*);
- *mobile phase B*: *water R, tetrahydrofuran R, acetonitrile R1* (20:30:50 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 16	70	30
16 - 42	70 → 30	30 → 70
42 - 49	30	70

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 245 nm and, for impurity J, at 210 nm.

Injection 20 µL of test solution (a) and reference solutions (a), (b), (c) and (e).

Identification of impurities Use the chromatogram supplied with *megestrol acetate for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, D, G, H, I, J and L; use the chromatogram supplied with *megestrol acetate for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities B, C and E; use the chromatogram obtained with reference solution (e) to identify the peak due to impurity K.

Relative retention With reference to megestrol acetate (retention time = about 22 min): impurity B = about 0.75; impurity E = about 0.80; impurity K = about 0.83; impurity C = about 0.9; impurity D = about 1.11; impurity A = about 1.14; impurity I = about 1.2; impurity G = about 1.3; impurity J = about 1.4; impurity H = about 1.5; impurity L = about 1.9.

System suitability Reference solution (b):
- *peak-to-valley ratio*: minimum 5.0, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity A.

Limits:
- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.2; impurity D = 0.4; impurity E = 0.4; impurity I = 0.5; impurity K = 0.2; impurity L = 0.6;
- *impurity A*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *impurities D, H*: for each impurity, not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
- *impurity J at 210 nm*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) at 210 nm (0.3 per cent);
- *impurity G*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *impurities B, C, E, I, K, L*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *sum of impurities other than J*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

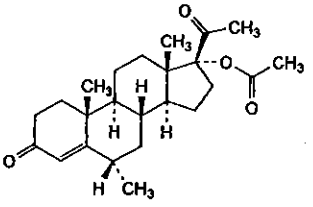
Injection Test solution (b) and reference solution (d).

Calculate the percentage content of $C_{24}H_{32}O_4$ taking into account the assigned content of *megestrol acetate CRS*.

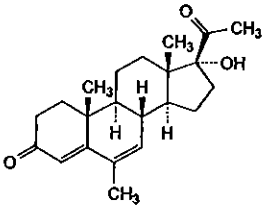
STORAGE
Protected from light.

IMPURITIES
Specified impurities *A, B, C, D, E, G, H, I, J, K, L.*

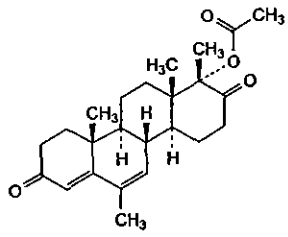
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *F.*



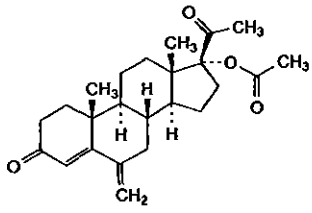
A. 6α-methyl-3,20-dioxopregn-4-en-17-yl acetate (medroxyprogesterone acetate),



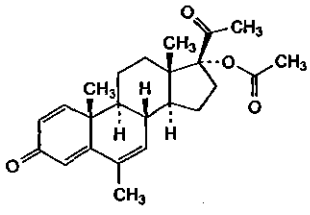
B. 6-methyl-17-hydroxypregna-4,6-diene-3,20-dione (megestrol),



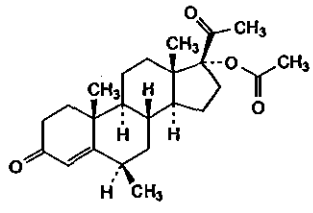
C. 6,17a-dimethyl-3,17-dioxo-*D*-homoandrosta-4,6-dien-17aα-yl acetate (*D-homo* megestrol acetate),



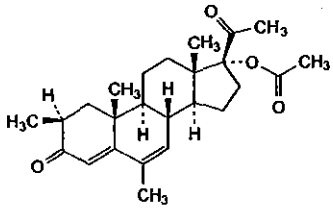
D. 6-methylene-3,20-dioxopregn-4-en-17-yl acetate (6-methylene hydroxyprogesterone acetate),



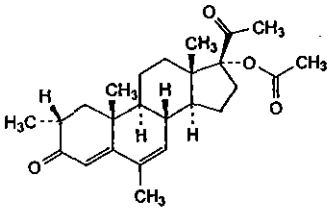
E. 6-methyl-3,20-dioxopregna-1,4,6-trien-17-yl acetate,



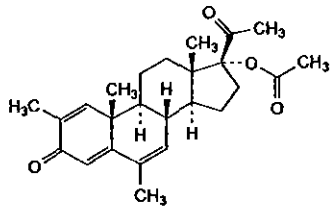
F. 6β-methyl-3,20-dioxopregn-4-en-17-yl acetate,



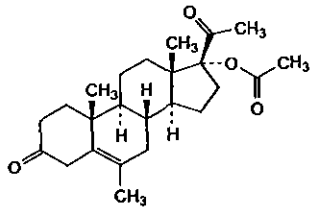
G. 2β,6-dimethyl-3,20-dioxopregna-4,6-dien-17-yl acetate,



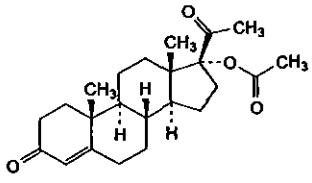
H. 2α,6-dimethyl-3,20-dioxopregna-4,6-dien-17-yl acetate,



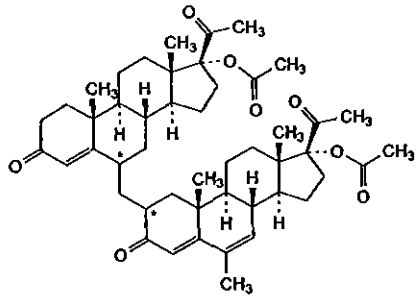
I. 2,6-dimethyl-3,20-dioxopregna-1,4,6-trien-17-yl acetate,



J. 6-methyl-3,20-dioxopregn-5-en-17-yl acetate,



K. 3,20-dioxopregn-4-en-17-yl acetate,

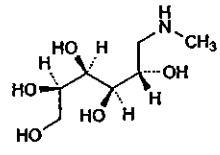


L. 2ξ-[[17-(acetyloxy)-3,20-dioxopregn-4-en-6ξ-yl]methyl]-6-methyl-3,20-dioxopregna-4,6-dien-17-yl acetate (megestrol acetate dimer).

Ph Eur

Meglumine

(Ph. Eur. monograph 2055)



$C_7H_{17}NO_5$ 195.2 *6284-40-8*

Action and use
Organic base used in the preparation of organic acids.

Ph Eur

DEFINITION
1-Deoxy-1-(methylamino)-D-glucitol.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water, sparingly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

mp
About 128 °C.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *meglumine CRS.*

TESTS

Solution S
Dissolve 20.0 g in *distilled water R* and dilute to 100.0 mL with the same solvent.

Appearance of solution
The solution is clear (*2.2.1*) and its absorbance (*2.2.25*) at 420 nm is not greater than 0.03.

Dissolve the residue obtained in the test for loss on drying in *water R* and dilute to 10 mL with the same solvent.

Specific optical rotation (*2.2.7*)
−17.0 to −16.0 (dried substance).

Dilute 12.5 mL of solution S to 25.0 mL with *water R.*

Reducing substances
Maximum 0.2 per cent, expressed as glucose.

Dilute 1.25 mL of solution S to 2.5 mL with *water R,* add 2 mL of *cupri-tartaric solution R* and heat on a water-bath for 10 min. Cool under running water for 1 min, then sonicate for 20 s. Immediately filter through a membrane filter 25 mm in diameter (nominal pore size 0.5 µm). Rinse with 10 mL of *water R.* Prepare a standard in the same manner using 2.5 mL of a solution obtained by dissolving 20 mg of *glucose R* in *water R* and diluting to 100 mL with the same solvent. Any precipitate on the membrane filter obtained with the test solution is not more intensely coloured than the precipitate obtained with the standard.

Chlorides (*2.4.4*)
Maximum 100 ppm.

To 2.5 mL of solution S add 12.5 mL of *water R.*

Sulfates (*2.4.13*)
Maximum 150 ppm.

To 5 mL of solution S add 10 mL of *distilled water R.*

Aluminium
Maximum 5 ppm.

Inductively coupled plasma-atomic emission spectrometry (ICP-AES) (*2.2.57*).

Test solution Dissolve 5.00 g in 30 mL of *water R,* add 10.0 mL of *lead-free hydrochloric acid R* and dilute to 50.0 mL with *water R.*

Reference solutions Prepare the reference solutions using *aluminium standard solution (10 ppm Al) R,* diluted as necessary with *water R.*

Wavelength 396.153 nm.

Iron
Maximum 10 ppm.

To 10 mL of solution S add about 0.8 mL of *hydrochloric acid R* and 0.05 mL of *bromine water R.* Allow to stand for 5 min, evaporate the excess of bromine in a current of air and add 3 mL of *potassium thiocyanate solution R.* Prepare a reference solution at the same time and in the same manner using 10 mL of *iron standard solution (2 ppm Fe) R,* to which 2 mL of *hydrochloric acid R* has been added. After 5 min, any red colour in the test solution is not more intense than that in the reference solution.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

Bacterial endotoxins (*2.6.14*)
Less than 1.5 IU/g, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

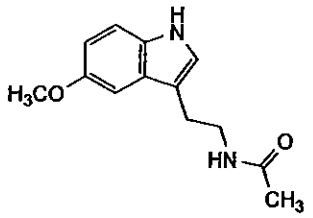
ASSAY

Dissolve 0.180 g in 30 mL of *water R.* Titrate with *0.05 M sulfuric acid,* determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.05 M sulfuric acid* is equivalent to 19.52 mg of $C_7H_{17}NO_5$.

Ph Eur

Melatonin



$C_{13}H_{16}N_2O_2$ 232.3 *73-31-4*

Action and use
Treatment of sleep onset insomnia.

Preparation
Melatonin Capsules

DEFINITION

Melatonin is *N*-acetyl-5-methoxytryptamine. It contains not less than 98.0% and not more than 102.0% of $C_{13}H_{16}N_2O_2$, calculated with reference to the anhydrous substance.

CHARACTERISTICS

A white to off-white crystalline powder. It melts at about 117°.

Slightly soluble in *water;* soluble in *acetone, ethyl acetate* and *methanol.*

IDENTIFICATION

The *infrared absorption spectrum,* Appendix II A, is concordant with the *reference spectrum* of melatonin (*RS 455*).

TESTS

Related substances
Carry out the method for *liquid chromatography,* Appendix III D, using the following solutions in a mixture of 20 volumes of *acetonitrile* and 80 volumes of *water.*

(1) Dissolve sufficient of the substance being examined to produce a solution containing 0.05% w/v of Melatonin.

(2) 0.0005% w/v of *melatonin BPCRS.*

(3) Dilute a mixture of 10 volumes of solution (2) and 1 volume of a 0.025% w/v solution of *5-methoxytryptamine BPCRS* to 100 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (1.8 µm) (Zorbax Eclipse Plus is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 25°.

(e) Use a detection wavelength of 225 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 0.245% w/v of *potassium dihydrogen orthophosphate*, adjusted to pH 3.0 with 20% v/v *orthophosphoric acid.*

Mobile phase B *acetonitrile.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-20	90→70	10→30	linear gradient
20-36	70→30	30→70	linear gradient
36-41	30→90	70→10	re-equilibration

When the chromatograms are recorded under the prescribed conditions the retention time of melatonin is about 7.3 minutes and the retention of 5-methoxtryptamine relative to that of melatonin is about 0.3.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to 5-methoxytryptamine is not greater than 0.2 times the area of the peak due to 5-methoxytryptamine in the chromatogram obtained with solution (3) (0.1%);

the area of any other *secondary peak* is not greater than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the sum of total impurities is not more than 1.0%.

Disregard any peak with an area less than 0.5 times the area of the peak due to melatonin in the chromatogram obtained with solution (3) (0.05%).

Water

Not more than 0.3%, Appendix IX C. Use 1 g.

Sulfated ash

Not more than 0.1%, Appendix IX A. Use 1 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) Dissolve 0.1 g of the substance being examined in 100 mL and dilute 1 volume of the resulting solution to 20 volumes.

(2) 0.005% w/v of *melatonin BPCRS.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (1.8 µm) (Zorbax Eclipse Plus is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 25°.

(e) Use a detection wavelength of 225 nm.

(f) Inject 5 µL of each solution.

MOBILE PHASE

20 volumes of *acetonitrile* and 80 volumes of a 0.245% w/v solution of *potassium dihydrogen orthophosphate*, the pH of which has been adjusted to 3.0 with 20% v/v *orthophosphoric acid.*

When the chromatograms are recorded under the prescribed conditions, the retention time of melatonin is about 2.3 minutes.

DETERMINATION OF CONTENT

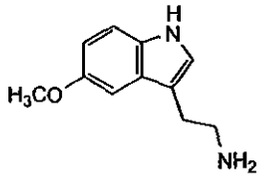
Calculate the content of $C_{13}H_{16}N_2O_2$ in the substance being examined using the declared content of $C_{13}H_{16}N_2O_2$ in *melatonin BPCRS.*

STORAGE

Melatonin should be protected from light.

IMPURITIES

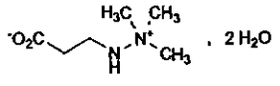
The impurities limited by the requirements of this monograph include:



A. 2-(5-methoxy-1*H*-indol-3-yl)ethanamine (5-methoxytryptamine).

Meldonium Dihydrate

(Ph. Eur. monograph 2624)



$C_6H_{14}N_2O_2,2H_2O$ 182.2 *86426-17-7*

Ph Eur

DEFINITION

3-(2,2,2-Trimethylhydrazin-2-ium-1-yl)propanoate dihydrate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white crystals or crystalline powder, deliquescent.

Solubility

Very soluble in water, freely soluble in methanol, practically insoluble in acetone.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *meldonium dihydrate CRS.*

TESTS

Solution S

Dissolve 10.0 g in 50 mL of *distilled water R.*

Appearance of solution
Solution S is not more opalescent than reference suspension I (*2.2.1*) and not more intensely coloured than reference solution B_9 (*2.2.2, Method II*).

Related substances
Liquid chromatography (*2.2.29*) coupled with mass spectrometry (*2.2.43*).

Test solution Dissolve 10.0 mg of the substance to be examined in mobile phase A and dilute to 10.0 mL with mobile phase A.

Reference solution (a) Dissolve 10.0 mg of *meldonium impurity A CRS*, 10.0 mg of *meldonium impurity B CRS*, 10.0 mg of *meldonium impurity C CRS*, 10.0 mg of *meldonium impurity D CRS*, 10.0 mg of *meldonium impurity E CRS* and 10.0 mg of *meldonium impurity F CRS* in mobile phase A and dilute to 100.0 mL with mobile phase A.

Reference solution (b) Dilute 1.5 mL of reference solution (a) to 100.0 mL with mobile phase A.

Reference solution (c) Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

Column:
— *size*: *l* = 0.15 m, Ø = 3.0 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3.5 µm).

Mobile phase:
— *mobile phase A*: 0.1 per cent *V/V* solution of *heptafluorobutyric acid R* in *water for chromatography R*;
— *mobile phase B*: 0.1 per cent *V/V* solution of *heptafluorobutyric acid R* in *methanol R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 7	90 → 65	10 → 35
7 - 12	65 → 40	35 → 60
12 - 18	40 → 25	60 → 75

Flow rate 0.2 mL/min.

Detection:
— triple-quadrupole mass spectrometer; the following settings have been found to be suitable and are given as examples; if the detector has different setting parameters, adjust the detector settings so as to comply with the system suitability criteria:
  — *ionisation*: ESI-positive;
  — *detection*:
  — multiple reaction monitoring (MRM) mode;
  — scanning mode: 50 - 300 *m/z*;
  — *ion source temperature*: 110 °C;
  — *desolvation temperature*: 220 °C;
  — *cone voltage*: 15 V;
  — *capillary voltage (Vcap)*: 3 kV;
  — *CID gas*: argon;
  — *CID gas pressure*: 2.7 × 10^{-3} mbar.
— MRM mode parameters:

Substance	MRM channels	Collision energy (eV) for the interval 0-25 min
Meldonium	147.00 → 59.00	18.0
Impurity A	59.97 → 44.98	18.0
Impurity B	74.99 → 59.04	15.0
Impurity F	115.19 → 71.92	19.0
Impurity C	161.19 → 59.00	23.0
Impurity D	175.19 → 58.07	23.0
Impurity E	189.26 → 58.01	22.0

Injection 20 µL of the test solution and reference solutions (b) and (c).

Relative retention With reference to meldonium (retention time = about 9 min): impurity F = about 0.7; impurity B = about 0.8; impurity A = about 0.9; impurity C = about 1.5; impurity D = about 1.8; impurity E = about 2.0.

System suitability Reference solution (b):
— *signal-to-noise ratio*: impurity A = minimum 10; impurity B = minimum 50; impurity C = minimum 200; impurity D = minimum 1000; impurity E = minimum 1000; impurity F = minimum 50;
— *repeatability*: maximum relative standard deviation of 10 per cent after 6 injections.

Calculation of percentage contents:
— for impurities A, B, C, D, E and F (MRM mode), use the concentration of the corresponding impurity in reference solution (b);
— for impurities other than A, B, C, D, E and F (complete spectrum mode), use the concentration of meldonium dihydrate in reference solution (c).

Limits:
— *impurities A, B, C, D, E, F*: for each impurity, maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.3 per cent;
— *reporting threshold*: 0.05 per cent.

Chlorides (*2.4.4*)
Maximum 100 ppm.

Dilute 5 mL of solution S to 30 mL with *distilled water R*.

Sulfates (*2.4.13*)
Maximum 100 ppm.

Dilute 10 mL of solution S to 20 mL with *distilled water R*.

Water (*2.5.12*)
19.7 per cent to 21.0 per cent, determined on 0.120 g.

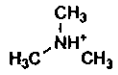
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.100 g in 40 mL of *glacial acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

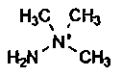
1 mL of *0.1 M perchloric acid* is equivalent to 14.62 mg of $C_6H_{14}N_2O_2$.

STORAGE
In an airtight container.

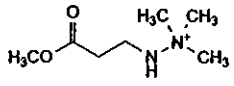
IMPURITIES
Specified impurities *A, B, C, D, E, F.*



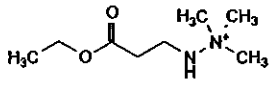
A. *N,N*-dimethylmethanaminium,



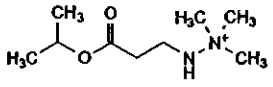
B. 1,1,1-trimethylhydrazin-1-ium,



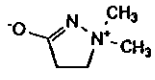
C. 2-(3-methoxy-3-oxopropyl)-1,1,1-trimethylhydrazin-1-ium,



D. 2-(3-ethoxy-3-oxopropyl)-1,1,1-trimethylhydrazin-1-ium,



E. 1,1,1-trimethyl-2-[3-(1-methylethoxy)-3-oxopropyl] hydrazin-1-ium,

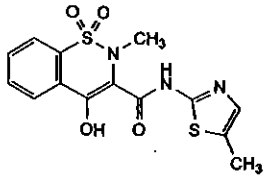


F. 1,1-dimethyl-4,5-dihydro-1*H*-pyrazol-1-ium-3-olate.

Ph Eur

Meloxicam

(Ph. Eur. monograph 2373)



$C_{14}H_{13}N_3O_4S_2$ 351.4 *71125-38-7*

Action and use
Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Preparations
Meloxicam Injection
Meloxicam Oral Suspension
Meloxicam Tablets

Ph Eur

DEFINITION
4-Hydroxy-2-methyl-*N*-(5-methylthiazol-2-yl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
Pale yellow powder.

Solubility
Practically insoluble in water, soluble in dimethylformamide, very slightly soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *meloxicam CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *acetone R*, evaporate to dryness and record new spectra using the residues.

TESTS
Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 40 mg of the substance to be examined in a mixture of 5 mL of *methanol R* and 0.3 mL of *1 M sodium hydroxide* and dilute to 20.0 mL with *methanol R*.

Reference solution (a) Dilute 2.0 mL of the test solution to 100.0 mL with *methanol R*. Dilute 5.0 mL of this solution to 100.0 mL with *methanol R*.

Reference solution (b) Dissolve 2 mg of the substance to be examined, 2 mg of *meloxicam impurity A CRS*, 2 mg of *meloxicam impurity B CRS*, 2 mg of *meloxicam impurity C CRS* and 2 mg of *meloxicam impurity D CRS* in a mixture of 5 mL of *methanol R* and 0.3 mL of *1 M sodium hydroxide* and dilute to 25 mL with *methanol R*. If complete dissolution cannot be achieved, filter the solution through a membrane filter (nominal pore size 0.45 µm) before injection.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 45 °C.

Mobile phase:
— *mobile phase A*: 1 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 6.0 with *1 M sodium hydroxide*;
— *mobile phase B*: *methanol R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	60	40
2 - 10	60 → 30	40 → 70
10 - 15	30	70

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 260 nm and 350 nm.

Injection 10 µL.

Relative retention With reference to meloxicam (retention time = about 7 min): impurity B = about 0.5; impurity A = about 1.4; impurity C = about 1.7; impurity D = about 1.9.

System suitability Reference solution (b):
— *resolution*: minimum 3.0 between the peaks due to meloxicam and impurity A at 350 nm; minimum 3.0 between the peaks due to impurity B and meloxicam at 260 nm.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity A by 2.0;

— *impurity A at 350 nm*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) at 350 nm (0.1 per cent);
— *impurity B at 260 nm*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) at 350 nm (0.1 per cent);
— *impurities C, D at 350 nm*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) at 350 nm (0.05 per cent);
— *unspecified impurities*: for each impurity, at the wavelength giving the higher value for the impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) at the same wavelength (0.10 per cent);
— *total*: not more than 0.3 per cent;
— *disregard limit*: 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) at the same wavelength (0.03 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

In order to avoid overheating during the titration, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.

Dissolve 0.250 g in a mixture of 5 mL of *anhydrous formic acid R* and 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

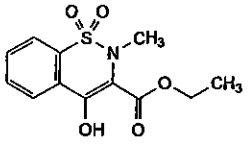
1 mL of *0.1 M perchloric acid* is equivalent to 35.14 mg of $C_{14}H_{13}N_3O_4S_2$.

STORAGE
Protected from light.

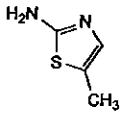
IMPURITIES

Specified impurities *A, B, C, D.*

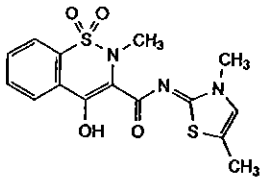
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *E, F.*



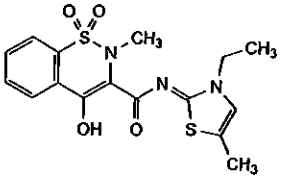
A. ethyl 4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide,



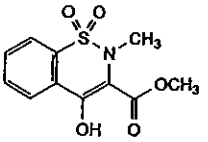
B. 5-methylthiazol-2-amine,



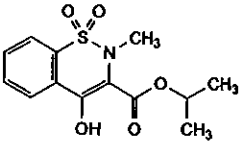
C. *N*-[(2*Z*)-3,5-dimethylthiazol-2(3*H*)-ylidene]-4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide,



D. *N*-[(2*Z*)-3-ethyl-5-methylthiazol-2(3*H*)-ylidene]-4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide,



E. methyl 4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide,

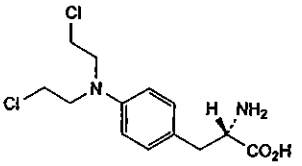


F. isopropyl 4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide.

Ph Eur

Melphalan

(Ph. Eur. monograph 1698)



$C_{13}H_{18}Cl_2N_2O_2$ 305.2 *148-82-3*

Action and use
Cytotoxic alkylating agent.

Preparations
Melphalan Tablets
Melphalan for Injection

Ph Eur

DEFINITION
4-[Bis(2-chloroethyl)amino]-L-phenylalanine.

Content
94.0 per cent to 102.0 per cent (anhydrous and diethylamine-free substance).

CHARACTERS

Appearance

White or almost white, hygroscopic powder.

Solubility

Practically insoluble in water, slightly soluble in methanol. It dissolves in dilute mineral acids.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of melphalan.*

TESTS

Appearance of solution

If intended for use in the manufacture of parenteral preparations, the solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 0.25 g in *dilute hydrochloric acid R* and dilute to 25 mL with the same acid.

Specific optical rotation (*2.2.7*)

−36.0 to −30.0 (anhydrous and diethylamine-free substance).

Dissolve 0.175 g at 45 °C for 10 min in *methanol R* and dilute to 25.0 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*). *Use freshly prepared solutions and protect from light.*

Test solution (a) Dissolve 50.0 mg of the substance to be examined in *methanol R1* and dilute to 50.0 mL with the same solvent.

Test solution (b) Dilute 1.0 mL of test solution (a) to 10.0 mL with *methanol R1*.

Reference solution (a) Dissolve 50.0 mg of *melphalan hydrochloride CRS* in *methanol R1* and dilute to 50.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with *methanol R1*.

Reference solution (b) Dilute 10.0 mL of test solution (a) to 100.0 mL with *methanol R1*.

Reference solution (c) Dilute 1.0 mL of reference solution (b) to 100.0 mL with *methanol R1*.

Reference solution (d) Dilute 5.0 mL of reference solution (b) to 100.0 mL with *methanol R1*.

Reference solution (e) In order to prepare impurity I *in situ*, dissolve 5 mg of *melphalan for system suitability CRS* (containing impurities B, D, G, H and J) in *methanol R1*, dilute to 5.0 mL with the same solvent and heat at 60 °C for 15 min.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A*: mixture of 5 volumes of *acetonitrile for chromatography R* and 95 volumes of *water R* containing 0.01 per cent *V/V* of *triethylamine R*, 0.05 per cent *m/m* of *ammonium acetate R* and 0.05 per cent *V/V* of *glacial acetic acid R*;
- *mobile phase B*: mixture of 40 volumes of *water R* containing 0.01 per cent *V/V* of *triethylamine R*, 0.05 per cent *m/m* of *ammonium acetate R* and 0.05 per cent *V/V* of *glacial acetic acid R*, and 60 volumes of *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	100 → 0	0 → 100
20 - 25	0	100

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 260 nm.

Injection 20 µL of test solution (a) and reference solutions (c), (d) and (e).

Identification of impurities Use the chromatogram supplied with *melphalan for system suitability CRS* and the chromatogram obtained with reference solution (e) to identify the peaks due to impurities B, D, G, H, I and J.

Relative retention With reference to melphalan (retention time = about 10 min): impurity B = about 0.3; impurity D = about 0.6; impurity I = about 0.8; impurity J = about 1.04; impurity G = about 1.4; impurity H = about 1.5.

System suitability Reference solution (e):
- *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to impurity J and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to melphalan.

Limits:
- *impurity D*: not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (d) (3.0 per cent);
- *impurity G*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (d) (1.0 per cent);
- *impurities J, H*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.5 per cent);
- *impurity B*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent);
- *impurity I*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);
- *total*: not more than 11 times the area of the principal peak in the chromatogram obtained with reference solution (d) (5.5 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Impurity K (diethylamine)

Gas chromatography (*2.2.28*).

Test solution Dissolve 0.125 g of substance to be examined in 0.15 mL of *hydrochloric acid R* and dilute to 5.0 mL with *dimethyl sulfoxide R*.

Reference solution Dilute 1 mL of *methanol R* and 0.125 g of *diethylamine R1* (impurity K) to 10.0 mL with *dimethyl sulfoxide R*. Dilute 1.0 mL of the solution to 100.0 mL with *dimethyl sulfoxide R*.

Column:
- *material*: glass;
- *size*: *l* = 1.6 m, Ø = 4 mm;
- *stationary phase*: *styrene-divinylbenzene copolymer R* coated with potassium carbonate (149-177 µm).

Carrier gas *nitrogen for chromatography R*.

Flow rate 42.5 mL/min.

Temperature:
— *column*: 170 °C;
— *injection port*: 190 °C;
— *detector*: 250 °C.

Detection Flame ionisation.

Injection 1 µL.

Elution order Methanol, impurity K.

System suitability Reference solution:
— *resolution*: minimum 2.0 between the peaks due to methanol and impurity K.

Limit:
— *impurity K*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.5 per cent).

Water (*2.5.12*)
Maximum 5.0 per cent, determined on 0.200 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (a).

Calculate the percentage content of $C_{13}H_{18}Cl_2N_2O_2$ taking into account the assigned content of *melphalan hydrochloride CRS* and a conversion factor of 0.8933.

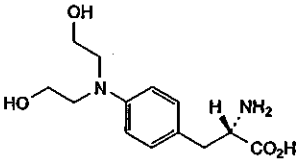
STORAGE

In an airtight container, protected from light.

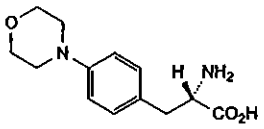
IMPURITIES

Specified impurities *B, D, G, H, I, J, K.*

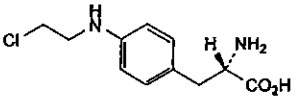
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, C, E, F.*



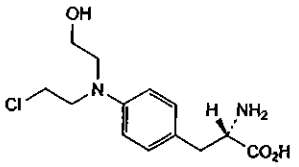
A. 4-[bis(2-hydroxyethyl)amino]-L-phenylalanine,



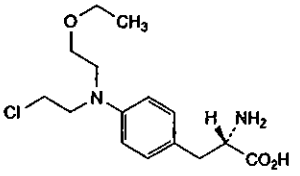
B. 4-morpholin-4-yl-L-phenylalanine,



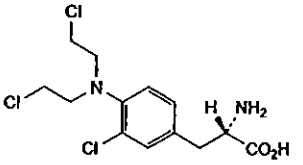
C. 4-[(2-chloroethyl)amino]-L-phenylalanine,



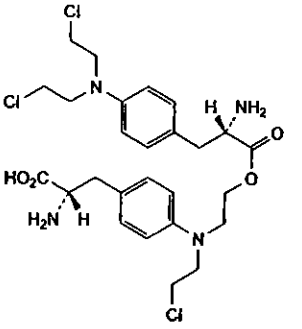
D. 4-[(2-chloroethyl)(2-hydroxyethyl)amino]-L-phenylalanine,



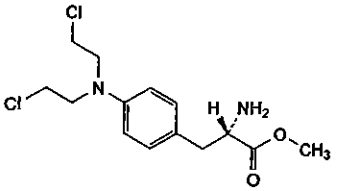
E. 4-[(2-chloroethyl)(2-ethoxyethyl)amino]-L-phenylalanine,



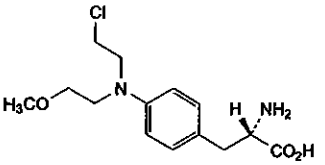
F. 4-[bis(2-chloroethyl)amino]-3-chloro-L-phenylalanine (3-chloromelphalan),



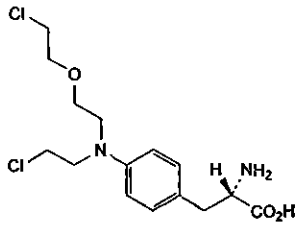
G. 4-[[2-[[4-[bis(2-chloroethyl)amino]-L-phenylalanyl]oxy] ethyl](2-chloroethyl)amino]-L-phenylalanine (melphalan dimer),



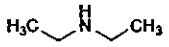
H. methyl 4-[bis(2-chloroethyl)amino]-L-phenylalaninate,



I. 4-[(2-chloroethyl)(2-methoxyethyl)amino]-L-phenylalanine,



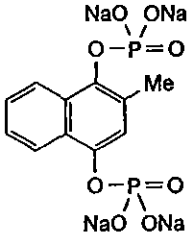
J. 4-[[2-(2-chloroethoxy)ethyl](2-chloroethyl)amino]-L-phenylalanine,



K. *N*-ethylethanamine (diethylamine).

Ph Eur

Menadiol Sodium Phosphate



$C_{11}H_8Na_4O_8P_2,6H_2O$ 530.2 *6700-42-1*

Action and use
Vitamin K analogue.

Preparation
Menadiol Phosphate Tablets

DEFINITION
Menadiol Sodium Phosphate is tetrasodium 2-methylnaphthalene-1,4-diyl di(orthophosphate) hexahydrate. It contains not less than 98.0% and not more than 100.5% of $C_{11}H_8Na_4O_8P_2$, calculated with reference to the anhydrous substance.

CHARACTERISTICS
A white to pink, crystalline powder; hygroscopic.

Very soluble in *water*; practically insoluble in *ethanol (96%)*.

IDENTIFICATION
A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of menadiol sodium phosphate *(RS 213)*.

B. To 10 mL of a 2% w/v solution add 10 mL of 1M *sulfuric acid*, 10 mL of 0.1M *cerium(IV) sulfate* and 1 mL of *hydrogen peroxide solution (20 vol)* and extract with two 10 mL quantities of *chloroform*. Evaporate the combined chloroform extracts to dryness on a water bath and dry the residue at 40° at a pressure not exceeding 0.7 kPa. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of menadione.

C. Dissolve 40 mg in 2 mL of *water*, heat gently with 2 mL of *sulfuric acid* until white fumes are evolved, add *nitric acid* dropwise until digestion is complete and cool. Add 2 mL of *water*, heat until white fumes are evolved again, cool, add a further 10 mL of *water* and neutralise to *litmus paper* with 5M *ammonia*. The solution yields reaction A characteristic of *sodium salts* and reaction B characteristic of *phosphates*, Appendix VI.

TESTS
Inorganic phosphate
Dissolve 25 mg in 10 mL of *water*, add 4 mL of 1M *sulfuric acid*, 1 mL of a 10% w/v solution of *ammonium molybdate* and 2 mL of *methylaminophenyl-sulfite reagent* and allow to stand for 15 minutes. The *absorbance* of the solution at 730 nm, Appendix II B, is not more than the *absorbance* of a solution prepared in the same manner but using 10 mL of a 0.0025% w/v solution of *potassium dihydrogen orthophosphate* in place of the solution of the substance being examined (0.7%).

Total phosphate
Dissolve 85 mg in 50 mL of *glacial acetic acid*, add 5 mL of *mercury(II) acetate solution* and carry out Method I for *non-aqueous titration*, Appendix VIII A, determining the end point potentiometrically. Not less than 7.7 mL and not more than 8.3 mL of *0.1M perchloric acid VS* is required.

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, in subdued light using the following solutions in *methanol (50%)*.

(1) 4.0% w/v of the substance being examined.

(2) 0.020% w/v of the substance being examined.

(3) 0.0080% w/v of *2-methyl-1,4-naphtha-quinone*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* GF_{254}.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1.5 volumes of *diethylamine*, 5 volumes of *butan-1-ol*, 50 volumes of *propan-1-ol and* 50 volumes of a 2% w/v solution of *ammonium chloride*.

LIMITS

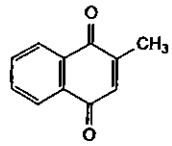
Any spot corresponding to 2-methyl-1,4-naphthaquinone (menadione) in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2).

Water
19.0 to 21.5% w/w, Appendix IX A. Use 0.25 g.

ASSAY
Dissolve 0.1 g in 25 mL of *water*, add 25 mL of *glacial acetic acid* and 25 mL of *3M hydrochloric acid* and titrate with 0.02M *cerium(IV) sulfate VS* using platinum and calomel electrodes and determining the end point potentiometrically. Each mL of 0.02M *cerium(IV) sulfate VS* is equivalent to 4.221 mg of $C_{11}H_8Na_4O_8P_2$.

Menadione

(Ph. Eur. monograph 0507)



$C_{11}H_8O_2$ 172.2 *58-27-5*

Action and use
Vitamin K analogue.

Ph Eur

DEFINITION
Menadione contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 2-methylnaphthalene-1,4-dione, calculated with reference to the dried substance.

CHARACTERS
A pale-yellow, crystalline powder, practically insoluble in water, freely soluble in toluene, sparingly soluble in alcohol and in methanol. It is unstable in light.

IDENTIFICATION
First identification: A, B.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 105 °C to 108 °C.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *menadione CRS*.

C. Dissolve about 1 mg in 5 mL of *alcohol R*, add 2 mL of *ammonia R* and 0.2 mL of *ethyl cyanoacetate R*. An intense bluish-violet colour develops. Add 2 mL of *hydrochloric acid R*. The colour disappears.

D. Dissolve about 10 mg in 1 mL of *alcohol R*, add 1 mL of *hydrochloric acid R* and heat in a water-bath. A red colour develops.

TESTS

Related substances
Carry out the test protected from bright light Examine by thin-layer chromatography (*2.2.27*), using *silica gel GF_{254} R* as the coating substance.

Test solution Dissolve 0.2 g of the substance to be examined in *acetone R* and dilute to 10 mL with the same solvent.

Reference solution Dilute 0.5 mL of the test solution to 100 mL with *acetone R*.

Apply separately to the plate 5 µL of each solution. Develop over a path of 15 cm using a mixture of 1 volume of *nitromethane R*, 2 volumes of *acetone R*, 5 volumes of *ethylene chloride R* and 90 volumes of *cyclohexane R*. Dry the plate in a current of hot air. Repeat the development and drying a further two times. Examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* for 4 h.

Sulfated ash (*2.4.14*)
Not more than 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.150 g in 15 mL of *glacial acetic acid R* in a flask with a stopper fitted with a valve. Add 15 mL of *dilute hydrochloric acid R* and 1 g of *zinc powder R*. Close the flask. Allow the mixture to stand for 60 min, protected from light, with occasional shaking. Filter the solution over a cotton wad, wash with three quantities, each of 10 mL, of *carbon dioxide-free water R*. Add 0.1 mL of *ferroin R* and immediately titrate the combined filtrate and washings with *0.1 M ammonium and cerium nitrate*.

1 mL of *0.1 M ammonium and cerium nitrate* is equivalent to 8.61 mg of $C_{11}H_8O_2$.

STORAGE
Store protected from light.

Ph Eur

Menotrophin

9002-68-0

Action and use
Gonadotrophin; treatment of infertility.

Preparation
Menotrophin Injection

DEFINITION
Menotrophin is a dry preparation containing glycoprotein gonadotrophins possessing follicle-stimulating and luteinising activities. The follicle-stimultating activity is of pituitary origin. The luteinising activity is predominantly of pituitary origin, but may also contain luteinising activity of placental origin. Menotrophin is obtained from the urine of post-menopausal women but, where necessary, chorionic gonadotrophin obtained from the urine of pregnant women may be added to achieve the ratio stated below.

Potency
It contains not less than 40 IU of follicle-stimulating hormone activity per mg. The ratio of IU of luteinising hormone (LH) activity to IU of follicle-stimulating hormone (FSH) activity is approximately 1. For each component the estimated potency is not less than 80% and not more than 125% of the stated potency.

PRODUCTION
Menotrophin is prepared by suitable collection and extraction procedures followed by purification steps. The method of preparation includes steps that have been shown to remove and/or inactivate extraneous agents, including viral agents as determined by a suitable risk-based approach. In addition, the process is designed to minimise microbial contamination.

CHARACTERISTICS
An almost white or slightly yellow powder.

Soluble in water.

IDENTIFICATION
Causes enlargement of the ovaries of immature female rats and increases the weight of the seminal vesicles and prostate gland of immature male rats when administered as directed in the Assay.

TESTS

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. The endotoxin limit is not more than 0.78 IU per IU of follicle stimulating hormone.

Water

Not more than 5.0% w/w, Appendix IX C, Method III. Use 4 mg.

ASSAY

Carry out the *biological assay of menotrophin* described below. The fiducial limits of error are not less than 64% and not more than 156% of the stated potency.

STORAGE

Menotrophin should be kept in an airtight, tamper-evident container and protected from light.

LABELLING

The label states (1) the number of IU (Units) of follicle-stimulating hormone activity and the number of IU (Units) of luteinising hormone activity in the container; (2) the number of IU (Units) of follicle-stimulating hormone activity per mg and the number of IU (Units) of luteinising hormone activity per mg; (3) where applicable, the number of IU (Units) of chorionic gonadotrophin activity per mg; (4) the date after which the material is not intended to be used; (5) the conditions under which it should be stored; (6) where applicable, that it is sterile.

Menotrophin intended for use in the manufacture of a parenteral dosage form without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility

Complies with the *test for sterility*, Appendix XVI A.

BIOLOGICAL ASSAY OF MENOTROPHIN

The potency of menotrophin with respect to its follicle-stimulating hormone activity is estimated by comparing its effect in enlarging the ovaries of immature female rats with that of the Standard Preparation of human urinary FSH and human urinary LH under the conditions of a suitable method of assay. The potency of menotrophin with respect to its luteinising hormone activity is estimated by comparing its effect in increasing the weight of the seminal vesicles or the prostate gland of immature male rats with that of the Standard Preparation of human urinary FSH and human urinary LH under the conditions of a suitable method of assay.

Standard Preparation

The Standard Preparation is the International Standard preparation of human urinary follicle-stimulating hormone and luteinising hormone, consisting of a freeze-dried extract of urine from post-menopausal women together with lactose, or another suitable preparation the potency of which has been determined in relation to the International Standard.

SUGGESTED METHOD

Follicle-stimulating hormone activity

Select female rats of the same strain, 19 to 28 days old and differing in age by not more than 3 days, and having weights such that the difference between the heaviest rat and the lightest rat is not more than 10 g. Assign the rats at random to six equal groups of at least five animals. If sets of six litter mates are available, allot one litter mate from each set to each group and mark according to litter.

Choose three doses of the Standard Preparation and three doses of the preparation being examined such that the smallest dose produces a positive response in some of the rats and the largest dose does not produce a maximum response in all of the rats. Use doses in geometric progression. As an initial approximation, total doses of 1.5, 3.0 and 6.0 IU may be tried although the dose will depend on the sensitivity of the animals used, which may vary widely.

Dissolve separately the total quantities of the preparation being examined, and of the Standard Preparation, corresponding to the daily doses to be used in sufficient *albumin-phosphate buffer pH 7.2* containing not less than 70 IU of *chorionic gonadotrophin* per mL so that the daily dose is about 0.2 mL. Add a suitable antimicrobial preservative such as 0.4% w/v of *phenol* or 0.002% w/v of *thiomersal*. Store the solutions at a temperature of 2° to 8°. Inject subcutaneously into each rat the daily dose allocated to its group. Repeat the procedure after 24 and 48 hours. About 24 hours after the last injection, kill the rats and remove the ovaries. Remove any extraneous fluid and tissue and immediately weigh the ovaries from each animal. Record the combined weight of both ovaries from each rat. Calculate the result of the assay by standard statistical methods using the weight of the ovaries as the response. (A suitable correction of the organ weight with reference to the weight of the animal from which it was taken may be applied; an analysis of covariance may be used).

Luteinising hormone activity

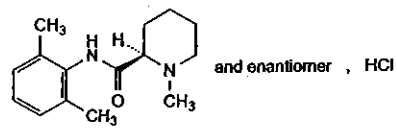
Select male rats of the same strain, approximately 19 to 28 days old and differing in age by not more than 3 days, and having weights such that the difference between the heaviest rat and the lightest rat is not more than 10 g. Assign the rats at random to six equal groups of at least five animals. If sets of six litter mates are available, allot one litter mate from each set to each group and mark according to litter.

Choose three doses of the Standard Preparation and three doses of the preparation being examined such that the smallest dose is sufficient to produce a positive response in some of the rats and the largest dose does not produce a maximum response in all of the rats. Use doses in geometric progression. As an initial approximation, total doses of 7, 14 and 28 IU may be tried although the dose will depend on the sensitivity of the animals used, which may vary widely.

Dissolve separately the total quantities of the preparation being examined, and of the Standard Preparation, corresponding to the daily doses to be used in sufficient *albumin-phosphate buffer pH 7.2* so that the daily dose is about 0.2 mL. Add a suitable antimicrobial preservative such as 0.4% w/v of *phenol* or 0.002% w/v of *thiomersal*. Store the solutions at a temperature of 2° to 8°. Inject subcutaneously into each rat the daily dose allocated to its group on 4 consecutive days at the same time each day. On the fifth day, about 24 hours after the last injection, kill the rats and remove the seminal vesicles or the prostate gland. Remove any extraneous fluid and tissue and weigh immediately the seminal vesicles or the prostate gland. Calculate the result of the assay by standard statistical methods, using the weight of the vesicles or the prostate gland as the response. (A suitable correction of the organ weight with reference to the weight of the animal from which it was taken may be applied; an analysis of covariance may be used.)

Mepivacaine Hydrochloride

(Ph. Eur. monograph 1242)



$C_{15}H_{23}ClN_2O$ 282.8 *1722-62-9*

Action and use
Local anaesthetic.

Ph Eur

DEFINITION

(2*RS*)-*N*-(2,6-Dimethylphenyl)-1-methylpiperidine-2-carboxamide hydrochloride.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water and in ethanol (96 per cent), very slightly soluble in methylene chloride.

mp
About 260 °C, with decomposition.

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification: A, C.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *mepivacaine hydrochloride CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and dry in an oven at 80 °C for 45 min. Record new spectra using the residues.

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 20 mg of the substance to be examined in *ethanol (96 per cent) R* and dilute to 5 mL with the same solvent.

Reference solution (a) Dissolve 20 mg of *mepivacaine hydrochloride CRS* in *ethanol (96 per cent) R* and dilute to 5 mL with the same solvent.

Reference solution (b) Dissolve 20 mg of *mepivacaine hydrochloride CRS* and 20 mg of *lidocaine hydrochloride CRS* in *ethanol (96 per cent) R* and dilute to 5 mL with the same solvent.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *concentrated ammonia R, methanol R, 1,1-dimethylethyl methyl ether R* (1:5:100 *V/V/V*).

Application 10 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):
— the chromatogram shows 2 clearly separated principal spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

C. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S
Dissolve 1.500 g in *carbon dioxide-free water R* and dilute to 30.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_7 (*2.2.2, Method II*).

pH (*2.2.3*)
4.0 to 5.0.

Dilute 2 mL of solution S to 5 mL with *carbon dioxide-free water R*.

Optical rotation (*2.2.7*)
−0.10° to + 0.10°, determined on solution S.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dissolve 20.0 mg of the substance to be examined and 30.0 mg of *mepivacaine impurity B CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 100.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.125 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 35 volumes of *acetonitrile R1* and 65 volumes of a 2.25 g/L solution of *phosphoric acid R*, previously adjusted to pH 7.6 with *strong sodium hydroxide solution R*.

Flow rate 1 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µL.

Run time 3 times the retention time of mepivacaine.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity B.

Relative retention With reference to mepivacaine (retention time = about 7 min): impurity B = about 0.5.

System suitability Reference solution (a):
— *resolution*: minimum 2.5 between the peaks due to impurity B and mepivacaine.

Limits:
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Impurity A
Head-space gas chromatography (*2.2.28*).

Test solution Introduce 60.0 mg of the substance to be examined into a 20 mL vial. Add 2.0 mL of a 103.0 g/L solution of *hydrochloric acid R* and 1.0 mL of a 126.0 g/L solution of *sodium hydroxide R*, and close the vial immediately.

Reference solution Dissolve 6.0 mg of *bupivacaine impurity F CRS* (impurity A) in a 103.0 g/L solution of *hydrochloric acid R* and dilute to 100.0 mL with the same acid solution. Dilute 1.0 mL of this solution to 100.0 mL with a 103.0 g/L solution of *hydrochloric acid R*. Introduce 2.0 mL of this solution into a 20 mL vial, add 1.0 mL of a 126.0 g/L solution of *sodium hydroxide R*, and close the vial immediately.

Column:
— *material*: fused silica;
— *size*: *l* = 30 m, Ø = 0.53 mm;
— *stationary phase*: *cyanopropyl(3)phenyl(3)methyl(94) polysiloxane R* (film thickness 3 µm).

Carrier gas *helium for chromatography R.*

Flow rate 4.0 mL/min.

Split ratio 1:1.

Static head-space conditions that may be used:
— *equilibration temperature*: 90 °C;
— *equilibration time*: 25 min;
— *pressurisation time*: 2 min.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 10	130 → 230
	10 - 15	230
Injection port		225
Detector		250

Detection Flame ionisation.

Retention time Impurity A = about 6 min.

System suitability Reference solution:
— *repeatability*: maximum relative standard deviation of 15.0 per cent determined on 6 injections.

Calculation of content:
— for impurity A, use the concentration of impurity A in the reference solution.

Limit:
— *impurity A*: maximum 20 ppm.

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

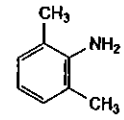
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.250 g in a mixture of 5.0 mL of *0.01 M hydrochloric acid* and 50 mL of *ethanol (96 per cent) R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

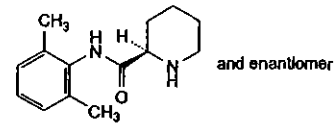
1 mL of *0.1 M sodium hydroxide* is equivalent to 28.28 mg of $C_{15}H_{23}ClN_2O$.

IMPURITIES
Specified impurities *A.*

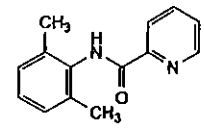
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D, E.*



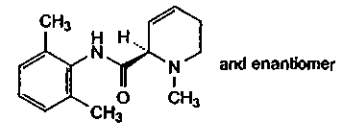
A. 2,6-dimethylaniline,



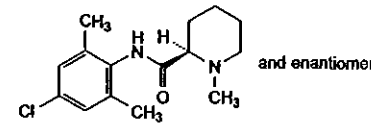
B. (2*RS*)-*N*-(2,6-dimethylphenyl)piperidine-2-carboxamide,



C. *N*-(2,6-dimethylphenyl)pyridine-2-carboxamide,



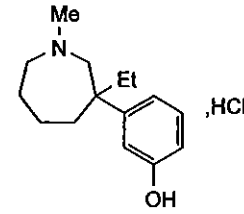
D. (2*RS*)-*N*-(2,6-dimethylphenyl)-1-methyl-1,2,5,6-tetrahydropyridine-2-carboxamide,



E. (2*RS*)-*N*-(4-chloro-2,6-dimethylphenyl)-1-methylpiperidine-2-carboxamide.

Ph Eur

Meptazinol Hydrochloride



$C_{15}H_{23}NO,HCl$ 269.8 *59263-76-2*

Action and use
Opioid receptor partial agonist; analgesic.

Preparations
Meptazinol Injection
Meptazinol Tablets

DEFINITION

Meptazinol Hydrochloride is 3-(3-ethyl-1-methylperhydroazepin-3-yl)phenol hydrochloride. It contains not less than 99.0% and not more than 101.0% of $C_{15}H_{23}NO,HCl$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white powder.

Very soluble in *water* and in *methanol*; freely soluble in *ethanol (96%)*; very slightly soluble in *acetone*. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.01% w/v solution in *ethanol (96%)* exhibits a maximum only at 275 nm. The *absorbance* at 275 nm is about 0.80.

B. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of meptazinol hydrochloride *(RS 215)*.

TESTS

Acidity or alkalinity

To 10 mL of a 2% w/v solution in *carbon dioxide-free water* add 0.2 mL of *0.01M sodium hydroxide VS* and 0.1 mL of *methyl red solution*; the solution is yellow. Add 0.4 mL of *0.01M hydrochloric acid VS*; the solution is red.

Colour of solution

A 10.0% w/v solution is not more intensely coloured than *reference solution* Y_6, Appendix IV B, Method II.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *methanol*.

(1) 1.0% w/v of the substance being examined.

(2) 0.01% w/v of the substance being examined.

(3) 0.005% w/v of the substance being examined.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, examine under *ultraviolet light (254 nm)*, expose to iodine vapour for 2 hours and examine again.

MOBILE PHASE

1 volume of 18M *ammonia*, 30 volumes of *chloroform* and 70 volumes of *ethyl acetate*.

LIMITS

By each method of visualisation, any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (1%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

Loss on drying

When dried at 105° for 3 hours, loses not more than 0.5% of its weight. Use 1 g.

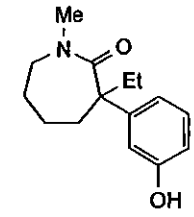
Sulfated ash

Not more than 0.1%, Appendix IX A.

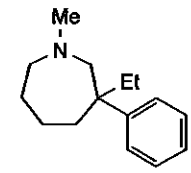
ASSAY

Dissolve 0.15 g in 50 mL of *anhydrous acetic acid* and add 5 mL of *mercury(II) acetate solution*. Carry out Method I for *non-aqueous titration*, Appendix VIII A, determining the end point potentiometrically. Each mL of *0.1M perchloric acid VS* is equivalent to 26.98 mg of $C_{15}H_{23}NO,HCl$.

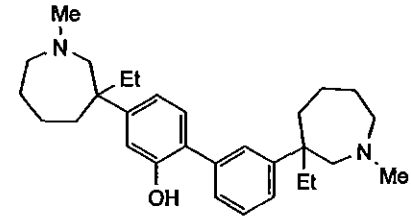
IMPURITIES



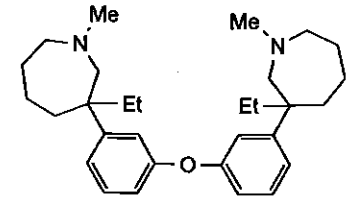
A. 3-ethyl-3-(3-hydroxyphenyl)-1-methylperhydroazepin-2-one



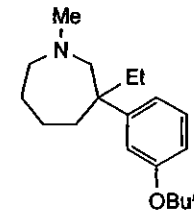
B. 3-ethyl-1-methyl-3-phenylperhydroazepine



C. 4-(3-ethyl-1-methylperhydroazepin-3-yl)-2-[3-(3-ethyl-1-methylperhydroazepin3-yl)phenyl]phenol



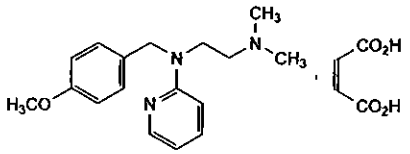
D. 3,3′-oxybis(3-ethyl-1-methylperhydroazepin-3-ylbenzene)



E. 3-ethyl-3-(3-butoxyphenyl)-1-methylperhydroazepine

Mepyramine Maleate

(Ph. Eur. monograph 0278)



$C_{21}H_{27}N_3O_5$ 401.5 *59-33-6*

Action and use
Anti-emetic.

Ph Eur

DEFINITION

N-(4-Methoxybenzyl)-*N'*,*N'*-dimethyl-*N*-(pyridin-2-yl) ethane-1,2-diamine (2*Z*)-but-2-enedioate.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or slightly yellowish, crystalline powder.

Solubility
Very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: B.

Second identification: A, C, D, E.

A. Melting point (*2.2.14*): 99 °C to 103 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation 50 g/L solutions in *methylene chloride R* using a 0.1 mm cell.

Comparison *mepyramine maleate CRS.*

C. Dissolve 0.100 g in *0.01 M hydrochloric acid* and dilute to 100.0 mL with the same acid. Dilute 1.0 mL of this solution to 100.0 mL with *0.01 M hydrochloric acid.* Examined between 220 nm and 350 nm (*2.2.25*), the solution shows 2 absorption maxima, at 239 nm and 316 nm. The specific absorbances at the absorption maxima are 431 to 477 and 196 to 220, respectively.

D. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 40 mg of the substance to be examined in *methylene chloride R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 40 mg of *mepyramine maleate CRS* in *methylene chloride R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel GF_{254} plate R.*

Mobile phase *diethylamine R, ethyl acetate R* (2:100 *V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

E. Triturate 0.1 g with 3 mL of *water R* and 1 mL of *strong sodium hydroxide solution R.* Shake with 3 quantities, each of 5 mL, of *ether R.* To 0.1 mL of the aqueous layer add a solution of 10 mg of *resorcinol R* in 3 mL of *sulfuric acid R.* Heat on a water-bath for 15 min; no colour develops. To the rest of the aqueous layer add 1 mL of *bromine water R.* Heat on a water-bath for 15 min and then heat to boiling and cool. To 0.2 mL of this solution add a solution of 10 mg of *resorcinol R* in 3 mL of *sulfuric acid R.* Heat on a water-bath for 15 min; a violet-pink colour develops.

TESTS

Solution S
Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent.

Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Dilute 5 mL of solution S to 25 mL with *carbon dioxide-free water R.*

pH (*2.2.3*)
4.9 to 5.2.

Dilute 1.0 mL of solution S to 10 mL with *carbon dioxide-free water R.*

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 5 mg of *anisaldehyde R* (impurity B), 5.0 mg of *mepyramine impurity A CRS* and 5.0 mg of *mepyramine impurity C CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 2.0 mL of the solution to 100.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: base-deactivated end-capped phenylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 0.1 volumes of *triethylamine R,* 40 volumes of a 0.771 g/L solution of *ammonium acetate R* and 60 volumes of *methanol R1.*

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µL.

Run time 3 times the retention time of mepyramine.

Relative retention With reference to mepyramine (retention time = about 13 min): maleic acid = about 0.2; impurity C = about 0.3; impurity B = about 0.4; impurity A = about 0.5.

System suitability Reference solution (a):
— *resolution*: minimum 3.0 between the peaks due to impurities C and B.

Limits:
— *impurities A, C*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard the peak due to maleic acid.

Chlorides (*2.4.4*)
Maximum 100 ppm.

Dilute 2.5 mL of solution S to 15 mL with *water R*.

Loss on drying (*2.2.32*)
Maximum 0.25 per cent, determined on 1.000 g by drying in an oven at 80 °C.

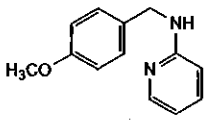
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on the residue obtained in the test for loss on drying.

ASSAY
Dissolve 0.150 g in 40 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

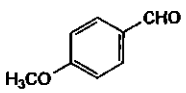
1 mL of *0.1 M perchloric acid* is equivalent to 20.07 mg of $C_{21}H_{27}N_3O_5$.

STORAGE
Protected from light.

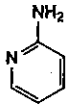
IMPURITIES
Specified impurities *A, B, C.*



A. *N*-(4-methoxybenzyl)pyridin-2-amine,



B. 4-methoxybenzaldehyde (anisaldehyde),



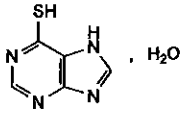
C. pyridin-2-amine.

Ph Eur

Mercaptopurine Monohydrate

Mercaptopurine

(Ph. Eur. monograph 0096)



$C_5H_4N_4S,H_2O$ 170.2 *6112-76-1*

Action and use
Thiopurine cytotoxic.

Preparations
Mercaptopurine Oral Suspension
Mercaptopurine Tablets

Ph Eur

DEFINITION
7*H*-Purine-6-thiol monohydrate.

Content
98.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance
Yellow, crystalline powder.

Solubility
Practically insoluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in heptane. It dissolves in solutions of alkali hydroxides.

IDENTIFICATION
A. Dissolve 20 mg in 5 mL of *dimethyl sulfoxide R* and dilute to 100 mL with a 10.3 g/L solution of *hydrochloric acid R*. Dilute 5 mL of this solution to 200 mL with a 10.3 g/L solution of *hydrochloric acid R*. Examined between 230 nm and 350 nm (*2.2.25*), the solution shows only 1 absorption maximum, at 325 nm.

B. Dissolve about 20 mg in 20 mL of *ethanol (96 per cent) R* heated to 60 °C and add 1 mL of a saturated solution of *mercuric acetate R* in *ethanol (96 per cent) R*. A white precipitate is formed.

C. Dissolve about 20 mg in 20 mL of *ethanol (96 per cent) R* heated to 60 °C and add 1 mL of a 10 g/L solution of *lead acetate R* in *ethanol (96 per cent) R*. A yellow precipitate is formed.

TESTS

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 12.0 mg of the substance to be examined in 2.5 mL of *methanol R* and dilute to 100.0 mL with a 0.1 per cent *V/V* solution of *anhydrous formic acid R*.

Reference solution (a) Dissolve 3.0 mg of *mercaptopurine impurity B CRS* and 4.5 mg of *mercaptopurine impurity A CRS* in mobile phase A and dilute to 250.0 mL with mobile phase A. Dilute 1.0 mL of the solution to 100.0 mL with mobile phase A.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with a 0.1 per cent *V/V* solution of *anhydrous formic acid R*.

Reference solution (c) Dissolve 3 mg of *mercaptopurine impurity D CRS* in 10 mL of *dimethyl sulfoxide R* and dilute to 250 mL with mobile phase A. Dilute 1 mL of the solution to 100 mL with mobile phase A.

Column:
— *size: l* = 0.10 m, Ø = 4.6 mm;
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (3 µm);
— *temperature*: 30 °C.

Mobile phase:
— *mobile phase A*: *methanol R*, 0.1 per cent *V/V* solution of *anhydrous formic acid R* (2:98 *V/V*);
— *mobile phase B*: *methanol R*, 0.1 per cent *V/V* solution of *anhydrous formic acid R* (50:50 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 8	100	0
8 - 20	100 → 0	0 → 100
20 - 25	0	100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 260 nm.

Injection 50 µL.

Autosampler Set at 4 °C.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A and B; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity D.

Relative retention With reference to mercaptopurine (retention time = about 6 min): impurity B = about 0.3; impurity A = about 0.5; impurity D = about 3.5.

System suitability Reference solution (a):
— *resolution*: minimum 5.0 between the peaks due to impurities B and A.

Calculation of percentage contents:
— *correction factor*: multiply the peak area of impurity D by 0.25;
— for impurities A and B, use the concentration of the corresponding impurity in reference solution (a);
— for impurities other than A and B, use the concentration of mercaptopurine monohydrate in reference solution (b).

Limits:
— *impurity A*: maximum 0.15 per cent;
— *impurities B, D*: for each impurity, maximum 0.10 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.3 per cent;
— *reporting threshold*: 0.05 per cent.

Water (*2.5.12*)
10.0 per cent to 12.0 per cent, determined on 0.250 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

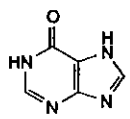
ASSAY
Dissolve 0.100 g in 50 mL of *dimethylformamide R*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M tetrabutylammonium hydroxide* is equivalent to 15.22 mg of $C_5H_4N_4S$.

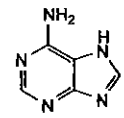
STORAGE
Protected from light.

IMPURITIES
Specified impurities *A, B, D.*

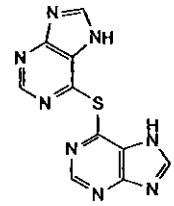
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C.*



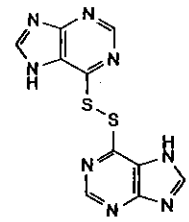
A. 1,7-dihydro-6*H*-purin-6-one (hypoxanthine),



B. 7*H*-purin-6-amine (adenine),



C. 6,6′-sulfanediyldi-7*H*-purine,



D. 6,6′-disulfanediyldi-7*H*-purine.

Ph Eur

Mercuric Chloride

(Ph. Eur. monograph 0120)

$HgCl_2$ 271.5 *7487-94-7*

Ph Eur

DEFINITION
Content
99.5 per cent to 100.5 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder or colourless or white or almost white crystals or heavy crystalline masses.

Solubility
Soluble in water and in glycerol, freely soluble in ethanol (96 per cent).

IDENTIFICATION
A. It gives reaction (a) of chlorides (*2.3.1*).

B. Solution S (see Tests) gives the reactions of mercury (*2.3.1*).

TESTS
Solution S
Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 mL with the same solvent.

Appearance of solution
Solution S is not more opalescent than reference suspension II (*2.2.1*) and is colourless (*2.2.2, Method II*).

Acidity or alkalinity
To 10 mL of solution S add 0.1 mL of *methyl red solution R*. The solution is red. Add 0.5 g of *sodium chloride R*. The solution becomes yellow. Not more than 0.5 mL of *0.01 M hydrochloric acid* is required to change the colour to red.

Mercurous chloride
Dissolve 1.0 g in 30 mL of *ether R*. The solution shows no opalescence.

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 2.00 g by drying *in vacuo* for 24 h.

ASSAY
Dissolve 0.500 g in 100 mL of *water R*. Add 20.0 mL of *0.1 M sodium edetate* and 5 mL of *buffer solution pH 10.9 R*. Allow to stand for 15 min. Add 0.1 g of *mordant black 11 triturate R* and titrate with *0.1 M zinc sulfate* until the colour changes to purple. Add 3 g of *potassium iodide R*, allow to stand for 2 min, add a further 0.1 g of *mordant black 11 triturate R* and titrate with *0.1 M zinc sulfate*.

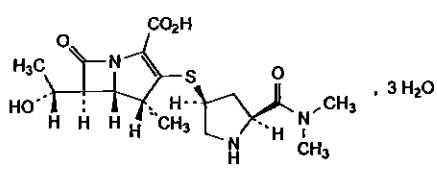
1 mL of *0.1 M zinc sulfate* used in the second titration is equivalent to 27.15 mg of $HgCl_2$.

STORAGE
Protected from light.

Ph Eur

Meropenem Trihydrate

(Ph. Eur. monograph 2234)



$C_{17}H_{25}N_3O_5S,3H_2O$ 437.5 *119478-56-7*

Action and use
Carbapenem antibacterial.

Ph Eur

DEFINITION
(4*R*,5*S*,6*S*)-3-[[(3*S*,5*S*)-5-[(Dimethylamino) carbonyl]pyrrolidin-3-yl]sulfanyl]-6-[(1*R*)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid trihydrate.

Semi-synthetic product derived from a fermentation product, or synthetic product.

Content
97.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or light yellow, crystalline powder.

Solubility
Sparingly soluble in water, practically insoluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *meropenem trihydrate CRS*.

TESTS
Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5 (*2.2.2, Method II*).

Dissolve 1.0 g in 20 mL of a 50 g/L solution of *sodium hydrogen carbonate R*.

pH (*2.2.3*)
4.0 to 6.0.

Dissolve 0.20 g in *carbon dioxide-free water R* and dilute to 20 mL with the same solvent.

Specific optical rotation (*2.2.7*)
−21 to −17 (anhydrous substance).

Dissolve 0.125 g in *water R* and dilute to 25.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*). *Prepare test solutions (a) and (b) and reference solution (c) immediately before use. Prepare and store reference solution (a) at 4 °C and use within 6 h.*

Solvent mixture To 1.0 mL of *triethylamine R* add 900 mL of *water for chromatography R*. Adjust to pH 5.0 with *dilute phosphoric acid R* and dilute to 1000.0 mL with *water for chromatography R*.

Test solution (a) Dissolve 0.100 g of the substance to be examined in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

Test solution (b) Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) In order to prepare impurities A and B *in-situ*, heat 10 mL of test solution (a) to 60 °C for about 20 min or, alternatively, allow 10 mL of test solution (a) to stand at room temperature for about 8 h.

Reference solution (c) Dissolve 50.0 mg of *meropenem trihydrate CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase.

Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 40 °C.

Mobile phase *acetonitrile R1*, solvent mixture (7:100 *V/V*).

Flow rate 1.6 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µL of test solution (a) and reference solutions (a) and (b).

Run time 4 times the retention time of meropenem.

Relative retention With reference to meropenem (retention time = about 7 min): impurity A = about 0.5; impurity B = about 2.2.

System suitability Reference solution (b):
— *resolution*: minimum 5.0 between the peaks due to impurity A and meropenem.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity A by 1.6;
— *impurity A*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *impurity B*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *sum of impurities other than A and B*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

For meropenem trihydrate produced by a fully synthetic process:
— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent);
— *disregard limit*: 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.03 per cent).

Water (*2.5.12*)
11.4 per cent to 13.4 per cent, determined on 0.100 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

Bacterial endotoxins (*2.6.14*)
Less than 0.125 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

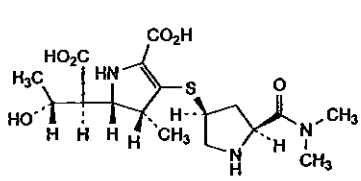
Injection Test solution (b) and reference solution (c).

Calculate the percentage content of $C_{17}H_{25}N_3O_5S$ taking into account the assigned content of *meropenem trihydrate CRS*.

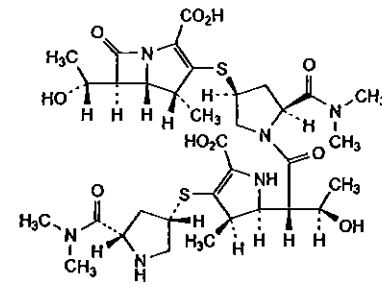
STORAGE
If the substance is sterile, store in a sterile, airtight, tamper-evident container.

LABELLING
The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

IMPURITIES
Specified impurities *A, B.*



A. (4*R*,5*S*)-5-[(1*S*,2*R*)-1-carboxy-2-hydroxypropyl]-3-[[(3*S*,5*S*)-5-[(dimethylamino)carbonyl]pyrrolidin-3-yl]sulfanyl]-4-methyl-4,5-dihydro-1*H*-pyrrole-2-carboxylic acid,

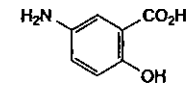


B. (4*R*,5*S*,6*S*)-3-[[(3*S*,5*S*)-1-[(2*S*,3*R*)-2-[(2*S*,3*R*)-5-carboxy-4-[[(3*S*,5*S*)-5-[(dimethylamino)carbonyl]pyrrolidin-3-yl]sulfanyl]-3-methyl-2,3-dihydro-1*H*-pyrrol-2-yl]-3-hydroxybutanoyl]-5-[(dimethylamino)carbonyl]pyrrolidin-3-yl]sulfanyl]-6-[(1*R*)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid.

Ph Eur

Mesalazine

(Ph. Eur. monograph 1699)



$C_7H_7NO_3$ 153.1 *89-57-6*

Action and use
Aminosalicylate; treatment of ulcerative colitis.

Preparations
Mesalazine Enema
Mesalazine Foam Enema
Mesalazine Prolonged-release Granules
Mesalazine Suppositories
Mesalazine Gastro-resistant Tablets
Mesalazine Prolonged-release Tablets

Ph Eur

DEFINITION
5-Amino-2-hydroxybenzoic acid.

Content
98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS
Appearance
Almost white or light grey or light pink powder or crystals.

Solubility
Very slightly soluble in water, practically insoluble in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides and in dilute hydrochloric acid.

IDENTIFICATION
First identification: B.

Second identification: A, C.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 50.0 mg in 10 mL of a 10.3 g/L solution of *hydrochloric acid R* and dilute to 100.0 mL with the same acid. Dilute 5.0 mL of this solution to 200.0 mL with a 10.3 g/L solution of *hydrochloric acid R*.

Spectral range 210-250 nm.

Absorption maximum At about 230 nm.

Specific absorbance at the absorption maximum 430 to 450.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *mesalazine CRS*.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 25 mg of the substance to be examined in 5 mL of a mixture of equal volumes of *glacial acetic acid R* and *water R* and dilute to 10.0 mL with *methanol R*.

Reference solution Dissolve 25 mg of *mesalazine CRS* in 5 mL of a mixture of equal volumes of *glacial acetic acid R* and *water R* and dilute to 10.0 mL with *methanol R*.

Plate A suitable silica gel as the coating substance.

Mobile phase *glacial acetic acid R, methanol R, methyl isobutyl ketone R* (10:40:50 *V/V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 365 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Appearance of solution

Maintain the solutions at 40 °C during preparation and measurements Dissolve 0.5 g in a 103.0 g/L solution of *hydrochloric acid R* and dilute to 20 mL with the same solution. The solution is clear (*2.2.1*). Immediately measure the absorbance (*2.2.25*) of the solution at 440 nm and 650 nm. The absorbance is not greater than 0.15 at 440 nm and 0.10 at 650 nm.

Reducing substances

Dissolve 0.10 g in *dilute hydrochloric acid R* and dilute to 25 mL with the same solvent. Add 0.2 mL of *starch solution R* and 0.25 mL of *0.01 M iodine*. Allow to stand for 2 min. The solution is blue or violet-brown.

Impurities A and C

Liquid chromatography (*2.2.29*). *Prepare the solutions and mobile phases immediately before use.*

Test solution Dissolve 50.0 mg of the substance to be examined in mobile phase A and dilute to 50.0 mL with mobile phase A.

Reference solution (a) Dissolve 5.0 mg of *mesalazine impurity C CRS* in mobile phase A and dilute to 100.0 mL with mobile phase A. Dilute 1.0 mL of the solution to 10.0 mL with mobile phase A.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 25.0 mL with mobile phase A.

Reference solution (c) Dissolve 5.0 mg of *mesalazine impurity A CRS* in mobile phase A and dilute to 250.0 mL with mobile phase A. Dilute 1.0 mL of the solution to 100.0 mL with mobile phase A.

Reference solution (d) Dilute 1 mL of the test solution to 200 mL with mobile phase A. To 5 mL of this solution add 5 mL of reference solution (a).

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
- *mobile phase A*: dissolve 1.0 g of *phosphoric acid R* and 2.2 g of *perchloric acid R* in *water for chromatography R* and dilute to 1000 mL with the same solvent;
- *mobile phase B*: dissolve 1.0 g of *phosphoric acid R* and 1.7 g of *perchloric acid R* in *acetonitrile R1* and dilute to 1000 mL with the same solvent;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 8	100	0
8 - 25	100 → 40	0 → 60

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µL of the test solution and reference solutions (b), (c) and (d).

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peak due to impurity A; use the chromatogram obtained with reference solution (b) to identify the peak due to impurity C.

Relative retention With reference to mesalazine (retention time = about 9 min): impurity A = about 0.5; impurity C = about 0.9.

System suitability Reference solution (d):
- *resolution*: minimum 3.0 between the peaks due to impurity C and mesalazine.

Limits:
- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (200 ppm);
- *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (200 ppm).

Impurity K

Liquid chromatography (*2.2.29*).

Test solution Dissolve 40.0 mg of the substance to be examined in the mobile phase and dilute to 20.0 mL with the mobile phase.

Reference solution Dissolve 27.8 mg of *aniline hydrochloride R* (equivalent to 20.0 mg of impurity K) in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 0.20 mL of the solution to 20.0 mL with the mobile phase. Dilute 0.20 mL of this solution to 20.0 mL with the mobile phase.

Column:
- *size*: *l* = 0.25 m, Ø = 4 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase Mix 15 volumes of *methanol R2* and 85 volumes of a solution containing 0.47 g/L of *disodium hydrogen phosphate dihydrate R* and 1.41 g/L of *potassium dihydrogen phosphate R* previously adjusted to pH 8.0 with a 42 g/L solution of *sodium hydroxide R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 205 nm.

Injection 50 µL.

Run time 1.5 times the retention time of impurity K.

Retention time Impurity K = about 14 min.

System suitability Reference solution:
- *signal-to-noise ratio*: minimum 10 for the principal peak.

Limit:
— *impurity K*: not more than the area of the principal peak in the chromatogram obtained with the reference solution (10 ppm).

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Solution A 1.03 g/L solution of *hydrochloric acid R*.

Test solution Dissolve 10.0 mg of the substance to be examined in solution A using sonication and dilute to 10.0 mL with solution A.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with solution A. Dilute 1.0 mL of this solution to 10.0 mL with solution A.

Reference solution (b) Dissolve 5 mg of *mesalazine for system suitability CRS* (containing impurities F, J and P) in solution A and dilute to 5 mL with solution A.

Reference solution (c) Dissolve 5 mg of *4-aminosalicylic acid R* (impurity E), 5 mg of *2,5-dihydroxybenzoic acid R* (impurity G), 15 mg of *salicylic acid R* (impurity H), 5 mg of *2-chlorobenzoic acid R* (impurity L), 5 mg of *2-chloro-5-nitrobenzoic acid R* (impurity M), 10 mg of *sulfanilic acid R* (impurity O) and 5 mg of *3-nitrosalicylic acid R* (impurity R) in solution A and dilute to 100 mL with solution A. Dilute 1 mL of the solution to 50 mL with solution A.

Reference solution (d) Dissolve 3.0 mg of *2-chlorobenzoic acid R* (impurity L) in solution A and dilute to 100.0 mL with solution A. Dilute 1.0 mL of the solution to 100.0 mL with solution A.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl amorphous organosilica polymer for chromatography R* (5 µm);
— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: dissolve 6.9 g of *sodium dihydrogen phosphate monohydrate R* in about 950 mL of *water for chromatography R*, adjust to pH 6.2 with *dilute sodium hydroxide solution R* and dilute to 1000 mL with *water for chromatography R*;
— *mobile phase B*: *acetonitrile for chromatography R*, mobile phase A (40:60 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 8	100	0
8 - 20	100 → 85	0 → 15
20 - 40	85 → 25	15 → 75
40 - 60	25 → 0	75 → 100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 240 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram supplied with *mesalazine for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities F, J and P; use the chromatogram obtained with reference solution (c) to identify the peaks due to impurities E, G, H, L, M, O and R.

Relative retention With reference to mesalazine (retention time = about 6 min): impurity O = about 0.5; impurity J = about 0.6; impurity E = about 0.8; impurity F = about 1.36; impurity G = about 1.44; impurity P = about 1.5; impurity L = about 2.0; impurity M = about 3.3; impurity H = about 3.5; impurity R = about 5.1.

System suitability:
— *peak-to-valley ratio*: minimum 3.0, where H_p = height above the baseline of the peak due to impurity F and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to mesalazine in the chromatogram obtained with reference solution (b);
— *signal-to-noise ratio*: minimum 10 for the peak due to impurity L in the chromatogram obtained with reference solution (d).

Limits:
— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity E = 1.3; impurity G = 1.4; impurity H = 1.4; impurity J = 2.0; impurity L = 4.5; impurity M = 1.7; impurity O = 0.6; impurity P = 0.6; impurity R = 1.3;
— *impurity H*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
— *impurities F, J, O, P*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *impurities E, G, L, M, R*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent);
— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *disregard limit*: 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.03 per cent).

Chlorides
Maximum 0.1 per cent.

Dissolve 1.50 g in 50 mL of *anhydrous formic acid R*. Add 100 mL of *water R* and 5 mL of *dilute nitric acid R*. Titrate with *0.005 M silver nitrate*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.005 M silver nitrate* is equivalent to 0.1773 mg of Cl.

Sulfates (*2.4.13*)
Maximum 200 ppm.

Shake 1.0 g with 20 mL of *distilled water R* for 1 min and filter. 15 mL of the filtrate complies with the test.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.2 per cent, determined on 1.0 g.

ASSAY
Dissolve 50.0 mg in 100 mL of boiling *water R*. Cool rapidly to room temperature and titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M sodium hydroxide* is equivalent to 15.31 mg of $C_7H_7NO_3$.

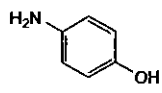
STORAGE

In an airtight container, protected from light.

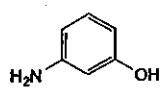
IMPURITIES

Specified impurities *A, C, E, F, G, H, J, K, L, M, O, P, R.*

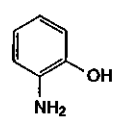
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, D, I, N, Q, S.*



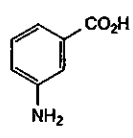
A. 4-aminophenol,



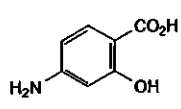
B. 3-aminophenol,



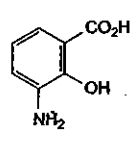
C. 2-aminophenol,



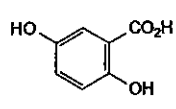
D. 3-aminobenzoic acid,



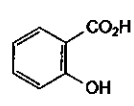
E. 4-amino-2-hydroxybenzoic acid (4-aminosalicylic acid),



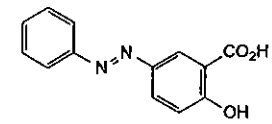
F. 3-amino-2-hydroxybenzoic acid (3-aminosalicylic acid),



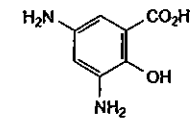
G. 2,5-dihydroxybenzoic acid,



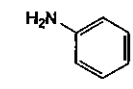
H. 2-hydroxybenzoic acid (salicylic acid),



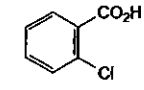
I. 2-hydroxy-5-(phenyldiazenyl)benzoic acid (phenylazosalicylic acid),



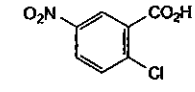
J. 3,5-diamino-2-hydroxybenzoic acid (3,5-diaminosalicylic acid),



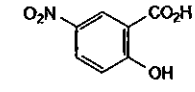
K. aniline,



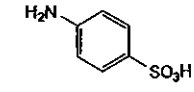
L. 2-chlorobenzoic acid,



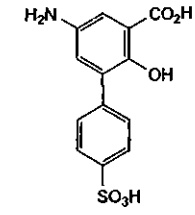
M.2-chloro-5-nitrobenzoic acid,



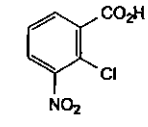
N. 2-hydroxy-5-nitrobenzoic acid (5-nitrosalicylic acid),



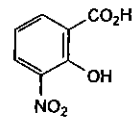
O. 4-aminobenzenesulfonic acid (sulfanilic acid),



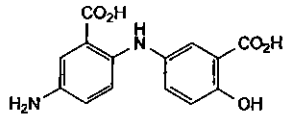
P. 5-amino-2-hydroxy-3-(4-sulfophenyl)benzoic acid (3-(4-sulfophenyl)-5-aminosalicylic acid),



Q. 2-chloro-3-nitrobenzoic acid,



R. 2-hydroxy-3-nitrobenzoic acid (3-nitrosalicylic acid),

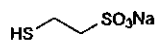


S. 2-hydroxy-5-[(2-carboxy-4-aminophenyl)amino]benzoic acid.

Ph Eur

Mesna

(Ph. Eur. monograph 1674)



$C_2H_5NaO_3S_2$ 164.2 *19767-45-4*

Action and use
Preventing adverse effects of cyclophosphamide and ifosfamide.

Ph Eur

DEFINITION
Sodium 2-sulfanylethanesulfonate.

Content
96.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
White or slightly yellow, crystalline powder, hygroscopic.

Solubility
Freely soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in cyclohexane.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *Ph. Eur. reference spectrum of mesna.*
B. It gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S
Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

pH (*2.2.3*)
4.5 to 6.0.
Dilute 10 mL of solution S to 20 mL with *carbon dioxide-free water R.*

Related substances
Liquid chromatography (*2.2.29*).
Test solution Dissolve 0.10 g of the substance to be examined in the mobile phase and dilute to 25.0 mL with the mobile phase.
Reference solution (a) Dissolve 4.0 mg of *mesna impurity C CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 2.0 mL of the solution to 20.0 mL with the mobile phase.
Reference solution (b) Dissolve 6.0 mg of *mesna impurity D CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase.
Reference solution (c) Dilute 3.0 mL of the test solution to 10.0 mL with the mobile phase.
Reference solution (d) Dilute 1.0 mL of reference solution (c) to 100.0 mL with the mobile phase.
Reference solution (e) Dilute 6.0 mL of reference solution (c) to 20.0 mL with the mobile phase. To 10 mL of the solution add 10 mL of reference solution (a).
Column:
— *size: l* = 0.25 m, Ø = 4.6 mm,
— *stationary phase: octadecylsilyl silica gel for chromatography R* (10 µm).
Mobile phase Dissolve 2.94 g of *potassium dihydrogen phosphate R*, 2.94 g of *dipotassium hydrogen phosphate R* and 2.6 g of *tetrabutylammonium hydrogen sulfate R* in about 600 mL of *water R*. Adjust to pH 2.3 with *phosphoric acid R*, add 335 mL of *methanol R* and dilute to 1000 mL with *water R.*
Flow rate 1 mL/min.
Detection Spectrophotometer at 235 nm.
Injection 20 µL.
Run time 4 times the retention time of mesna.
Relative retention With reference to mesna (retention time = about 4.8 min): impurities A and B = about 0.6; impurity E = about 0.8; impurity C = about 1.4; impurity D = about 2.3.
System suitability Reference solution (e):
— *resolution*: minimum 3.0 between the peaks due to mesna and impurity C.
Limits:
— *correction factors*: for the calculation of content, multiply the peak areas of impurities A, B and E by 0.01,
— *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.2 per cent),
— *impurity D*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (3.0 per cent),
— *impurities A, B, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.3 per cent),
— *any other impurity*: for each impurity, not more than one third of the area of the principal peak in the chromatogram obtained with reference solution (d) (0.1 per cent),
— *sum of other impurities*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.3 per cent),
— *disregard limit*: 0.15 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.045 per cent).

Chlorides (*2.4.4*)
Maximum 250 ppm.
Dilute 1 mL of solution S to 15 mL with *water R.*

Sulfates (*2.4.13*)
Maximum 300 ppm.

Dilute 5 mL of solution S to 30 mL with *distilled water R*. 15 mL of the solution complies with the test.

Disodium edetate
Maximum 500 ppm.

Dissolve 4.000 g in 90 mL of *water R* and adjust to pH 4.5 using *0.1 M hydrochloric acid*. Add 10 mL of *acetate buffer solution pH 4.5 R* and 50 mL of *2-propanol R*. Add 2 mL of a 0.25 g/L solution of *dithizone R* in *2-propanol R*. Titrate with *0.01 M zinc sulfate* until the colour changes from bluish-grey to pink.

1 mL of *0.01 M zinc sulfate* is equivalent to 3.72 mg of $C_{10}H_{14}N_2Na_2O_8,2H_2O$.

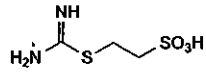
Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g under high vacuum at 60 °C for 2 h.

ASSAY
Dissolve 0.120 g in 10 mL of *water R*. Add 10 mL of *dilute sulfuric acid R* and 10.0 mL of 0.1 M iodine. Titrate with *0.1 M sodium thiosulfate* adding 1 mL of *starch solution R* near the endpoint. Carry out a blank titration.

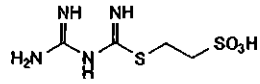
1 mL of *0.1 M sodium thiosulfate* is equivalent to 16.42 mg of $C_2H_5NaO_3S_2$.

STORAGE
In an airtight container.

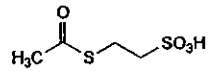
IMPURITIES
Specified impurities *A, B, C, D, E.*



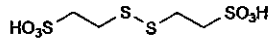
A. 2-(carbamimidoylsulfanyl)ethanesulfonic acid,



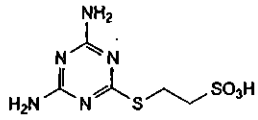
B. 2-[[(guanidino)(imino)methyl]sulfanyl]ethanesulfonic acid,



C. 2-(acetylsulfanyl)ethanesulfonic acid,



D. 2,2′-(disulfanediyl)bis(ethanesulfonic acid),

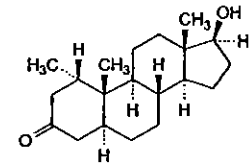


E. 2-(4,6-diamino-1,3,5-triazin-2-yl)sulfanylethanesulfonic acid.

Ph Eur

Mesterolone

(Ph. Eur. monograph 1730)



$C_{20}H_{32}O_2$ 304.5 *1424-00-6*

Action and use
Androgen.

Ph Eur

DEFINITION
17β-Hydroxy-1α-methyl-5α-androstan-3-one.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
White or yellowish crystalline powder.

Solubility
Practically insoluble in water, sparingly soluble in acetone, in ethyl acetate and in methanol.

IDENTIFICATION
A. Melting point (*2.2.14*): 206 °C to 211 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *mesterolone CRS.*

TESTS

Specific optical rotation (*2.2.7*)
+ 20 to + 24 (dried substance).

Dissolve 0.200 g in *methylene chloride R* and dilute to 10.0 mL with the same solvent.

Impurity B
Thin-layer chromatography (*2.2.27*).

Solvent mixture *methanol R, methylene chloride R* (50:50 *V/V*).

Test solution Dissolve 0.100 g of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 200.0 mL with the solvent mixture.

Reference solution (b) Dissolve 5 mg of *mesterolone impurity A CRS* in reference solution (a) and dilute to 100 mL with the same solution.

Plate *TLC silica gel plate R.*

Mobile phase *methanol R, acetone R, toluene R* (2:15:85 *V/V/V*).

Application 10 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 366 nm; spray with a 200 g/L solution of *toluenesulfonic acid R* in *ethanol (96 per cent) R* and heat at 120 °C for 10 min.

System suitability The chromatogram obtained with reference solution (b) shows 2 clearly separated spots (blue spot due to mesterolone and yellow spot due to impurity A).

Limit:
— *impurity B*: any blue spot, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture *water R, acetonitrile R* (20:80 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

Reference solution (a) Dissolve 50.0 mg of *mesterolone CRS* in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *mesterolone for system suitability CRS* (containing impurity C) in the solvent mixture and dilute to 5 mL with the solvent mixture.

Reference solution (c) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase *acetonitrile R1, water for chromatography R, methanol R2* (20:40:60 *V/V/V*).

Flow rate 0.9 mL/min.

Detection Spectrophotometer at 200 nm.

Injection 50 µL of the test solution and reference solutions (b) and (c).

Run time 3 times the retention time of mesterolone.

Identification of impurities Use the chromatogram supplied with *mesterolone for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity C.

Relative retention With reference to mesterolone (retention time = about 22 min): impurity C = about 0.9.

System suitability Reference solution (b):
— *resolution*: minimum 2.5 between the peaks due to impurity C and mesterolone.

Calculation of percentage contents:
— *correction factor*: multiply the peak area of impurity C by 0.2;
— for each impurity, use the concentration of mesterolone in reference solution (c).

Limits:
— *impurity C*: maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.3 per cent;
— *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

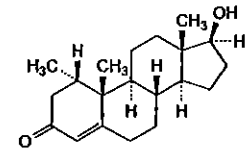
ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection 10 µL of the test solution and reference solution (a).

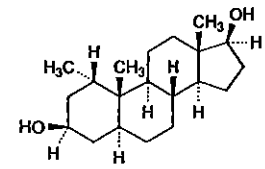
Calculate the percentage content of $C_{20}H_{32}O_2$ taking into account the assigned content of *mesterolone CRS*.

IMPURITIES
Specified impurities *B, C.*

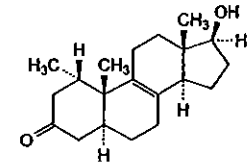
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A.*



A. 17β-hydroxy-1α-methylandrost-4-en-3-one,



B. 1α-methyl-5α-androstane-3β,17β-diol,

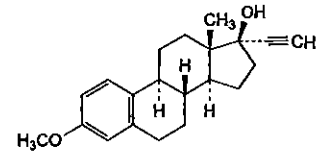


C. 17β-hydroxy-1α-methyl-5α-androst-8-en-3-one.

Ph Eur

Mestranol

(Ph. Eur. monograph 0509)



$C_{21}H_{26}O_2$ 310.4 *72-33-3*

Action and use
Estrogen.

Ph Eur

DEFINITION
Mestranol contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of 3-methoxy-19-nor-17α-pregna-1,3,5(10)-trien-20-yn-17-ol, calculated with reference to the dried substance.

CHARACTERS
A white or almost white, crystalline powder, practically insoluble in water, sparingly soluble in alcohol.

IDENTIFICATION

First identification: B.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 150 °C to 154 °C.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *mestranol CRS*.

C. Examine the chromatograms obtained in the test for related substances in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour, fluorescence and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 5 mg in 1 mL of *sulfuric acid R*. A red colour develops with a greenish-yellow fluorescence in ultraviolet light at 365 nm. Add the solution to 10 mL of *water R* and mix. The solution becomes pink and a pink to violet precipitate is formed on standing.

TESTS

Specific optical rotation (*2.2.7*)

Dissolve 0.100 g in *anhydrous pyridine R* and dilute to 10.0 mL with the same solvent. The specific optical rotation is −20 to −24, calculated with reference to the dried substance.

Absorbance (*2.2.25*)

Dissolve 25.0 mg in *alcohol R* and dilute to 25.0 mL with the same solvent. Dilute 10.0 mL of this solution to 100.0 mL with *alcohol R*. Examined between 260 nm and 310 nm, the solution shows two absorption maxima, at 279 nm and 288 nm, and a minimum at 286 nm. The specific absorbances at the maxima are 62 to 68 and 59 to 64, respectively.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution (a) Dissolve 0.10 g of the substance to be examined in *chloroform R* and dilute to 10 mL with the same solvent.

Test solution (b) Dilute 1 mL of test solution (a) to 10 mL with *chloroform R*.

Reference solution (a) Dissolve 10 mg of *mestranol CRS* in *chloroform R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dilute 1 mL of test solution (b) to 10 mL with *chloroform R*.

Reference solution (c) Dilute 5 mL of reference solution (b) to 10 mL with *chloroform R*.

Apply separately to the plate 5 µL of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *alcohol R* and 90 volumes of *toluene R*. Allow the plate to dry in air until the solvent has evaporated. Heat at 110 °C for 10 min. Spray the hot plate with *alcoholic solution of sulfuric acid R*. Heat again at 110 °C for 10 min. Examine in daylight and in ultraviolet light at 365 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.0 per cent) and at most one such spot is more intense than the spot in the chromatogram obtained with reference solution (c) (0.5 per cent).

Loss on drying (*2.2.32*)

Not more than 1.0 per cent, determined on 0.500 g by drying in an oven at 105 °C for 3 h.

ASSAY

Dissolve 0.200 g in 40 mL of *tetrahydrofuran R* and add 5 mL of a 100 g/L solution of *silver nitrate R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 mL of *0.1 M sodium hydroxide* is equivalent to 31.04 mg of $C_{21}H_{26}O_2$.

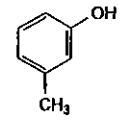
STORAGE

Store protected from light.

Ph Eur

Metacresol

(Ph. Eur. monograph 2077)



C_7H_8O 108.1 *108-39-4*

Action and use

Antiseptic; antimicrobial preservative.

Ph Eur

DEFINITION

3-Methylphenol.

CHARACTERS

Appearance

Colourless or yellowish liquid.

Solubility

Sparingly soluble in water, miscible with ethanol (96 per cent) and with methylene chloride.

Relative density

About 1.03.

mp

About 11 °C.

bp

About 202 °C.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of metacresol.

TESTS

Solution S

Dissolve 1.5 g in *carbon dioxide-free water R* and dilute to 100 mL with the same solvent.

Appearance of solution

Freshly prepared solution S is not more opalescent than reference suspension III (*2.2.1*) and not more intensely coloured than reference solution BY_7 (*2.2.2, Method II*).

Acidity

To 25 mL of solution S add 0.15 mL of *methyl red solution R*. The solution is red. Not more than 0.5 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to yellow.

Related substances

Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution Dissolve 1.00 g of the substance to be examined in *methanol R* and dilute to 100.0 mL with the same solvent.

Reference solution (a) Dissolve 0.10 g of *cresol R*, 0.10 g of *p-cresol R* and 0.10 g of the substance to be examined in *methanol R* and dilute to 20.0 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *methanol R*. Dilute 1.0 mL of this solution to 20.0 mL with *methanol R*.

Column:
- *material*: fused silica,
- *size*: *l* = 25 m, Ø = 0.25 mm,
- *stationary phase*: *cyanopropyl(25)phenyl(25)methyl(50) polysiloxane R* (0.2 µm).

Carrier gas *helium for chromatography R.*

Flow rate 1.8 mL/min.

Split ratio 1:30.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 35	100
	35 - 40	100 → 150
	40 - 50	150
Injection port		200
Detector		200

Detection Flame ionisation.

Injection 1.0 µL.

Relative retention With reference to metacresol (retention time = about 28 min): impurity B = about 0.75; impurity C = about 0.98.

System suitability Reference solution (a):
- *resolution*: minimum 1.4 between the peaks due to impurity C and metacresol.

Limits:
- *impurities B, C*: for each impurity, not more than 0.5 per cent,
- *any other impurity*: for each impurity, not more than 0.1 per cent,
- *total*: not more than 1.0 per cent.
- *disregard limit*: the area of the peak due to metacresol in the chromatogram obtained with reference solution (b) (0.05 per cent).

Residue on evaporation

Maximum 0.1 per cent.

Evaporate 2.0 g to dryness on a water-bath in a fume cupboard and dry at 100-105 °C for 1 h. The residue weighs a maximum of 2 mg.

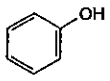
STORAGE

In an airtight container, protected from light.

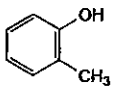
IMPURITIES

Specified impurities *B, C.*

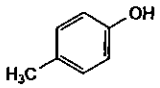
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, D, E, F, G, H, I, J, K, L, M.*



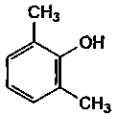
A. phenol,



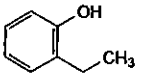
B. 2-methylphenol (*o*-cresol, cresol),



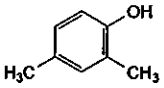
C. 4-methylphenol (*p*-cresol),



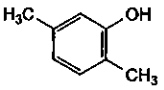
D. 2,6-dimethylphenol (2,6-xylenol),



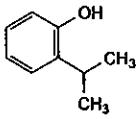
E. 2-ethylphenol (*o*-ethylphenol),



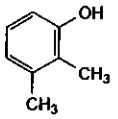
F. 2,4-dimethylphenol (2,4-xylenol),



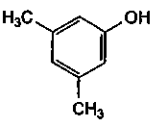
G. 2,5-dimethylphenol (2,5-xylenol),



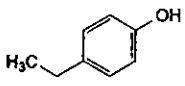
H. 2-(1-methylethyl)phenol,



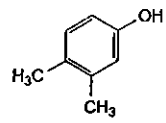
I. 2,3-dimethylphenol (2,3-xylenol),



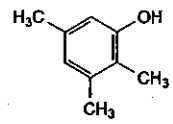
J. 3,5-dimethylphenol (3,5-xylenol),



K. 4-ethylphenol (*p*-ethylphenol),



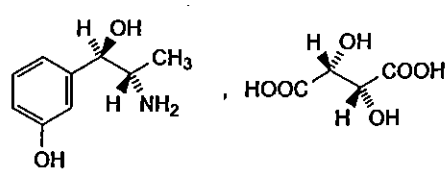
L. 3,4-dimethylphenol (3,4-xylenol),



M. 2,3,5-trimethylphenol.

Ph Eur

Metaraminol Tartrate



$C_9H_{13}NO_2,C_4H_6O_6$ 317.3 *17171-57-2*

Action and use
Adrenoceptor agonist.

Preparation
Metaraminol Injection

DEFINITION
Metaraminol Tartrate is (1*R*,2*S*)-2-amino-1-(3-hydroxyphenyl)propan-1-ol hydrogen (2*R*,3*R*)-tartrate.
It contains not less than 99.0% and not more than 101.0% of $C_9H_{13}NO_2,C_4H_6O_6$, calculated with reference to the dried substance.

CHARACTERISTICS
A white, crystalline powder.

Freely soluble in *water*; sparingly soluble in *ethanol (96%)*; practically insoluble in *ether*.

IDENTIFICATION
A. In the test for Related substances the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (4).

B. To 0.5 mL of a 0.05% w/v solution add 0.5 mL of *phosphomolybdotungstic reagent* and 5 mL of *dilute sodium carbonate solution* and allow to stand for 5 minutes.
An intense blue colour is produced.

C. To 4 mL of a 0.05% w/v solution add 5 mL of *borate buffer pH 9.6* and 1 mL of a freshly prepared 0.5% w/v solution of *sodium 1,2-naphthaquinone-4-sulfonate* and allow to stand for 1 minute. Add 0.2 mL of a 2% v/v solution of *benzalkonium chloride solution* and 5 mL of *toluene* and shake.
A mauve colour is immediately produced in the toluene layer (distinction from phenylephrine).

TESTS
Acidity
pH of a 5% w/v solution, 3.2 to 3.5, Appendix V L.

Phenones
Absorbance of a 0.2% w/v solution at 310 nm, not more than 0.2, calculated with reference to the dried substance, Appendix II B.

Related substances
Carry out in subdued light the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *methanol*.

(1) 1.0% w/v of the substance being examined.

(2) 0.050% w/v of the substance being examined.

(3) 0.0050% w/v of the substance being examined.

(4) 0.050% w/v of *metaraminol tartrate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel precoated plate (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and spray with a solution prepared in the following manner. Mix 25 mL of a 0.45% w/v solution of *sulfanilic acid* in 1M *hydrochloric acid* with 1.5 mL of a 5% w/v solution of *sodium nitrite*, allow to stand for 5 minutes and mix cautiously with 25 mL of 2M *sodium carbonate*.

MOBILE PHASE

10 volumes of 13.5M *ammonia*, 80 volumes of *chloroform* and 80 volumes of *methanol*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

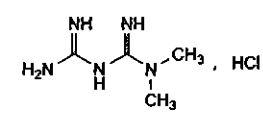
Loss on drying
When dried to constant weight at 105°, loses not more than 0.5% of its weight. Use 1 g.

Sulfated ash
Not more than 0.1%, Appendix IX A.

ASSAY
Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.6 g and *crystal violet solution* as indicator. Each mL of *0.1M perchloric acid VS* is equivalent to 31.73 mg of $C_9H_{13}NO_2,C_4H_6O_6$.

Metformin Hydrochloride

(Ph. Eur. monograph 0931)



$C_4H_{12}ClN_5$ 165.6 *1115-70-4*

Action and use
Biguanide; treatment of diabetes mellitus.

Preparations
Metformin Oral Solution
Metformin Tablets
Metformin Prolonged-release Tablets

Ph Eur

DEFINITION

1,1-Dimethylbiguanide hydrochloride.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white crystals.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in acetone and in methylene chloride.

IDENTIFICATION

First identification: B, E.

Second identification: A, C, D, E.

A. Melting point (*2.2.14*): 222 °C to 226 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *metformin hydrochloride CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 20 mg of the substance to be examined in *water R* and dilute to 5 mL with the same solvent.

Reference solution Dissolve 20 mg of *metformin hydrochloride CRS* in *water R* and dilute to 5 mL with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *glacial acetic acid R, butanol R, water R* (10:40:50 *V/V/V*); use the upper layer.

Application 5 µL.

Development Over 3/4 of the plate.

Drying At 100-105 °C for 15 min.

Detection Spray with a mixture of equal volumes of a 100 g/L solution of *sodium nitroprusside R*, a 100 g/L solution of *potassium ferricyanide R* and a 100 g/L solution of *sodium hydroxide R*, prepared 20 min before use.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve about 5 mg in *water R* and dilute to 100 mL with the same solvent. To 2 mL of the solution add 0.25 mL of *strong sodium hydroxide solution R* and 0.10 mL of *α-naphthol solution R*. Mix and allow to stand in iced water for 15 min. Add 0.5 mL of *sodium hypobromite solution R* and mix. A pink colour develops.

E. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 2.0 g in *water R* and dilute to 20 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*). Heat the solution to 50 °C and cool to room temperature.

Impurity F

Liquid chromatography (*2.2.29*).

Derivatisation solution *Prepare the solution immediately before use.* Dilute 1 mL of *fluorodinitrobenzene R* in 100.0 mL of *acetonitrile R*.

Blank solution To 5.0 mL of *acetonitrile R* add 100 µL of *triethylamine R1* and 1.0 mL of the derivatisation solution. Shake well and heat at 60 °C for 30 min. After cooling, dilute to 10.0 mL with *acetonitrile R*.

Test solution *Prepare the solution immediately before use.* Suspend 10.0 mg of the substance to be examined in 5.0 mL of *acetonitrile R* and sonicate for 5 min. Add 100 µL of *triethylamine R1* and 1.0 mL of the derivatisation solution. Shake well and heat at 60 °C for 30 min. After cooling, dilute to 10.0 mL with *acetonitrile R*. Filter or centrifuge at 800 *g* for 5 min before use.

Reference solution Dilute 1.0 mL of *metformin impurity F CRS* in 100.0 mL of *acetonitrile R*. Dilute 2.5 mL of the solution to 100.0 mL with *acetonitrile R*. To 1.0 mL of this solution add successively 5.0 mL of *acetonitrile R*, 100 µL of *triethylamine R1* and 1.0 mL of the derivatisation solution. Shake well and heat at 60 °C for 30 min. After cooling, dilute to 10.0 mL with *acetonitrile R*.

Column:

— *size*: *l* = 0.125 m, Ø = 3 mm;
— *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R1* (5 µm);
— *temperature*: 30 °C.

Mobile phase:

— *mobile phase A*: *phosphoric acid R, water for chromatography R* (0.1:99.9 *V/V*);
— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	60 → 45	40 → 55
10 - 11	45 → 25	55 → 75
11 - 15	25	75

Flow rate 0.7 mL/min.

Detection Spectrophotometer at 380 nm.

Injection 5 µL.

Identification of impurities Use the chromatograms obtained with the blank solution and the reference solution to identify the peak due to the impurity F derivative.

Retention time Impurity F derivative = about 4 min.

System suitability Reference solution:

— *resolution*: minimum 3.0 between the peak due to the impurity F derivative and the nearby eluting peaks due to the derivatisation reagent.

Limit:

— *impurity F*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.05 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dissolve 20.0 mg of *metformin impurity A CRS* in *water R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of the solution to 200.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 50.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 20.0 mL with the mobile phase.

Reference solution (c) Dissolve 10 mg of *melamine R* (impurity D) in about 90 mL of *water R*. Add 5 mL of the test solution and dilute to 100 mL with *water R*. Dilute 1 mL of this solution to 50 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *strong cation-exchange silica gel for chromatography R* (10 µm).

Mobile phase 17 g/L solution of *ammonium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 218 nm.

Injection 20 µL.

Run time Twice the retention time of metformin.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity A; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity D.

Relative retention With reference to metformin (retention time = about 14 min): impurity A = about 0.3; impurity D = about 0.4.

System suitability Reference solution (c):
— *resolution*: minimum 10 between the peaks due to impurity D and metformin.

Limits:
— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.02 per cent);
— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent);
— *total*: maximum 0.2 per cent;
— *disregard limit*: 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.03 per cent); do not disregard the peak due to impurity A.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 5 h.

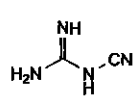
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.100 g in 4 mL of *anhydrous formic acid R*. Add 80 mL of *acetonitrile R*. Carry out the titration immediately. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

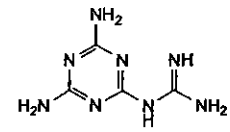
1 mL of *0.1 M perchloric acid* is equivalent to 16.56 mg of $C_4H_{12}ClN_5$.

IMPURITIES
Specified impurities *A, F.*

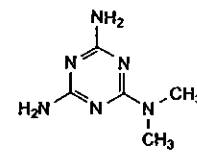
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D, E.*



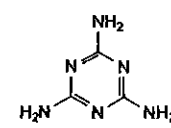
A. cyanoguanidine,



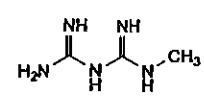
B. (4,6-diamino-1,3,5-triazin-2-yl)guanidine,



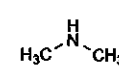
C. N^2,N^2-dimethyl-1,3,5-triazine-2,4,6-triamine (*N*,*N*-dimethylmelamine),



D. 1,3,5-triazine-2,4,6-triamine (melamine),



E. 1-methylbiguanide,



F. *N*-methylmethanamine (dimethylamine).

Ph Eur

Basic Butylated Methacrylate Copolymer

(Ph. Eur. monograph 1975)

Action and use
Excipient.

Ph Eur

DEFINITION
Copolymer of 2-(dimethylamino)ethyl methacrylate, butyl methacrylate and methyl methacrylate having a mean relative molecular mass of about 47 000. The ratio of 2-(dimethylamino)ethyl methacrylate groups to butyl methacrylate and methyl methacrylate groups is about 2:1:1.

Content of (dimethylamino)ethyl groups 20.8 per cent to 25.5 per cent (dried substance).

CHARACTERS
Appearance
Colourless or yellowish granules or white or almost white powder, slightly hygroscopic.

Solubility
Practically insoluble in water, freely soluble in methylene chloride. It dissolves slowly in ethanol (96 per cent).

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *basic butylated methacrylate copolymer CRS.*
B. It complies with the limits of the assay.

TESTS

Solution S

Dissolve 12.5 g in a mixture of 35.0 g of *acetone R* and 52.5 g of *2-propanol R*.

Viscosity (*2.2.10*)

3 mPa·s to 6 mPa·s, determined on solution S.

Apparatus Rotating viscometer.

Dimensions:

— *spindle*: diameter = 25.15 mm, height = 90.74 mm, shaft diameter = 4 mm;

— *cylinder*: diameter = 27.62 mm, height = 0.135 m.

Rotating speed 30 r/min.

Volume of solution 16 mL of solution S.

Temperature 20 °C.

Absorbance (*2.2.25*)

Maximum 0.30 at 420 nm, determined on solution S.

Appearance of a film

Spread 1.0 mL of solution S evenly on a glass plate. Upon drying a clear film is formed.

Monomers

Maximum 0.1 per cent for each monomer (butyl methacrylate, methyl methacrylate and 2-(dimethylamino) ethyl methacrylate), determined by procedures A and B.

A. Butyl methacrylate and methyl methacrylate. Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R1, phosphate buffer solution pH 2.0 R* (40:60 *V/V*).

Test solution Dissolve 1.00 g of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution Dissolve 20.0 mg of *butyl methacrylate CRS* (impurity A) and 10.0 mg of *methyl methacrylate CRS* (impurity B) in 3.0 mL of *butanol R* and dilute to 10.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 250.0 mL with the solvent mixture.

Column:

— *size*: *l* = 0.125 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (7 µm).

Mobile phase *phosphate buffer solution pH 2.0 R, methanol R2* (45:55 *V/V*).

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 205 nm.

Injection 50 µL.

System suitability Reference solution:

— *resolution*: minimum 5 between the peaks due to impurities A and B.

Calculate the percentage contents of impurities A and B using the following expression:

$$100 \times 10^{-6} \times 50 \times \frac{C}{M} \times \frac{A_T}{A_R}$$

C = concentration of the monomer in the reference solution, in micrograms per millilitre;

M = mass of substance to be examined in the test solution, in grams;

A_T = area of the peak due to the monomer in the chromatogram obtained with the test solution;

A_R = area of the peak due to the monomer in the chromatogram obtained with the reference solution.

B. 2-(Dimethylamino)ethyl methacrylate. Liquid chromatography (*2.2.29*).

Test solution Dissolve 1.00 g of the substance to be examined in *tetrahydrofuran R* and dilute to 50.0 mL with the same solvent.

Reference solution Dissolve 10.0 mg of *2-(dimethylamino)ethyl methacrylate CRS* (impurity C) in *tetrahydrofuran R* and dilute to 50.0 mL with the same solvent. Dilute 2.0 mL of the solution to 50.0 mL with *tetrahydrofuran R*.

Column:

— *size*: *l* = 0.125 m, Ø = 4.6 mm;

— *stationary phase*: *aminopropylsilyl silica gel for chromatography R* (7 µm).

Mobile phase Mix 25 volumes of a 3.404 g/L solution of *potassium dihydrogen phosphate R* and 75 volumes of *tetrahydrofuran R*.

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 50 µL.

Calculate the percentage content of impurity C as described under procedure A.

Loss on drying (*2.2.32*)

Maximum 2.0 per cent, determined on 1.000 g by drying in an oven at 110 °C for 3 h.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

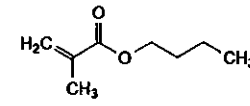
Dissolve 0.200 g in a mixture of 4 mL of *water R* and 96 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 7.21 mg of $C_4H_{10}N$.

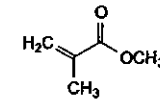
STORAGE

In an airtight container.

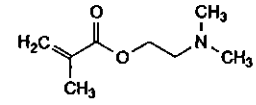
IMPURITIES



A. butyl 2-methylprop-2-enoate (butyl methacrylate),



B. methyl 2-methylprop-2-enoate (methyl methacrylate),



C. 2-(dimethylamino)ethyl 2-methylprop-2-enoate (2-(dimethylamino)ethyl methacrylate).

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute

to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for basic butylated methacrylate copolymer used as film former in tablets.

Viscosity
(see Tests).

Appearance of a film
(see Tests).

Solubility of a film
Take the film obtained in the test for appearance of a film (see Tests), place it in a flask containing a 10.3 g/L solution of *hydrochloric acid R* and stir. It dissolves within 1 h. Take another film, place it in a flask containing phosphate buffer solution pH 6.8 R and stir. It does not dissolve within 2 h.

Ph Eur

Methacrylic Acid - Ethyl Acrylate Copolymer (1:1)

(Ph. Eur. monograph 1128)

Action and use
Pharmaceutical aid.

Ph Eur

DEFINITION
Copolymer of methacrylic acid and ethyl acrylate having a mean relative molecular mass of about 250 000. The ratio of carboxylic groups to ester groups is about 1:1. The substance is in the acid form (type A) or partially neutralised using sodium hydroxide (type B). It may contain suitable surface-active agents such as sodium dodecyl sulfate and polysorbate 80.

Content
— *type A*: 46.0 per cent to 50.6 per cent of methacrylic acid units (dried substance);
— *type B*: 43.0 per cent to 48.0 per cent of methacrylic acid units (dried substance).

CHARACTERS
Appearance
White or almost white, free-flowing powder.

Solubility
Practically insoluble in water (type A) or dispersible in water (type B), freely soluble in anhydrous ethanol, practically insoluble in ethyl acetate. It is freely soluble in a 40 g/L solution of sodium hydroxide.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Dissolve 0.1 g of the substance to be examined in 1 mL of *ethanol (90 per cent V/V) R* and place 2 drops of the solution on a sodium chloride plate; dry to allow the formation of a film and cover with another sodium chloride plate.

Comparison *methacrylic acid - ethyl acrylate copolymer (1:1) - type A CRS* or *methacrylic acid - ethyl acrylate copolymer (1:1) - type B CRS*.

B. It complies with the limits of the assay.

C. Sulfated ash (see Tests).

TESTS
Viscosity (*2.2.10*)
— *Type A*: 100 mPa·s to 200 mPa·s.

Dissolve a quantity of the substance to be examined corresponding to 37.5 g of the dried substance in a mixture of 7.9 g of *water R* and 254.6 g of *2-propanol R*. Determine the viscosity at 20 °C using a rotating viscometer at a shear rate of 10 s^{-1}.
— *Type B*: maximum 100 mPa·s.

Disperse a quantity of the substance to be examined corresponding to 80.0 g of the dried substance in *water R* and make up to 400 g with the same solvent. Stir for 3 h and determine the viscosity at 23 °C using a rotating viscometer and a spindle rotating at 100 r/min.

Dimensions of the spindle Diameter = 47.0 mm; height = 27.0 mm; shaft diameter = 3.18 mm.

Appearance of a film
Spread 1 mL of the solution (type A) or dispersion (type B) prepared for the test for viscosity on a glass plate and allow to dry. A clear, brittle film is formed.

Ethyl acrylate and methacrylic acid
Liquid chromatography (*2.2.29*).

Solution A Dissolve 3.5 g of *sodium perchlorate R* in *water R* and dilute to 100.0 mL with the same solvent.

Blank solution *methanol R*, solution A (50:50 *V/V*).

Test solution Dissolve 3.0 g of the substance to be examined in *methanol R* and dilute to 50.0 mL with the same solvent. To 5.0 mL of solution A add 5.0 mL of the solution dropwise whilst stirring continuously. Centrifuge until a clear supernatant is obtained. Use the clear supernatant as the test solution.

Reference solution Dissolve 50.0 mg of *ethyl acrylate R* and 50.0 mg of *methacrylic acid R* in 5 mL of *butanol R* and dilute to 100.0 mL with *methanol R*. Dilute 1.0 mL of the solution to 100.0 mL with *methanol R*. Dilute 2.0 mL of this solution to 10.0 mL with *methanol R*. Mix 5.0 mL of this solution and 5.0 mL of solution A.

Column:
— *size*: *l* = 0.125 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (7 µm).

Mobile phase Mix 20 volumes of *methanol R2* and 80 volumes of *water for chromatography R* previously adjusted to pH 2.0 with *phosphoric acid R*.

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 202 nm.

Injection 20 µL.

Retention time Methacrylic acid = about 3 min; ethyl acrylate = about 9 min.

System suitability:
— *resolution*: minimum 5.0 between the peaks due to methacrylic acid and ethyl acrylate in the chromatogram obtained with the reference solution;
— the chromatogram obtained with the blank solution does not show peaks with the same retention times as ethyl acrylate or methacrylic acid.

Calculation of percentage contents:
— for ethyl acrylate and methacrylic acid, use the respective concentration of these substances in the reference solution.

Limit:
— *sum of the contents of ethyl acrylate and methacrylic acid*: maximum 0.01 per cent;
— *reporting threshold*: 1 ppm.

Loss on drying (*2.2.32*)
Maximum 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 6 h.

Sulfated ash (*2.4.14*)
Maximum 0.4 per cent (type A) or 0.5 per cent to 3.0 per cent (type B), determined on 1.0 g.

ASSAY
Dissolve 1.000 g in a mixture of 40 mL of *water R* and 60 mL of *2-propanol R*. Titrate slowly while stirring with *0.5 M sodium hydroxide*, using *phenolphthalein solution R* as indicator.

1 mL of *0.5 M sodium hydroxide* is equivalent to 43.05 mg of $C_4H_6O_2$ (methacrylic acid units).

LABELLING
The label states the type (type A or type B).

FUNCTIONALITY-RELATED CHARACTERISTICS
This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for methacrylic acid - ethyl acrylate copolymer (1:1) used as gastro-resistant coating agent.

Viscosity
(see Tests).

Appearance of a film
(see Tests).

Solubility of a film
Take a piece of the film obtained in the test for appearance of a film (see Tests), place it in a flask containing a 10.3 g/L solution of *hydrochloric acid R* and stir; it does not dissolve within 2 h. Take another piece of the film, place it in a flask containing *phosphate buffer solution pH 6.0 R* and stir; it dissolves within 1 h.

Ph Eur

Methacrylic Acid - Ethyl Acrylate Copolymer (1:1) Dispersion 30 per cent

(Ph. Eur. monograph 1129)

Action and use
Excipient.

Ph Eur

DEFINITION
Dispersion in water of a copolymer of methacrylic acid and ethyl acrylate having a mean relative molecular mass of about 250 000. The ratio of carboxylic groups to ester groups is about 1:1.

Content
46.0 per cent to 50.6 per cent of methacrylic acid units (residue on evaporation).

It may contain suitable surface-active agents such as sodium dodecyl sulfate and polysorbate 80.

CHARACTERS
Appearance
Opaque, white or almost white, slightly viscous liquid.

Solubility
Miscible with water. On addition of solvents such as acetone, anhydrous ethanol or 2-propanol, a precipitate is formed which dissolves on addition of excess solvent. It is miscible with a 40 g/L solution of sodium hydroxide.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *Ph. Eur. reference spectrum of methacrylic acid - ethyl acrylate copolymer (1:1) dispersion 30 per cent.*

B. It complies with the limits of the assay.

TESTS
Viscosity (*2.2.10*)
Maximum 15 mPa·s, determined using a rotating viscometer at 20 °C and at a shear rate of 50 s^{-1}.

Appearance of a film
Place 1 mL on a glass plate and allow to dry. A clear, brittle film is formed.

Particulate matter
Filter 100.0 g through a tared stainless steel sieve (90). Rinse with *water R* until a clear filtrate is obtained and dry at 100-105 °C. The residue weighs a maximum of 1.00 g.

Ethyl acrylate and methacrylic acid
Liquid chromatography (*2.2.29*).

Solution A Dissolve 3.5 g of *sodium perchlorate R* in *water for chromatography R* and dilute to 100.0 mL with the same solvent.

Blank solution Solution A, *methanol R1* (1:2 *V/V*).

Test solution Dissolve 10.0 g of the dispersion to be examined in *methanol R1* and dilute to 50.0 mL with the same solvent. To 5.0 mL of solution A add 10.0 mL of the solution dropwise whilst stirring continuously. Centrifuge until a clear supernatant is obtained. Use the clear supernatant as the test solution.

Reference solution Dissolve 50.0 mg of *ethyl acrylate R* and 50.0 mg of *methacrylic acid R* in 5 mL of *butanol R* and dilute to 50.0 mL with *methanol R1*. Dilute 1.0 mL of the solution to 100.0 mL with *methanol R1*. Dilute 2.0 mL of this solution to 10.0 mL with *methanol R1*. Mix 10.0 mL of this solution and 5.0 mL of solution A.

Column:
— *size: l* = 0.125 m, Ø = 4.6 mm;
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (7 µm).

Mobile phase Mix 20 volumes of *methanol R1* and 80 volumes of *water for chromatography R* previously adjusted to pH 2.0 with *phosphoric acid R.*

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 202 nm.

Injection 20 µL.

Retention time Methacrylic acid = about 3 min, ethyl acrylate = about 8 min.

System suitability:
— *resolution:* minimum 5.0 between the peaks due to methacrylic acid and ethyl acrylate in the chromatogram obtained with the reference solution;
— the chromatogram obtained with the blank solution does not show peaks with the same retention times as ethyl acrylate or methacrylic acid.

Calculation of percentage contents:
— for ethyl acrylate and methacrylic acid, use the respective concentration of these substances in the reference solution.

Limit:
— *sum of the contents of ethyl acrylate and methacrylic acid:* maximum 0.01 per cent;
— *reporting threshold:* 1 ppm.

Residue on evaporation
28.5 per cent to 31.5 per cent.

Dry 1.000 g at 110 °C for 5 h. The residue weighs not less than 0.285 g and not more than 0.315 g.

Sulfated ash (*2.4.14*)
Maximum 0.2 per cent, determined on 1.0 g.

Microbial contamination
TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*).
TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

ASSAY
Dissolve 1.500 g in a mixture of 40 mL of *water R* and 60 mL of *2-propanol R.* Titrate slowly while stirring with *0.5 M sodium hydroxide,* using *phenolphthalein solution R* as indicator.

1 mL of *0.5 M sodium hydroxide* is equivalent to 43.05 mg of $C_4H_6O_2$ (methacrylic acid units).

STORAGE
At a temperature of 5 °C to 25 °C, protected from freezing.

FUNCTIONALITY-RELATED CHARACTERISTICS
This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for methacrylic acid-ethyl acrylate copolymer (1:1) dispersion 30 per cent used as a gastro-resistant coating agent.

Viscosity
(see Tests).

Appearance of a film
(see Tests).

Solubility of a film
Take a piece of the film obtained in the test for appearance of a film (see Tests), place it in a flask containing a 10.3 g/L solution of *hydrochloric acid R* and stir; it does not dissolve within 2 h. Take another piece of the film, place it in a flask containing phosphate buffer solution pH 6.0 R and stir; it dissolves within 1 h.

Ph Eur

Methacrylic Acid - Methyl Methacrylate Copolymer (1:1)

(Ph. Eur. monograph 1127)

Action and use
Excipient.

Ph Eur

DEFINITION
Copolymer of methacrylic acid and methyl methacrylate having a mean relative molecular mass of about 135 000. The ratio of carboxylic groups to ester groups is about 1:1.

Content
46.0 per cent to 50.6 per cent of methacrylic acid units (dried substance).

CHARACTERS
Appearance
White or almost white, free-flowing powder.

Solubility
Practically insoluble in water, freely soluble in anhydrous ethanol and in 2-propanol, practically insoluble in ethyl acetate. It is freely soluble in a 40 g/L solution of sodium hydroxide.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Dissolve 0.1 g of the substance to be examined in 1 mL of *2-propanol R* and place 2 drops of the solution on a sodium chloride plate; dry to allow the formation of a film and cover with another sodium chloride plate.

Comparison methacrylic acid - methyl methacrylate copolymer (1:1) CRS.

B. It complies with the limits of the assay.

TESTS
Viscosity (*2.2.10*)
50 mPa·s to 200 mPa·s.

Dissolve a quantity of the substance to be examined corresponding to 37.5 g of the dried substance in a mixture of 7.9 g of *water R* and 254.6 g of *2-propanol R.* Determine the viscosity using a rotating viscometer at 20 °C and at a shear rate of 10 s^{-1}.

Appearance of a film
Place 1 mL of the solution prepared in the test for viscosity on a glass plate and allow to dry. A clear, brittle film is formed.

Methyl methacrylate and methacrylic acid
Liquid chromatography (*2.2.29*).

Blank solution Mix 3.0 mL of *methanol R1* and 10.0 mL of *0.125 M phosphate buffer solution pH 2.0 R*.

Test solution Dissolve 1.00 g of the substance to be examined in *methanol R1* and dilute to 50.0 mL with the same solvent. To 10.0 mL of *0.125 M phosphate buffer solution pH 2.0 R* add 3.0 mL of the solution dropwise whilst stirring continuously. Centrifuge until a clear supernatant is obtained. Use the clear supernatant as the test solution.

Reference solution Dissolve 50.0 mg of *methacrylic acid R* and 50.0 mg of *methyl methacrylate R* in 5 mL of *butanol R* and dilute to 100.0 mL with *methanol R1*. Dilute 1.0 mL of the solution to 100.0 mL with *methanol R1*. Mix 3.0 mL of this solution and 10.0 mL of *0.125 M phosphate buffer solution pH 2.0 R*.

Column:
— *size*: *l* = 0.125 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (7 µm).

Mobile phase Mix 20 volumes of *methanol R1* and 80 volumes of *water for chromatography R* previously adjusted to pH 2.0 with *phosphoric acid R*.

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 202 nm.

Injection 20 µL.

Retention time Methacrylic acid = about 3 min; methyl methacrylate = about 9 min.

System suitability:
— *resolution*: minimum 5.0 between the peaks due to methacrylic acid and methyl methacrylate in the chromatogram obtained with the reference solution;
— the chromatogram obtained with the blank solution does not show peaks with the same retention times as methacrylic acid or methyl methacrylate.

Calculation of percentage contents:
— for methacrylic acid and methyl methacrylate, use the respective concentration of these substances in the reference solution.

Limits:
— *methacrylic acid*: maximum 0.03 per cent;
— *methyl methacrylate*: maximum 0.02 per cent.

Loss on drying (*2.2.32*)
Maximum 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 6 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 1.000 g in a mixture of 40 mL of *water R* and 60 mL of *2-propanol R*. Titrate slowly while stirring with *0.5 M sodium hydroxide*, using *phenolphthalein solution R* as indicator.

1 mL of *0.5 M sodium hydroxide* is equivalent to 43.05 mg of $C_4H_6O_2$ (methacrylic acid units).

FUNCTIONALITY-RELATED CHARACTERISTICS
This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for methacrylic acid-methyl methacrylate copolymer (1:1) used as gastro-resistant coating agent.

Viscosity
(see Tests).

Appearance of a film
(see Tests).

Solubility of a film
Take a piece of the film obtained in the test for appearance of a film (see Tests), place it in a flask containing a 10.3 g/L solution of *hydrochloric acid R* and stir; it does not dissolve within 2 h. Take another piece of the film, place it in a flask containing phosphate buffer solution pH 6.8 R and stir; it dissolves within 1 h.

Ph Eur

Methacrylic Acid - Methyl Methacrylate Copolymer (1:2)

(Ph. Eur. monograph 1130)

Action and use
Excipient.

Ph Eur

DEFINITION
Copolymer of methacrylic acid and methyl methacrylate having a mean relative molecular mass of about 135 000. The ratio of carboxylic groups to ester groups is about 1:2.

Content
27.6 per cent to 30.7 per cent of methacrylic acid units (dried substance).

CHARACTERS
Appearance
White or almost white, free-flowing powder.

Solubility
Practically insoluble in water, freely soluble in anhydrous ethanol and in 2-propanol, practically insoluble in ethyl acetate. It is freely soluble in a 40 g/L solution of sodium hydroxide.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Dissolve 0.1 g of the substance to be examined in 1 mL of *2-propanol R* and place 2 drops of the solution on a sodium chloride plate; dry to allow the formation of a film and cover with another sodium chloride plate.

Comparison *methacrylic acid - methyl methacrylate copolymer (1:2) CRS*.

B. It complies with the limits of the assay.

TESTS

Viscosity (*2.2.10*)

50 mPa·s to 200 mPa·s.

Dissolve a quantity of the substance to be examined corresponding to 37.5 g of the dried substance in a mixture of 7.9 g of *water R* and 254.6 g of *2-propanol R*. Determine the viscosity using a rotating viscometer at 20 °C and at a shear rate of 10 s^{-1}.

Appearance of a film

Place 1 mL of the solution prepared in the test for viscosity on a glass plate and allow to dry. A clear, brittle film is formed.

Methyl methacrylate and methacrylic acid

Liquid chromatography (*2.2.29*).

Blank solution Mix 3.0 mL of *methanol R1* and 10.0 mL of *0.125 M phosphate buffer solution pH 2.0 R*.

Test solution Dissolve 1.00 g of the substance to be examined in *methanol R1* and dilute to 50.0 mL with the same solvent. To 10.0 mL of *0.125 M phosphate buffer solution pH 2.0 R* add 3.0 mL of the solution dropwise whilst stirring continuously. Centrifuge until a clear supernatant is obtained. Use the clear supernatant as the test solution.

Reference solution Dissolve 50.0 mg of *methacrylic acid R* and 50.0 mg of *methyl methacrylate R* in 5 mL of *butanol R* and dilute to 100.0 mL with *methanol R1*. Dilute 1.0 mL of the solution to 100.0 mL with *methanol R1*. Mix 3.0 mL of this solution and 10.0 mL of *0.125 M phosphate buffer solution pH 2.0 R*.

Column:
- *size*: *l* = 0.125 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (7 µm).

Mobile phase Mix 20 volumes of *methanol R1* and 80 volumes of *water for chromatography R* previously adjusted to pH 2.0 with *phosphoric acid R*.

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 202 nm.

Injection 20 µL.

Retention time Methacrylic acid = about 3 min; methyl methacrylate = about 9 min.

System suitability:
- *resolution*: minimum 5.0 between the peaks due to methacrylic acid and methyl methacrylate in the chromatogram obtained with the reference solution;
- the chromatogram obtained with the blank solution does not show peaks with the same retention times as methacrylic acid or methyl methacrylate.

Calculation of percentage contents:
- for methacrylic acid and methyl methacrylate, use the respective concentration of these substances in the reference solution.

Limits:
- *methacrylic acid*: maximum 0.03 per cent;
- *methyl methacrylate*: maximum 0.02 per cent.

Loss on drying (*2.2.32*)

Maximum 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 6 h.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 1.000 g in a mixture of 40 mL of *water R* and 60 mL of *2-propanol R*. Titrate slowly while stirring with *0.5 M sodium hydroxide*, using *phenolphthalein solution R* as indicator.

1 mL of *0.5 M sodium hydroxide* is equivalent to 43.05 mg of $C_4H_6O_2$ (methacrylic acid units).

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for methacrylic acid-methyl methacrylate copolymer (1:2) used as gastro-resistant coating agent.

Viscosity

(see Tests).

Appearance of a film

(see Tests).

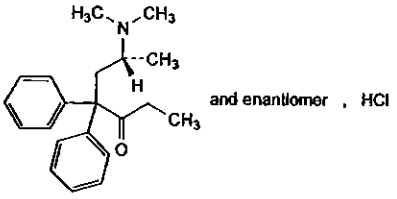
Solubility of a film

Take a piece of the film obtained in the test for appearance of a film (see Tests), place it in a flask containing a 10.3 g/L solution of *hydrochloric acid R* and stir; it does not dissolve within 2 h. Take another piece of the film, place it in a flask containing phosphate buffer solution pH 6.8 R and stir; it does not dissolve within 2 h. Take another piece of the film, place it in a flask containing 0.2 M phosphate buffer solution pH 7.5 R and stir; it dissolves within 1 h.

Ph Eur

Methadone Hydrochloride

(Ph. Eur. monograph 0408)



$C_{21}H_{28}ClNO$ 345.9 *1095-90-5*

Action and use

Opioid receptor agonist; analgesic; treatment of opioid dependence.

Preparations

Methadone Concentrate for Oral Solution

Methadone Injection

Methadone Oral Solution (1 mg per ml)

Methadone Tablets

Ph Eur

DEFINITION

(6*RS*)-6-(Dimethylamino)-4,4-diphenylheptan-3-one hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: A, C, D.

Second identification: A, B, D.

A. Optical rotation (see Tests).

B. Melting point (*2.2.14*): 233 °C to 236 °C.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of methadone hydrochloride.*

D. Dilute 1 mL of solution S (see Tests) to 5 mL with *water R* and add 1 mL of *dilute ammonia R1*. Mix, allow to stand for 5 min and filter. The filtrate gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 50.0 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

Dilute 10 mL of solution S to 25 mL with *carbon dioxide-free water R*. To 10 mL of the solution add 0.2 mL of *methyl red solution R* and 0.2 mL of *0.01 M sodium hydroxide*. The solution is yellow. Add 0.4 mL of *0.01 M hydrochloric acid*. The solution is red.

Optical rotation (*2.2.7*)

−0.05° to + 0.05°, determined on solution S in a 2 dm tube.

Related substances

Gas chromatography (*2.2.28*).

Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 10.0 mL with *methanol R*. Dilute 1.0 mL of this solution to 100.0 mL with *methanol R*.

Reference solution (b) Dissolve 5 mg of *imipramine hydrochloride CRS* and 5 mg of *cyclobenzaprine hydrochloride CRS* in 100.0 mL of *methanol R*.

Column:

- *material*: fused silica;
- *size*: *l* = 50 m, Ø = 0.32 mm;
- *stationary phase*: *phenyl(5)methyl(95)polysiloxane R* (film thickness 1.05 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.2 mL/min.

Injection liner Packed with deactivated glass wool to wipe the needle.

Split ratio 1:100.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 4	150 → 250
	4 - 35	250
Injection port		200
Detector		250

Detection Flame ionisation.

Injection 2 µL.

Run time 1.5 times the retention time of methadone.

Relative retention With reference to methadone (retention time = about 25 min): impurity E = about 0.44; impurity C = about 0.81; impurity B = about 0.89; impurity D = about 0.98; impurity A = about 1.14; imipramine = about 1.19; cyclobenzaprine = about 1.24.

System suitability Reference solution (b):

- *resolution*: minimum 3.0 between the peaks due to imipramine and cyclobenzaprine.

Limits:

- *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in a mixture of 5 mL of *0.01 M hydrochloric acid* and 50 mL of *anhydrous ethanol R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion. Carry out a blank titration.

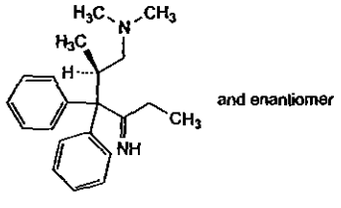
1 mL of *0.1 M sodium hydroxide* is equivalent to 34.59 mg of $C_{21}H_{28}ClNO$.

STORAGE

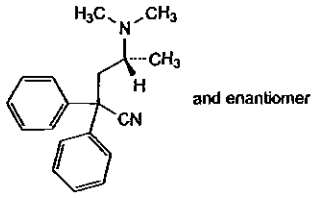
Protected from light.

IMPURITIES

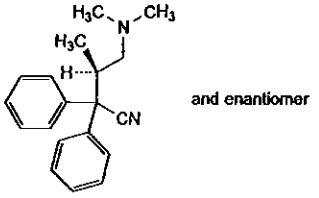
Specified impurities *A, B, C, D, E.*



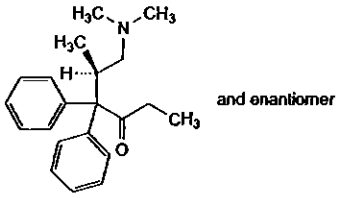
A. (2*RS*)-4-imino-*N*,*N*,2-trimethyl-3,3-diphenylhexan-1-amine (isomethadone ketimine),



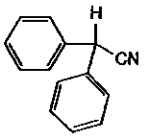
B. (4*RS*)-4-(dimethylamino)-2,2-diphenylpentanenitrile (didiavalo),



C. (3*RS*)-4-(dimethylamino)-3-methyl-2,2-diphenylbutanenitrile (isodidiavalo),



D. (5*RS*)-6-(dimethylamino)-5-methyl-4,4-diphenylhexan-3-one (isomethadone),



E. diphenylacetonitrile.

Ph Eur

Methane

(Ph. Eur. monograph 2413)

CH_4 16.04 *74-82-8*

Ph Eur

DEFINITION

Content

Minimum 99.5 per cent *V/V* of CH_4.

This monograph applies to methane obtained from natural gas and intended for medicinal use.

CHARACTERS

Appearance

Colourless gas, flammable in air.

Solubility

At 20 °C and at a pressure of 101 kPa, 1 volume dissolves in about 27 volumes of water.

IDENTIFICATION

Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the gas to be examined is similar in retention time to the principal peak in the chromatogram obtained with reference gas (b).

TESTS

Nitrogen

Gas chromatography (*2.2.28*).

Gas to be examined The substance to be examined.

Reference gas Mixture containing 500 ppm *V/V* of *nitrogen R1* in *methane R1*.

Column:
— *material*: stainless steel;
— *size*: *l* = 2 m, Ø = 2 mm;
— *stationary phase*: *molecular sieve for chromatography R* (0.5 nm).

Carrier gas *helium for chromatography R*.

Flow rate 30 mL/min.

Temperature:
— *column*: 80 °C;
— *detector*: 150 °C.

Detection Thermal conductivity.

Injection 1 mL.

Relative retention With reference to methane (retention time = about 3 min): nitrogen = about 0.7.

System suitability Reference gas:
— *resolution*: minimum 1.5 between the peaks due to nitrogen and methane.

Calculation of percentage content:
— use the concentration of nitrogen in the reference gas.

Limit:
— maximum 500 ppm *V/V*.

C_2-C_4 Hydrocarbons

Gas chromatography (*2.2.28*).

Gas to be examined The substance to be examined.

Reference gas Use the following mixture of gases in *methane R*: *ethane R* (impurity C) (100 ppm *V/V*), *propane R* (impurity D) (100 ppm *V/V*), *i-butane R* (impurity E) (100 ppm *V/V*) and *n-butane R* (impurity F) (100 ppm *V/V*).

Column:
— *material*: fused silica;
— *size*: *l* = 30 m, Ø = 0.53 mm;
— *stationary phase*: *deactivated aluminium oxide for chromatography R*.

Carrier gas *helium for chromatography R*.

Flow rate 113 mL/min.

Split ratio 1:10.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 1	30
	1 - 3.4	30 → 150
	3.4 - 4.5	150
Detector		300

Detection Flame ionisation. Use appropriate fuel gases: *hydrocarbon-free air R* and *hydrogen for chromatography R*.

Injection 0.1 mL.

Relative retention With reference to methane (retention time = about 1.2 min): impurity C = about 1.2; impurity D = about 1.8; impurity E = about 2.5; impurity F = about 2.6.

System suitability Reference gas:
— *resolution*: minimum 1.5 between the peaks due to methane and impurity C; minimum 1.5 between the peaks due to impurity E and impurity F.

Calculation of percentage contents:
— for each of the impurities C, D, E and F, use the corresponding concentration in the reference gas.

Limit:
— *total* (sum of impurities C, D, E and F): maximum 100 ppm *V/V*.

Water (*2.5.28*)
Maximum 10.0 ppm *V/V*.

ASSAY
Gas chromatography (*2.2.28*).

Gas to be examined The substance to be examined.

Reference gas (a) *Methane R1*.

Reference gas (b) Mixture containing 1 per cent *V/V* of *nitrogen R1* in *methane R1*.

Column:
— *material*: stainless steel;
— *size*: *l* = 2 m, Ø = 2 mm;
— *stationary phase*: *molecular sieve for chromatography R* (0.5 nm).

Carrier gas *helium for chromatography R*.

Flow rate 30 mL/min.

Temperature:
— *column*: 80 °C;
— *detector*: 150 °C.

Detection Thermal conductivity.

Injection 10 µL.

Relative retention With reference to methane (retention time = about 3 min): nitrogen = about 0.7.

System suitability Reference gas (b):
— *resolution*: minimum 1.5 between the peaks due to nitrogen and methane.

Calculate the percentage content of CH_4, using the chromatogram obtained with reference gas (a).

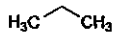
STORAGE
As a compressed gas, in appropriate containers complying with the legal regulations.

IMPURITIES
Specified impurities *A, B, C, D, E, F.*

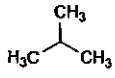
A. N_2: nitrogen,

B. H_2O: water,

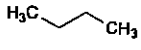
C. ethane,



D. propane,



E. 2-methylpropane (*i*-butane),



F. butane (*n*-butane).

Ph Eur

Methane Intermix (2 per cent) in Nitrogen

(Ph. Eur. monograph 2905)

Ph Eur

DEFINITION
A mixture containing 2 per cent *V/V* of *Methane (2413)* in *Low-oxygen nitrogen (1685)*.

Content
1.9 per cent *V/V* to 2.1 per cent *V/V* of methane (CH_4) in nitrogen (N_2).

This monograph applies to methane intermix (2 per cent) in nitrogen used in the preparation of lung function test gas mixtures for medicinal use.

CHARACTERS
Appearance
Colourless gas.

IDENTIFICATION
A. It complies with the limits of the assay.

B. Gas chromatography (*2.2.28*).

Gas to be examined The substance to be examined.

Reference gas *Nitrogen R1*.

Column:
— *material*: stainless steel;
— *size*: *l* = 2 m, Ø = 2 mm;
— *stationary phase*: *molecular sieve for chromatography R* (0.5 nm).

Carrier gas *helium for chromatography R*.

Flow rate 20 mL/min.

Temperature:
— *column*: 80 °C;
— *detector*: 130 °C.

Detection Thermal conductivity.

Injection 10 µL.

Retention time Nitrogen = about 2 min.

Results The principal peak in the chromatogram obtained with the gas to be examined is similar in retention time to the principal peak in the chromatogram obtained with the reference gas.

TESTS
Water (*2.5.28*)
Maximum 10 ppm *V/V*.

ASSAY
Gas chromatography (*2.2.28*).

Gas to be examined The substance to be examined.

Reference gas Mixture containing 2.0 per cent *V/V* of *methane R1* in *nitrogen R1*.

Column:
— *material*: stainless steel;
— *size*: *l* = 2 m, Ø = 2 mm;
— *stationary phase*: 3 per cent *squalane R* on alumina.

Carrier gas *helium for chromatography R*.

Flow rate 20 mL/min.

Temperature:
— *column*: 100 °C;
— *detector*: 250 °C.

Detection Flame ionisation.

Injection 100 µL.

Retention time Methane = about 0.5 min.

Calculate the percentage content of CH_4.

STORAGE

As a compressed gas, in appropriate high-pressure cylinders complying with the legal regulations.

LABELLING

The label states the nominal content, in per cent *V/V*, of methane in nitrogen.

IMPURITIES

Specified impurities A.

A. water.

Ph Eur

Methanol

Methyl Alcohol

(Ph. Eur. monograph 1989)

CH_4O	32.04	*67-56-1*

Ph Eur

DEFINITION

Methyl alcohol.

CHARACTERS

Appearance

Clear, colourless, volatile, hygroscopic liquid.

Solubility

Miscible with water and with methylene chloride.

bp

About 64 °C.

It is flammable.

IDENTIFICATION

A. Refractive index (*2.2.6*): 1.328 to 1.330.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of methanol.*

TESTS

Appearance

It is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 25 mL add 25 mL of *water R* and 0.25 mL of *phenolphthalein solution R1*. The solution is colourless. Not more than 0.9 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink.

Relative density (*2.2.5*)

0.791 to 0.793.

Absorbance (*2.2.25*)

Maximum 0.15 at 230 nm, maximum 0.05 at 250 nm, maximum 0.02 at 270 nm and maximum 0.01 at 290 nm.

Examine between 230 nm and 290 nm using *water R* as the compensation liquid. The absorption curve is smooth.

Impurity A

Gas chromatography (*2.2.28*).

Test solution (a) The substance to be examined.

Test solution (b) Dilute 1.0 mL of *4-methylpentan-2-ol R* to 50.0 mL with test solution (a). Dilute 5.0 mL of this solution to 100.0 mL with test solution (a).

Reference solution (a) To 50 µL of *anhydrous ethanol R* add 50 µL of *acetone R* and dilute to 50.0 mL with test solution (a). Dilute 100 µL of this solution to 10.0 mL with test solution (a).

Reference solution (b) Dilute 100 µL of *benzene R* to 100.0 mL with test solution (a). Dilute 0.20 mL to 100.0 mL with test solution (a).

Column:

— *material*: fused silica,

— *size*: *l* = 30 m, Ø = 0.32 mm,

— *stationary phase*: *cyanopropyl(3)phenyl(3)methyl(94) polysiloxane R* (film thickness 1.8 µm).

Carrier gas *helium for chromatography R.*

Linear velocity 35 cm/s.

Split ratio 1:20.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 12	40
	12 - 32	40 → 240
	32 - 42	240
Injection port		200
Detector		280

Detection Flame ionisation.

Injection 1 µL.

System suitability Reference solution (a):

— *resolution*: minimum 4.0 between the peaks due to impurity B (1st peak) and impurity C (2nd peak).

Limit:

— *impurity A*: maximum 2 ppm *V/V*.

Calculate the content of impurity A in parts per million *V/V* using the following expression:

$$\frac{2 \times A_1}{A_2 - A_1}$$

A_1 = area of the peak due to impurity A in the chromatogram obtained with test solution (a),

A_2 = area of the peak due to impurity A in the chromatogram obtained with reference solution (b).

If necessary, the identity of impurity A can be confirmed using another suitable chromatographic system (stationary phase with a different polarity).

Related substances

Gas chromatography (*2.2.28*) as described in the test for impurity A.

Limits:

— *any impurity*: for each impurity, not more than the area of the peak due to 4-methylpentan-2-ol in the chromatogram obtained with test solution (b) (0.1 per cent),

— *total*: not more than 3 times the area of the peak due to 4-methylpentan-2-ol in the chromatogram obtained with test solution (b) (0.3 per cent),

— *disregard limit*: 0.05 times the area of the peak due to 4-methylpentan-2-ol in the chromatogram obtained with test solution (b) (50 ppm).

Reducing substances

To 20 mL add 0.1 mL of *0.02 M potassium permanganate*. The pink colour is not completely discharged within 5 min.

Residue on evaporation
Maximum 10 ppm.

Evaporate 100 g to dryness on a water bath and dry in an oven at 100-105 °C. The residue weighs a maximum of 1 mg.

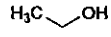
Water (*2.5.12*)
Maximum 0.10 per cent, determined on 10.0 g.

STORAGE
In an airtight container.

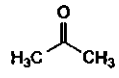
IMPURITIES
Specified impurities A.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) B, C.

A. benzene,



B. ethanol,

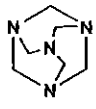


C. propanone (acetone).

Ph Eur

Methenamine

(Ph. Eur. monograph 1545)



$C_6H_{12}N_4$ 140.2 *100-97-0*

Action and use
Anti-infective.

Ph Eur

DEFINITION
1,3,5,7-Tetraazotricyclo[3.3.1.1^{3,7}]decane.

Content
99.0 per cent to 100.5 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder or colourless crystals.

Solubility
Freely soluble in water, soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION
First identification: A.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison methenamine CRS.

B. To 1 mL of solution S (see Tests) add 1 mL of *sulfuric acid R* and immediately heat to boiling. Allow to cool. To 1 mL of the solution add 4 mL of *water R* and 5 mL of *acetylacetone reagent R1*. Heat on a water-bath for 5 min. An intense yellow colour develops.

C. To 1 mL of solution S add 1 mL of *dilute sulfuric acid R* and immediately heat to boiling. The solution gives the reaction of ammonium salts and salts of volatile bases (*2.3.1*).

D. Dissolve 10 mg in 5 mL of *water R* and acidify with *dilute hydrochloric acid R*. Add 1 mL of *potassium iodobismuthate solution R*. An orange precipitate is formed immediately.

TESTS
Solution S
Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity
To 5 mL of solution S add 0.1 mL of *phenolphthalein solution R*. Not more than 0.2 mL of *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Free formaldehyde
Maximum 50 ppm.

Dissolve 0.8 g in *water R* and dilute to 8 mL with the same solvent. Add 2 mL of *ammoniacal silver nitrate solution R*. After 5 min, any grey colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner with a mixture of 8 mL of freshly prepared *formaldehyde standard solution (5 ppm CH_2O) R* and 2 mL of *ammoniacal silver nitrate solution R*.

Chlorides (*2.4.4*)
Maximum 100 ppm.

Dilute 5 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 100 ppm, determined on solution S.

Ammonium (*2.4.1*)
Maximum 50 ppm.

Dilute 2 mL of freshly prepared solution S to 13 mL with *water R*. Add 2 mL of *dilute sodium hydroxide solution R*.

Loss on drying (*2.2.32*)
Maximum 2.0 per cent, determined on 1.000 g by drying in a desiccator.

ASSAY
Dissolve 0.100 g in 30 mL of *methanol R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

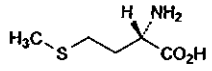
1 mL of *0.1 M perchloric acid* is equivalent to 14.02 mg of $C_6H_{12}N_4$.

STORAGE
Protected from light.

Ph Eur

Methionine

(Ph. Eur. monograph 1027)



$C_5H_{11}NO_2S$ 149.2 *63-68-3*

Action and use
Amino acid.

Ph Eur

DEFINITION
(2*S*)-2-Amino-4-(methylsulfanyl)butanoic acid.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder or colourless crystals.

Solubility
Soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION
First identification: A, B.
Second identification: A, C, D.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: methionine CRS.

C. Thin-layer chromatography (*2.2.27*).
Test solution Dissolve 10 mg of the substance to be examined in *dilute hydrochloric acid R* and dilute to 50 mL with the same acid.
Reference solution Dissolve 10 mg of *methionine CRS* in *dilute hydrochloric acid R* and dilute to 50 mL with the same acid.
Plate *TLC silica gel plate R.*
Mobile phase *glacial acetic acid R, water R, butanol R* (20:20:60 *V/V/V*).
Application 5 µL.
Development Over 2/3 of the plate.
Drying In air.
Detection Spray with *ninhydrin solution R* and heat at 105 °C for 15 min.
Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve 0.1 g of the substance to be examined and 0.1 g of *glycine R* in 4.5 mL of *dilute sodium hydroxide solution R*. Add 1 mL of a 25 g/L solution of *sodium nitroprusside R*. Heat to 40 °C for 10 min. Allow to cool and add 2 mL of a mixture of 1 volume of *phosphoric acid R* and 9 volumes of *hydrochloric acid R*. A dark red colour develops.

TESTS

Solution S
Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)
5.5 to 6.5 for solution S.

Specific optical rotation (*2.2.7*)
+ 22.5 to + 24.0 (dried substance).
Dissolve 1.00 g in *hydrochloric acid R1* and dilute to 50.0 mL with the same acid.

Related substances
Liquid chromatography (*2.2.29*).
Test solution Dissolve 0.30 g of the substance to be examined in mobile phase A and dilute to 10.0 mL with mobile phase A.
Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.
Reference solution (b) Dissolve 5 mg of the substance to be examined and 10 mg of *L-methionine sulfoxide R* (impurity A) in mobile phase A and dilute to 10.0 mL with mobile phase A.
Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature:* 30 °C.
Mobile phase:
— *mobile phase A: acetonitrile R1,* 23 g/L solution of *phosphoric acid R, water for chromatography R* (0.5:12.5:87 *V/V/V*);
— *mobile phase B:* 23 g/L solution of *phosphoric acid R, water for chromatography R, acetonitrile R1* (12.5:40:47.5 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 6	100	0
6 - 50	100 → 0	0 → 100
50 - 60	0	100

Flow rate 1.0 mL/min.
Detection Spectrophotometer at 205 nm.
Injection 50 µL.
Relative retention With reference to methionine (retention time = about 6 min): impurity A = about 0.5.
System suitability Reference solution (b):
— *resolution:* minimum 5.0 between the peaks due to impurity A and methionine.
Calculation of percentage contents:
— for each impurity, use the concentration of methionine in reference solution (a).
Limits:
— *unspecified impurities:* for each impurity, maximum 0.10 per cent;
— *total:* maximum 0.3 per cent;
— *reporting threshold:* 0.05 per cent.

Chlorides
Maximum 200 ppm.
Dissolve 0.5 g of the substance to be examined in 5 mL of *dilute nitric acid R* and dilute to 10 mL with the same acid. Add 10 mL of *strong hydrogen peroxide solution R* and heat on a water bath for 30 min. Cool and dilute to 50 mL with *water R*. Add 1 mL of *silver nitrate solution R2* and mix. Allow to stand protected from light for 5 min. Any opalescence in the solution is not more intense than that in a standard prepared at the same time and in the same manner using

2 mL of *chloride standard solution (50 ppm Cl) R*. Examine the tubes laterally against a black background.

Sulfates (*2.4.13*)
Maximum 300 ppm.

Dissolve 0.5 g in 3 mL of *dilute hydrochloric acid R* and dilute to 15 mL with *distilled water R*.

Ammonium (*2.4.1, Method B*)
Maximum 200 ppm, determined on 0.10 g.

Prepare the standard using 0.2 mL of *ammonium standard solution (100 ppm NH_4) R*.

Iron (*2.4.9*)
Maximum 10 ppm.

In a separating funnel, dissolve 1.0 g in 10 mL of *dilute hydrochloric acid R*. Shake with 3 quantities, each of 10 mL, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined upper layers, add 10 mL of *water R* and shake for 3 min. Use the lower layer.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

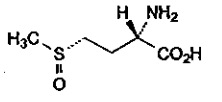
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.125 g in 5 mL of *anhydrous formic acid R*. Add 30 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

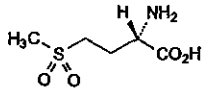
1 mL of *0.1 M perchloric acid* is equivalent to 14.92 mg of $C_5H_{11}NO_2S$.

STORAGE
Protected from light.

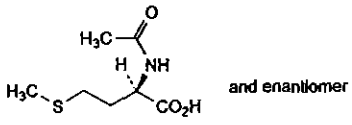
IMPURITIES
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, E.*



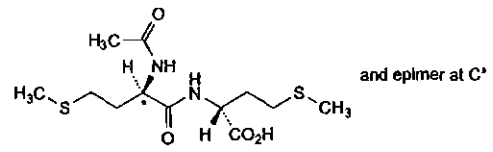
A. (2*S*)-2-amino-4-[(*RS*)-methylsulfinyl]butanoic acid (L-methionine sulfoxide),



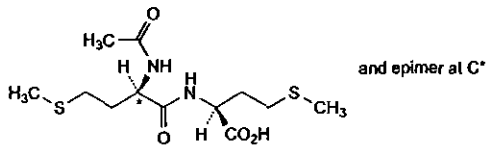
B. (2*S*)-2-amino-4-(methylsulfonyl)butanoic acid,



C. (2*RS*)-2-(acetylamino)-4-(methylsulfanyl)butanoic acid,



D. (2*R*)-2-[[(2*RS*)-2-(acetylamino)-4-(methylsulfanyl)butanoyl]amino]-4-(methylsulfanyl) butanoic acid,

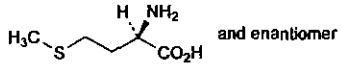


E. (2*S*)-2-[[(2*RS*)-2-(acetylamino)-4-(methylsulfanyl)butanoyl]amino]-4-(methylsulfanyl) butanoic acid.

Ph Eur

DL-Methionine

(Ph. Eur. monograph 0624)



$C_5H_{11}NO_2S$ 149.2 *59-51-8*

Action and use
Used in treatment of paracetamol overdose.

Ph Eur

DEFINITION
DL-Methionine contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (2*RS*)-2-amino-4-(methylsulfanyl)butanoic acid, calculated with reference to the dried substance.

CHARACTERS
Almost white, crystalline powder or small flakes, sparingly soluble in water, very slightly soluble in alcohol. It dissolves in dilute acids and in dilute solutions of the alkali hydroxides.

It melts at about 270 °C (instantaneous method).

IDENTIFICATION
First identification: A, C.

Second identification: B, C, D.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *DL-methionine CRS*. Dry the substances at 105 °C.

B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 2.50 g in *1 M hydrochloric acid* and dilute to 50.0 mL with the same acid. The angle of optical rotation (*2.2.7*) is −0.05° to + 0.05°.

D. Dissolve 0.1 g of the substance to be examined and 0.1 g of *glycine R* in 4.5 mL of *dilute sodium hydroxide solution R*. Add 1 mL of a 25 g/L solution of *sodium nitroprusside R*. Heat to 40 °C for 10 min. Allow to cool and add 2 mL of a

mixture of 1 volume of *phosphoric acid R* and 9 volumes of *hydrochloric acid R*. A deep-red colour develops.

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)

The pH of solution S is 5.4 to 6.1.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution (a) Dissolve 0.2 g in *water R* and dilute to 10 mL with the same solvent.

Test solution (b) Dilute 1 mL of test solution (a) to 50 mL with *water R*.

Reference solution (a) Dissolve 20 mg of *DL-methionine CRS* in *water R* and dilute to 50 mL with the same solvent.

Reference solution (b) Dilute 1 mL of reference solution (a) to 10 mL with *water R*.

Apply separately to the plate 5 µL of each solution. Develop over a path of 10 cm using a mixture of 20 volumes of *glacial acetic acid R*, 20 volumes of *water R* and 60 volumes of *butanol R*. Allow the plate to dry in air and spray with *ninhydrin solution R*. Heat the plate at 100 °C to 105 °C for 15 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent).

Chlorides

Dissolve 0.25 g in 35 mL of *water R*. Add 5 mL of *dilute nitric acid R* and 10 mL of *silver nitrate solution R2*. Allow to stand protected from light for 5 min. Any opalescence in the solution is not more intense than that in a standard prepared at the same time in the same manner using a mixture of 10 mL of *chloride standard solution (5 ppm Cl) R* and 25 mL of *water R* (200 ppm). Examine the tubes laterally against a black background.

Sulfates (*2.4.13*)

Dissolve 1.0 g in 20 mL of *distilled water R*, heating to 60 °C. Cool to 10 °C and filter. 15 mL of the solution complies with the limit test for sulfates (200 ppm).

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.140 g in 3 mL of *anhydrous formic acid R*. Add 30 mL of *anhydrous acetic acid R*. Immediately after dissolution, titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 14.92 mg of $C_5H_{11}NO_2S$.

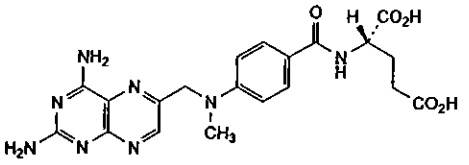
STORAGE

Store protected from light.

Ph Eur

Methotrexate

(Ph. Eur. monograph 0560)



$C_{20}H_{22}N_8O_5$ 454.4 *59-05-2*

Action and use

Dihydrofolate reductase inhibitor; cytostatic.

Preparations

Methotrexate Injection

Methotrexate Oral Solution

Methotrexate Tablets

Ph Eur

DEFINITION

(2*S*)-2-[[4-[[(2,4-Diaminopteridin-6-yl)methyl]methylamino] benzoyl]amino]pentanedioic acid.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Yellow or orange, crystalline, hygroscopic powder.

Solubility

Practically insoluble in water, in ethanol (96 per cent) and in methylene chloride. It dissolves in dilute mineral acids and in dilute solutions of alkali hydroxides and carbonates.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison methotrexate CRS.

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 40.0 mg of the substance to be examined in a mixture of 0.5 mL of *dilute ammonia R1* and 5 mL of mobile phase A and dilute to 100.0 mL with mobile phase A.

Test solution (b) Dissolve 25.0 mg of the substance to be examined in a mixture of 0.5 mL of *dilute ammonia R1* and 5 mL of mobile phase A and dilute to 50.0 mL with mobile phase A. Dilute 5.0 mL of this solution to 50.0 mL with mobile phase A.

Reference solution (a) Dissolve 25.0 mg of *methotrexate CRS* in a mixture of 0.5 mL of *dilute ammonia R1* and 5 mL of mobile phase A and dilute to 50.0 mL with mobile phase A. Dilute 5.0 mL of this solution to 50.0 mL with mobile phase A.

Reference solution (b) Dilute 5.0 mL of test solution (a) to 100.0 mL with mobile phase A. Dilute 5.0 mL of this solution to 50.0 mL with mobile phase A.

Reference solution (c) Dilute 5.0 mL of reference solution (b) to 25.0 mL with mobile phase A.

Reference solution (d) Dissolve 5 mg of the substance to be examined, 5 mg of 4-aminofolic acid R (impurity B), 5 mg of *methotrexate impurity C CRS*, 5 mg of *methotrexate impurity D CRS* and 5 mg of *methotrexate impurity E CRS* in a

mixture of 0.5 mL of *dilute ammonia R1* and 5 mL of mobile phase A and dilute to 100 mL with mobile phase A.

Reference solution (e) Dissolve 8 mg of *methotrexate for peak identification CRS* (containing impurities H and I) in a mixture of 0.1 mL of *dilute ammonia R1* and 1 mL of mobile phase A and dilute to 20 mL with mobile phase A.

Column:
- *size*: *l* = 0.25 m, Ø = 4.0 mm;
- *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A*: mix 5 volumes of *acetonitrile for chromatography R* and 95 volumes of a 3.4 g/L solution of *anhydrous sodium dihydrogen phosphate R* previously adjusted to pH 6.0 with a 42 g/L solution of *sodium hydroxide R*;
- *mobile phase B*: mix 50 volumes of *acetonitrile for chromatography R* and 50 volumes of a 3.4 g/L solution of *anhydrous sodium dihydrogen phosphate R* previously adjusted to pH 6.0 with a 42 g/L solution of *sodium hydroxide R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	100	0
10 - 20	100 → 95	0 → 5
20 - 28	95 → 50	5 → 50
28 - 37	50	50

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µL of test solution (a) and reference solutions (b), (c), (d) and (e).

Identification of impurities Use the chromatogram supplied with *methotrexate for peak identification CRS* and the chromatogram obtained with reference solution (e) to identify the peaks due to impurities H and I; use the chromatogram obtained with reference solution (d) to identify the peaks due to impurities B, C and E.

Relative retention With reference to methotrexate (retention time = about 18 min): impurity B = about 0.3; impurity C = about 0.4; impurity D = about 0.9; impurity E = about 1.4; impurity I = about 1.5; impurity H = about 1.6.

System suitability:
- *resolution*: minimum 2.0 between the peaks due to impurities B and C and minimum 1.5 between the peaks due to impurity D and methotrexate, in the chromatogram obtained with reference solution (d); minimum 1.5 between the peaks due to impurities I and H in the chromatogram obtained with reference solution (e); if the resolution between impurity D and methotrexate does not comply, increase the flow rate to meet the requirement.

Limits:
- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity E = 0.8; impurity I = 1.4;
- *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *impurities B, E*: for each impurity, not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
- *impurities H, I*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);
- *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent);
- *sum of impurities other than B, C and E*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *disregard limit*: 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.03 per cent).

Enantiomeric purity

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve 4.0 mg of *methotrexate for system suitability CRS* (containing impurity F) in the mobile phase and dilute to 20.0 mL with the mobile phase.

Column:
- *size*: *l* = 0.15 m, Ø = 4.0 mm;
- *stationary phase*: *bovine albumin R* bound to *silica gel for chromatography R* (7 µm) with a pore size of 30 nm.

Mobile phase Add 500 mL of a 7.1 g/L solution of *anhydrous disodium hydrogen phosphate R* to 600 mL of a 6.9 g/L solution of *sodium dihydrogen phosphate monohydrate R*, mix, and adjust to pH 6.9 with *dilute sodium hydroxide solution R*; to 920 mL of this mixture add 80 mL of *propanol R*.

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 302 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity F.

Relative retention With reference to methotrexate (retention time = about 4 min): impurity F = about 1.6.

System suitability Reference solution (b):
- *resolution*: minimum 2.0 between the peaks due to methotrexate and impurity F.

Limit:
- *impurity F*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.0 per cent).

Water (*2.5.12*)

Maximum 13.0 per cent, determined on 0.10 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (a).

Calculate the percentage content of $C_{20}H_{22}N_8O_5$ taking into account the assigned content of *methotrexate CRS*.

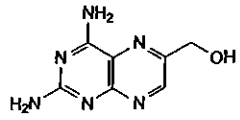
STORAGE

In an airtight container, protected from light.

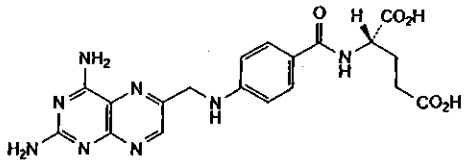
IMPURITIES

Specified impurities *B, C, E, F, H, I.*

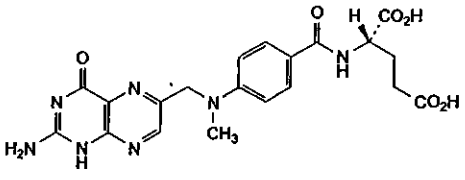
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, D, G, J, K, L.*



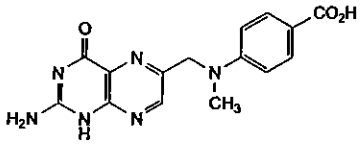
A. (2,4-diaminopteridin-6-yl)methanol,



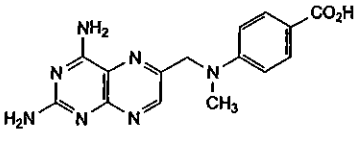
B. (2*S*)-2-[[4-[[(2,4-diaminopteridin-6-yl)methyl] amino]benzoyl]amino]pentanedioic acid (4-aminofolic acid, aminopterin),



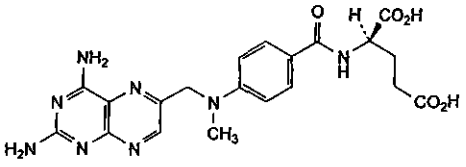
C. (2*S*)-2-[[4-[[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]methylamino]benzoyl]amino]pentanedioic acid (*N*-methylfolic acid, methopterin),



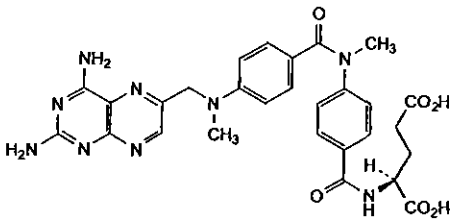
D. 4-[[(2-amino-4-oxo-1,4-dihydropteridin-6-yl) methyl]methylamino]benzoic acid (N^{10}-methylpteroic acid),



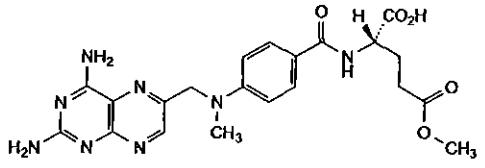
E. 4-[[(2,4-diaminopteridin-6-yl)methyl] methylamino]benzoic acid (4-amino-N^{10}-methylpteroic acid, APA),



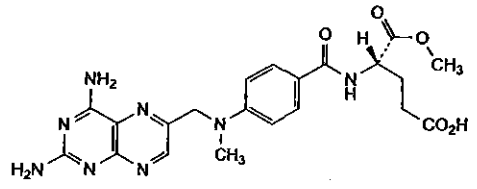
F. (2*R*)-2-[[4-[[(2,4-diaminopteridin-6-yl)methyl] methylamino]benzoyl]amino]pentanedioic acid ((*R*)-methotrexate),



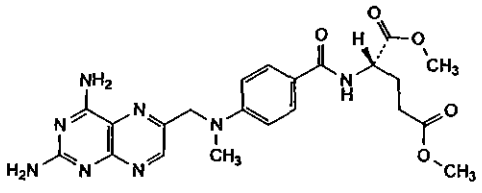
G. (2*S*)-2-[[4-[[4-[[(2,4-diaminopteridin-6-yl) methyl]methylamino]benzoyl]methylamino]benzoyl] amino]pentanedioic acid,



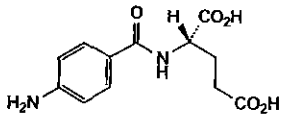
H. (2*S*)-2-[[4-[[(2,4-diaminopteridin-6-yl)methyl] methylamino]benzoyl]amino]-5-methoxy-5-oxopentanoic acid (methotrexate 5-methyl ester),



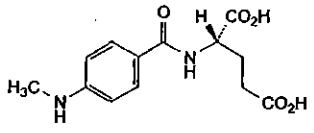
I. (4*S*)-4-[[4-[[(2,4-diaminopteridin-6-yl)methyl] methylamino]benzoyl]amino]-5-methoxy-5-oxopentanoic acid (methotrexate 1-methyl ester),



J. dimethyl (2*S*)-2-[[4-[[(2,4-diaminopteridin-6-yl) methyl]methylamino]benzoyl]amino]pentanedioate (methotrexate dimethyl ester),



K. (2*S*)-2-[(4-aminobenzoyl)amino]pentanedioic acid,



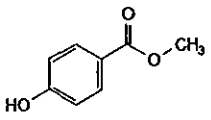
L. (2*S*)-2-[[4-(methylamino)benzoyl]amino]pentanedioic acid.

Ph Eur

Methyl Hydroxybenzoate

Methylparaben

(Methyl Parahydroxybenzoate, Ph. Eur. monograph 0409)



$C_8H_8O_3$ 152.1 *99-76-3*

Action and use
Antimicrobial preservative.

Ph Eur

DEFINITION
Methyl 4-hydroxybenzoate.

Content
98.0 per cent to 102.0 per cent.

CHARACTERS

Appearance
White or almost white, crystalline powder or colourless crystals.

Solubility
Very slightly soluble in water, freely soluble in ethanol (96 per cent) and in methanol.

IDENTIFICATION
First identification: A, B.

Second identification: A, C.

A. Melting point (*2.2.14*): 125 °C to 128 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *methyl parahydroxybenzoate CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution (a) Dissolve 0.10 g of the substance to be examined in *acetone R* and dilute to 10 mL with the same solvent.

Test solution (b) Dilute 1 mL of test solution (a) to 10 mL with *acetone R.*

Reference solution (a) Dissolve 10 mg of *methyl parahydroxybenzoate CRS* in *acetone R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 10 mg of *ethyl parahydroxybenzoate CRS* in 1 mL of test solution (a) and dilute to 10 mL with *acetone R.*

Plate *TLC octadecylsilyl silica gel F_{254} plate R.*

Mobile phase *glacial acetic acid R, water R, methanol R* (1:30:70 *V/V/V*).

Application 2 µL of test solution (b) and reference solutions (a) and (b).

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):
— the chromatogram shows 2 clearly separated principal spots.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Solution S
Dissolve 1.0 g in *ethanol (96 per cent) R* and dilute to 10 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Acidity
To 2 mL of solution S add 3 mL of *ethanol (96 per cent) R*, 5 mL of *carbon dioxide-free water R* and 0.1 mL of *bromocresol green solution R.* Not more than 0.1 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator to blue.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in 2.5 mL of *methanol R* and dilute to 50.0 mL with the mobile phase. Dilute 10.0 mL of this solution to 100.0 mL with the mobile phase.

Reference solution (a) Dissolve 5 mg of *4-hydroxybenzoic acid R* (impurity A) and 5 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 50.0 mg of *methyl parahydroxybenzoate CRS* in 2.5 mL of *methanol R* and dilute to 50.0 mL with the mobile phase. Dilute 10.0 mL of this solution to 100.0 mL with the mobile phase.

Reference solution (c) Dilute 1.0 mL of the test solution to 20.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase 6.8 g/L solution of *potassium dihydrogen phosphate R, methanol R* (35:65 *V/V*).

Flow rate 1.3 mL/min.

Detection Spectrophotometer at 272 nm.

Injection 10 µL of the test solution and reference solutions (a) and (c).

Run time 5 times the retention time of methyl parahydroxybenzoate.

Relative retention With reference to methyl parahydroxybenzoate (retention time = about 2.3 min): impurity A = about 0.6.

System suitability Reference solution (a):
— *resolution*: minimum 2.0 between the peaks due to impurity A and methyl parahydroxybenzoate.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity A by 1.4;
— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

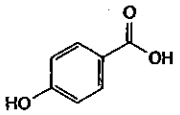
ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (b).

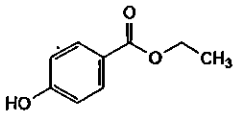
Calculate the percentage content of $C_8H_8O_3$ from the declared content of *methyl parahydroxybenzoate CRS*.

IMPURITIES
Specified impurities *A.*

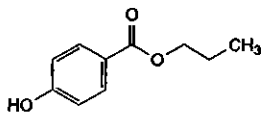
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D.*



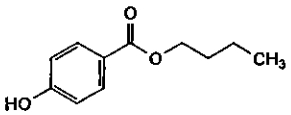
A. 4-hydroxybenzoic acid,



B. ethyl 4-hydroxybenzoate (ethyl parahydroxybenzoate),



C. propyl 4-hydroxybenzoate (propyl parahydroxybenzoate),

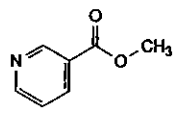


D. butyl 4-hydroxybenzoate (butyl parahydroxybenzoate).

Ph Eur

Methyl Nicotinate

(Ph. Eur. monograph 2129)



$C_7H_7NO_2$ 137.1 *93-60-7*

Action and use
Vasodilator.

Ph Eur

DEFINITION
Methyl pyridine-3-carboxylate.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white powder.

Solubility
Very soluble in water, in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION
First identification: B.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 40 °C to 42 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *methyl nicotinate CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 2 mL with the same solvent.

Reference solution Dissolve 10 mg of *methyl nicotinate CRS* in *methanol R* and dilute to 2 mL with the same solvent.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *methanol R, toluene R* (10:90 *V/V*).

Application 2 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. To 0.5 g add 0.1 g of *citric acid monohydrate R* and 0.2 mL of *acetic anhydride R*. Heat cautiously for 1 min. A yellow colour is produced which turns first to orange, then to red and then to violet.

TESTS
Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 25 mg of the substance to be examined in the mobile phase and dilute to 25.0 mL with the mobile phase.

Reference solution (a) Dissolve 25 mg of *nicotinic acid R* in the mobile phase and dilute to 25.0 mL with the mobile phase. To 0.5 mL of this solution add 0.5 mL of the test solution and dilute to 100 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4 mm,
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetic acid R, water R, acetonitrile R* (1:29:70 *V/V/V*).

Flow rate 1 mL/min.

Detection Spectrophotometer at 261 nm.

Injection 20 µL.

Run time 3 times the retention time of methyl nicotinate.

Retention time Methyl nicotinate = about 3.3 min.

System suitability Reference solution (a):
— *resolution*: minimum 2 between the peaks due to impurity A and methyl nicotinate.

Limits:
— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),
— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Chlorides (*2.4.4*)
Maximum 200 ppm.

Dissolve 0.25 g in *water R* and dilute to 15 mL with the same solvent.

Water (*2.5.12*)
Maximum 0.5 per cent, determined on 2.000 g.

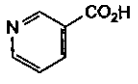
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.120 g in 50 mL of *glacial acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 13.71 mg of $C_7H_7NO_2$.

STORAGE
Protected from light.

IMPURITIES
Specified impurities *A*.

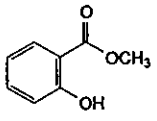


A. pyridine-3-carboxylic acid (nicotinic acid).

Ph Eur

Methyl Salicylate

(Ph. Eur. monograph 0230)



$C_8H_8O_3$ 152.1 *119-36-8*

Action and use
Counter-irritant.

Preparations
Kaolin Poultice
Methyl Salicylate Liniment
Methyl Salicylate Ointment
Surgical Spirit

Ph Eur

DEFINITION
Methyl 2-hydroxybenzoate.

Content
99.0 per cent *m/m* to 101.0 per cent *m/m*.

CHARACTERS
Appearance
Colourless or slightly yellow liquid.

Solubility
Very slightly soluble in water, miscible with ethanol (96 per cent) and with fatty and essential oils.

IDENTIFICATION
First identification: A.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *methyl salicylate CRS.*

B. Heat 0.25 mL with 2 mL of *dilute sodium hydroxide solution R* on a water-bath for 5 min. Add 3 mL of *dilute sulfuric acid R*. A crystalline precipitate is formed. Filter. The precipitate, washed with *water R* and dried at 100-105 °C, melts (*2.2.14*) at 156 °C to 161 °C.

C. To 10 mL of a saturated solution add 0.05 mL of *ferric chloride solution R1*. A violet colour develops.

TESTS
Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

To 2 mL add 10 mL of *ethanol (96 per cent) R*.

Acidity
Dissolve 5.0 g in a mixture of 0.2 mL of *bromocresol green solution R* and 50 mL of *ethanol (96 per cent) R* previously neutralised to a blue colour by addition of *0.1 M sodium hydroxide*. Not more than 0.4 mL of *0.1 M sodium hydroxide* is required to restore the blue colour.

Relative density (*2.2.5*)
1.182 to 1.188.

Refractive index (*2.2.6*)
1.535 to 1.538.

Related substances
Gas chromatography (*2.2.28*).

Test solution The substance to be examined.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *acetone R*. Dilute 1.0 mL of this solution to 10.0 mL with *acetone R*.

Reference solution (b) Dissolve 59.0 mg of *phenol R* (impurity B) in *acetone R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with *acetone R*.

Reference solution (c) Dissolve 5 mg of *methyl 2-methoxybenzoate R* (impurity M) and 5 mg of *methyl 4-methoxybenzoate R* (impurity D) in *acetone R* and dilute to 100 mL with the same solvent.

Column:
— *material*: fused silica;
— *size*: *l* = 30 m, Ø = 0.53 mm;
— *stationary phase*: *phenyl(50)methyl(50)polysiloxane R* (film thickness 1 µm).

Carrier gas *helium for chromatography R.*

Flow rate 5.0 mL/min.

Split ratio 1:10.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 32.5	100 → 230
	32.5 - 40.5	230
Injection port		200
Detector		250

Detection Flame ionisation.

Injection 0.5 µL.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity B; use the chromatogram obtained with reference solution (c) to identify the peaks due to impurities D and M.

Relative retention With reference to methyl salicylate (retention time = about 9 min): methanol = about 0.15; anisole = about 0.37; impurity B = about 0.47; impurity M = about 1.56; impurity D = about 1.59.

System suitability Reference solution (c):
— *resolution*: minimum 1.5 between the peaks due to impurities M and D.

Calculation of contents:
— for impurity B, use the concentration of impurity B in reference solution (b);
— for impurities other than B, use the concentration of methyl salicylate in reference solution (a).

Limits:
— *impurity B*: maximum 50 ppm;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.5 per cent;
— *reporting threshold*: 0.05 per cent, except for impurity B; disregard the peaks due to anisole and methanol.

ASSAY

Dissolve 0.500 g in 25 mL of *ethanol (96 per cent) R*. Add 0.05 mL of *phenol red solution R* and neutralise with *0.1 M sodium hydroxide*. To the neutralised solution add 50.0 mL of *0.1 M sodium hydroxide* and heat under a reflux condenser on a water-bath for 30 min. Cool and titrate with *0.1 M hydrochloric acid*. Calculate the volume of *0.1 M sodium hydroxide* used in the saponification. Carry out a blank titration.

1 mL of *0.1 M sodium hydroxide* is equivalent to 15.21 mg of $C_8H_8O_3$.

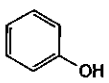
STORAGE

Protected from light.

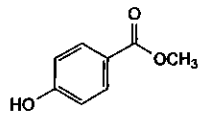
IMPURITIES

Specified impurities *B.*

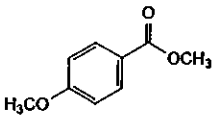
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C, D, E, F, I, J, K, L, M.*



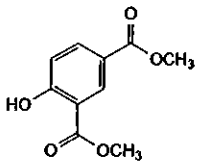
B. phenol,



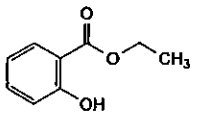
C. methyl 4-hydroxybenzoate (methyl parahydroxybenzoate),



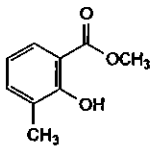
D. methyl 4-methoxybenzoate,



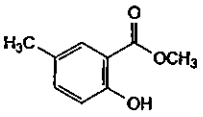
E. dimethyl 4-hydroxybenzene-1,3-dicarboxylate,



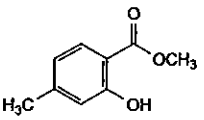
F. ethyl 2-hydroxybenzoate,



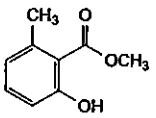
I. methyl 2-hydroxy-3-methylbenzoate,



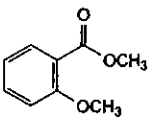
J. methyl 2-hydroxy-5-methylbenzoate,



K. methyl 2-hydroxy-4-methylbenzoate,



L. methyl 2-hydroxy-6-methylbenzoate,



M.methyl 2-methoxybenzoate.

Industrial Methylated Spirit

Industrial Methylated Spirits; IMS

Industrial Denatured Alcohol

DEFINITION

Industrial Methylated Spirit is a mixture of nineteen volumes of ethanol of an appropriate strength with one volume of approved wood naphtha. Two strengths are available containing 99% by volume and 95% by volume of alcohol (also known as 74 OP and 66 OP respectively).

CHARACTERISTICS

A colourless, clear, mobile, volatile liquid, boiling at about 78°.

IDENTIFICATION

Mix 0.1 mL with 0.05 mL of an 11% w/w solution of *orthophosphoric acid* and 0.25 mL of *dilute potassium permanganate solution*. After 1 minute add a few mg of *sodium metabisulfite* and shake until the mixture is decolorised. Add 1.5 mL of a 50% v/v solution of *sulfuric acid* and a few mg of finely powdered *chromotropic acid sodium salt*, shake well and heat on a water bath for 5 minutes. A deep violet colour is produced.

TESTS

Acidity or alkalinity

25 mL requires not more than 0.2 mL of *0.1M sodium hydroxide VS* to produce a pink colour with *phenolphthalein solution R1* and not more than 1.0 mL of *0.1M hydrochloric acid VS* to produce a red colour with *methyl red solution*.

Clarity of solution

Dilute 5.0 mL to 100 mL with *water*. The solution is *clear*, Appendix IV A.

Apparent density

For '66 OP' grade, not greater than 811.6 kg m^{-3}, and for '74 OP' grade, not greater than 792.8 kg m^{-3}, Appendix V G.

Aldehydes

To 5.0 mL add 5 mL of *water* and 1 mL of *decolorised fuchsin solution* and allow to stand for 30 minutes. Any colour produced is not more intense than that obtained by treating in the same manner 5 mL of a 0.005% w/v solution of redistilled *acetaldehyde* in *aldehyde-free ethanol (96%)* (50 ppm).

Non-volatile matter

When evaporated and dried at 105°, leaves not more than 0.01% w/v of residue.

Industrial Methylated Spirit (Ketone-free)

Industrial Denatured Alcohol (Ketone-free)

DEFINITION

Industrial Methylated Spirit (Ketone-free) is a mixture of nineteen volumes of ethanol of an appropriate strength with one volume of approved wood naphtha substantially free from ketones. Two strengths are available containing 99% by volume and 95% by volume of alcohol (also known as 74 OP and 66 OP respectively).

CHARACTERISTICS; IDENTIFICATION; TESTS

Acidity or alkalinity; Clarity of solution; Apparent density; Aldehydes; Non-volatile matter

Complies with the requirements stated under Industrial Methylated Spirit.

Ketones

Dilute 5 mL to 10 mL with *water*, add 1 mL of a 1.0% w/v solution of *2-nitrobenzaldehyde* in *ethanol (50%)* followed by 1 mL of a 15% w/v solution of *sodium hydroxide* in *water* and allow to stand for 15 minutes. Any colour produced is not more intense than that produced by treating in the same manner 10 mL of a 0.025% v/v solution of *acetone* in *ethanol (50%)* (500 ppm).

Methylcellulose[1]

(Ph. Eur. monograph 0345)

9004-67-5

Action and use

Bulk-forming laxative; excipient.

Preparation

Methylcellulose Tablets

Ph Eur

DEFINITION

Partly *O*-methylated cellulose. Methyl ether of cellulose.

Content

26.0 per cent to 33.0 per cent of methoxy groups ($-OCH_3$; M_r 31.03) (dried substance).

♦CHARACTERS

Appearance

White, yellowish-white or greyish-white powder or granules, hygroscopic after drying.

Solubility

Practically insoluble in hot water, in acetone, in anhydrous ethanol and in toluene. It dissolves in cold water giving a colloidal solution.♦

IDENTIFICATION

A. Evenly distribute 1.0 g onto the surface of 100 mL of *water R* in a beaker, tapping the top of the beaker gently if necessary to ensure a uniform layer on the surface. Allow to stand for 1-2 min: the powdered material aggregates on the surface.

B. Evenly distribute 1.0 g into 100 mL of boiling *water R*, and stir the mixture using a magnetic stirrer with a bar 25 mm long: a slurry is formed and the particles do not dissolve. Allow the slurry to cool to 5 °C and stir using a magnetic stirrer: a clear or slightly turbid solution occurs with its thickness dependent on the viscosity grade.

C. To 0.1 mL of the solution obtained in identification test B add 9 mL of a 90 per cent *V/V* solution of *sulfuric acid R*, shake, heat on a water-bath for exactly 3 min, immediately cool in an ice-bath, carefully add 0.6 mL of a 20 g/L solution of *ninhydrin R*, shake and allow to stand at 25 °C: a red colour develops and does not change to purple within 100 min.

D. Place 2-3 mL of the solution obtained in identification test B on a glass slide as a thin film and allow the water to evaporate: a coherent, clear film forms on the glass slide.

E. Add 50.0 mL of the solution obtained in identification test B to 50.0 mL of *water R* in a beaker. Insert a thermometer into the solution. Stir the solution on a

[1] *This monograph has undergone pharmacopoeial harmonisation. See chapter 5.8 Pharmacopoeial harmonisation.*

magnetic stirrer/hot plate and begin heating, increasing the temperature at a rate of 2-5 °C per minute. Determine the temperature at which a turbidity increase begins to occur and designate the temperature as the flocculation temperature: the flocculation temperature is higher than 50 °C.

TESTS

◊Appearance of solution

The solution is not more opalescent than reference suspension III (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

While stirring, introduce a quantity of the substance to be examined equivalent to 1.0 g of the dried substance into 50 g of *carbon dioxide-free water R* heated to 90 °C. Allow to cool, adjust the mass of the solution to 100 g with *carbon dioxide-free water R* and stir until dissolution is complete. Allow to stand at 2-8 °C for 1 h before carrying out the test.◊

pH (*2.2.3*)

5.0 to 8.0 for the solution prepared as described under Viscosity.

Read the pH after the probe has been immersed for 5 ± 0.5 min.

Viscosity

80 per cent to 120 per cent of the nominal value for samples with a viscosity of less than 600 mPa·s (Method 1);
75 per cent to 140 per cent of the nominal value for samples with a viscosity of 600 mPa·s or higher (Method 2).

Method 1, to be applied to samples with a viscosity of less than 600 mPa·s Weigh a quantity of the substance to be examined equivalent to 4.000 g of the dried substance. Transfer into a wide-mouthed bottle, and adjust the total mass of the sample and the water to 200.0 g with hot *water R* (90-99 °C). Capping the bottle, stir by mechanical means at 400 ± 50 r/min for 10-20 min until the particles are thoroughly dispersed and wetted. Scrape down the insides of the bottle with a spatula if necessary, to ensure that there is no undissolved material on the sides of the bottle, and continue the stirring in a cooling water-bath maintained at a temperature below 5 °C for another 20-40 min. Adjust the solution mass if necessary to 200.0 g using cold *water R*. Centrifuge the solution if necessary to expel any entrapped air bubbles. Using a spatula, remove any foam. Determine the kinematic viscosity (ν) of this solution using the capillary viscometer method (*2.2.9*). Separately, determine the density (ρ) (*2.2.5*) of the solution and calculate the dynamic viscosity (η), as $\eta = \rho\nu$.

Method 2, to be applied to samples with a viscosity of 600 mPa·s or higher Weigh a quantity of the substance to be examined equivalent to 10.00 g of the dried substance. Transfer into a wide-mouthed bottle, and adjust the total mass of the sample and the water to 500.0 g with hot *water R* (90-99 °C). Capping the bottle, stir by mechanical means at 400 ± 50 r/min for 10-20 min until the particles are thoroughly dispersed and wetted. Scrape down the insides of the bottle with a spatula if necessary, to ensure that there is no undissolved material on the sides of the bottle, and continue the stirring in a cooling water-bath maintained at a temperature below 5 °C for another 20-40 min. Adjust the solution mass if necessary to 500.0 g using cold *water R*. Centrifuge the solution if necessary to expel any entrapped air bubbles. Using a spatula, remove any foam. Determine the viscosity (*2.2.10*) of this solution at 20 ± 0.1 °C using a rotating viscometer.

Apparatus Single-cylinder type spindle viscometer.

Rotor number, revolution and calculation multiplier Apply the conditions specified in Table 0345.-1.

Table 0345.-1.

Nominal viscosity* (mPa·s)	Rotor number	Revolution (r/min)	Calculation multiplier
600 to less than 1400	3	60	20
1400 to less than 3500	3	12	100
3500 to less than 9500	4	60	100
9500 to less than 99 500	4	6	1000
99 500 or more	4	3	2000

*the nominal viscosity is based on the manufacturer's specifications.

Allow the spindle to rotate for 2 min before taking the measurement. Allow a rest period of at least 2 min between subsequent measurements. Repeat the measurement twice and determine the mean of the 3 readings.

Loss on drying (*2.2.32*)

Maximum 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 1 h.

Sulfated ash (*2.4.14*)

Maximum 1.5 per cent, determined on 1.0 g.

ASSAY

Gas chromatography (*2.2.28*).

Apparatus:

- *reaction vial*: a 5 mL pressure-tight vial, 50 mm in height, 20 mm in external diameter and 13 mm in internal diameter at the mouth, equipped with a pressure-tight butyl rubber membrane stopper coated with polytetrafluoroethylene and secured with an aluminium crimped cap or another sealing system providing a sufficient air-tightness;
- *heater*: a heating module with a square aluminium block having holes 20 mm in diameter and 32 mm in depth, so that the reaction vials fit; mixing of the contents of the vial is effected using a magnetic stirrer equipped in the heating module or using a reciprocal shaker that performs approximately 100 cycles/min.

Internal standard solution 30 g/L solution of *octane R* in *o-xylene R*.

Test solution Weigh 65.0 mg of the substance to be examined, place in a reaction vial, add 0.06-0.10 g of *adipic acid R*, 2.0 mL of the internal standard solution and 2.0 mL of *hydriodic acid R*, immediately cap and seal the vial, and weigh accurately. Mix the contents of the vial continuously for 60 min while heating the block so that the temperature of the contents is maintained at 130 ± 2 °C. If a reciprocal shaker or magnetic stirrer cannot be used, shake the vial thoroughly by hand at 5 min intervals during the initial 30 min of the heating time. Allow the vial to cool, and again weigh accurately. If the loss of mass is less than 26 mg and there is no evidence of a leak, use the upper layer of the mixture as the test solution.

Reference solution Place 0.06-0.10 g of *adipic acid R*, 2.0 mL of the internal standard solution and 2.0 mL of *hydriodic acid R* in another reaction vial, cap and seal the vial, and weigh accurately. Add 45 µL of *methyl iodide R* through the septum with a syringe, and weigh accurately. Shake the reaction vial thoroughly and use the upper layer as the reference solution.

Use a precolumn if needed.

Column:
— *material:* fused silica;
— *size:* *l* = 30 m, Ø = 0.53 mm;
— *stationary phase:* *methylpolysiloxane R* (3 µm).

Carrier gas *helium for chromatography R.*

Flow rate 4.3 mL/min.

Split ratio 1:40.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 3	50
	3 - 8	50 → 100
	8 - 12.3	100 → 250
	12.3 - 20.3	250
Injection port		250
Detector		280

Detection Flame ionisation or thermal conductivity.

Injection 1-2 µL.

Relative retention With reference to octane (retention time = about 10 min): methyl iodide = about 0.4.

System suitability Reference solution:
— *resolution:* minimum 5.0 between the peaks due to methyl iodide and octane;
— *repeatability:* maximum relative standard deviation of 2.0 per cent for the ratio of the area of the peak due to methyl iodide to that due to octane, determined on 6 injections.

Calculate the ratio (Q) of the area of the peak due to methyl iodide to the area of the peak due to the internal standard in the chromatogram obtained with the test solution, and the ratio (Q_1) of the area of the peak due to methyl iodide to the area of the peak due to the internal standard in the chromatogram obtained with the reference solution.

Calculate the percentage content of methoxy groups using the following expression:

$$\frac{Q \times m_1}{Q_1 \times m} \times 21.864$$

m_1 = mass of methyl iodide in the reference solution, in milligrams;
m = mass of the sample (dried substance), in milligrams.

LABELLING

The label states the viscosity in millipascal seconds.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for methylcellulose used as binder, viscosity-enhancing agent or film former.

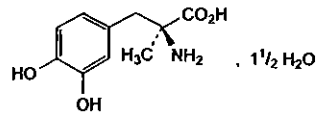
Viscosity
See Tests.

Degree of substitution
See Assay.

Ph Eur

Methyldopa

(Ph. Eur. monograph 0045)



$C_{10}H_{13}NO_4,1½H_2O$ 238.2 *41372-08-1*

Action and use
Alpha$_2$-adrenoceptor agonist; treatment of hypertension.

Preparation
Methyldopa Tablets

Ph Eur

DEFINITION

(2*S*)-2-Amino-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid sesquihydrate (L-methyldopa sesquihydrate).

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or yellowish-white, crystalline powder or colourless or almost colourless crystals.

Solubility
Slightly soluble in water, very slightly soluble in ethanol (96 per cent). It is freely soluble in dilute mineral acids.

IDENTIFICATION

Carry out either tests A, B or tests A, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *methyldopa CRS.*

B. Enantiomeric purity (see Tests).

C. Specific optical rotation (*2.2.7*): −28.0 to −25.0.

Dissolve a quantity equivalent to 2.20 g of the anhydrous substance in *aluminium chloride solution R* and dilute to 50.0 mL with the same solution.

TESTS

Appearance of solution
Dissolve 1.0 g in *1 M hydrochloric acid* and dilute to 25 mL with the same solvent. The solution is not more intensely coloured than reference solution BY_6 or B_6 (*2.2.2, Method II*).

Acidity
Dissolve 1.0 g with heating in 100 mL of *carbon dioxide-free water R.* Add 0.1 mL of *methyl red solution R.* Not more than 0.5 mL of *0.1 M sodium hydroxide* is required to produce the pure yellow colour of the indicator.

Absorbance (*2.2.25*)

Test solution Dissolve 40.0 mg in *0.1 M hydrochloric acid* and dilute to 100.0 mL with the same acid. Dilute 10.0 mL of the solution to 100.0 mL with *0.1 M hydrochloric acid.*

Spectral range 230-350 nm.

Absorption maximum At 280 nm.

Specific absorbance at the absorption maximum 122 to 137 (anhydrous substance).

Enantiomeric purity

Liquid chromatography (*2.2.29*).

Test solution Dissolve 25 mg of the substance to be examined in the mobile phase and dilute to 25.0 mL with the mobile phase.

Reference solution (a) Dilute 5.0 mL of the test solution to 20.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution (b) Dissolve 2 mg of *racemic methyldopa CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase.

Column:
- *size*: *l* = 0.15 m, Ø = 3.9 mm;
- *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve separately 0.200 g of *copper acetate R* and 0.387 g of *N,N-dimethyl-L-phenylalanine R* in *water R*; mix the 2 solutions and adjust immediately to pH 4.3 with *acetic acid R*; add 50 mL of *methanol R* and dilute to 1000 mL with *water R*; mix and filter.

Equilibrate the column with the mobile phase for about 2 h.

If necessary, decrease the concentration of *methanol R* so the peak corresponding to D-methyldopa is clearly separated from the negative system peak that appears at about 6 min.

Flow rate 1 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µL.

Run time Twice the retention time of L-methyldopa.

Relative retention With reference to L-methyldopa (retention time = about 14 min): D-methyldopa = about 0.7.

System suitability Reference solution (b):
- *resolution*: minimum 5.0 between the peaks due to D-methyldopa and L-methyldopa.

Limit:
- *D-methyldopa (impurity D)*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent).

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 0.100 g of the substance to be examined in *0.1 M hydrochloric acid* and dilute to 25.0 mL with the same acid.

Reference solution (a) Dilute 1.0 mL of the test solution to 50.0 mL with *0.1 M hydrochloric acid.* Dilute 5.0 mL of this solution to 100.0 mL with *0.1 M hydrochloric acid.*

Reference solution (b) Dissolve the contents of a vial of *methyldopa for system suitability CRS* (containing impurities A, B and C) in 1.0 mL of *0.1 M hydrochloric acid.*

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: spherical *di-isobutyloctadecylsilyl silica gel for chromatography R* (5 µm) with a pore size of 8 nm.

Mobile phase *methanol R*, 0.1 M phosphate buffer solution pH 3.0 R (15:85 *V/V*).

Flow rate 1 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µL.

Run time 6 times the retention time of methyldopa.

Identification of impurities Use the chromatogram supplied with *methyldopa for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B and C.

Relative retention With reference to methyldopa (retention time = about 5 min): impurity A = about 1.9; impurity B = about 4.3; impurity C = about 4.9.

System suitability Reference solution (b):
- *resolution*: minimum 2.0 between the peaks due to impurities B and C.

Limits:
- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 2.6; impurity C = 1.3;
- *impurities A, B, C*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.03 per cent).

Water (*2.5.12*)

10.0 per cent to 13.0 per cent, determined on 0.20 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.180 g, heating if necessary, in 50 mL of *glacial acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

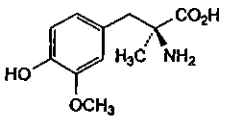
1 mL of *0.1 M perchloric acid* is equivalent to 21.12 mg of $C_{10}H_{13}NO_4$.

STORAGE

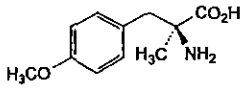
Protected from light.

IMPURITIES

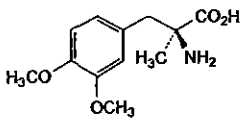
Specified impurities *A, B, C, D.*



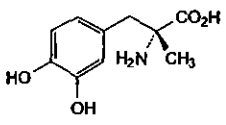
A. (2*S*)-2-amino-3-(4-hydroxy-3-methoxyphenyl)-2-methylpropanoic acid (3-methoxymethyldopa),



B. (2*S*)-2-amino-3-(4-methoxyphenyl)-2-methylpropanoic acid,



C. (2*S*)-2-amino-3-(3,4-dimethoxyphenyl)-2-methylpropanoic acid,

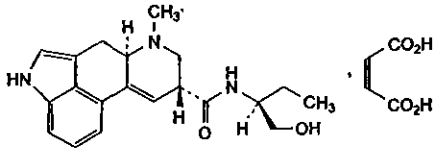


D. (2*R*)-2-amino-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid (D-methyldopa).

Ph Eur

Methylergometrine Maleate

(Ph. Eur. monograph 1788)



$C_{24}H_{29}N_3O_6$ 455.5 *57432-61-8*

Action and use
Oxytocic.

Ph Eur

DEFINITION
(6a*R*,9*R*)-*N*-[(1*S*)-1-(Hydroxymethyl)propyl]-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide (*Z*)-butenedioate.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, hygroscopic, crystalline powder.

Solubility
Soluble in water, slightly soluble in anhydrous ethanol.

IDENTIFICATION
A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *methylergometrine maleate CRS.*

TESTS

Solution S
Dissolve 0.100 g in *carbon dioxide-free water R* and dilute to 20.0 mL with the same solvent.

pH (*2.2.3*)
4.4 to 5.2.

Dilute 2.0 mL of solution S to 50.0 mL with *carbon dioxide-free water R.*

Specific optical rotation (*2.2.7*)
+ 44.0 to + 50.0 (dried substance), determined on solution S.

Related substances
Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Test solution Dissolve 25 mg of the substance to be examined in 15 mL of mobile phase B and dilute to 50.0 mL with *water R.*

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *water R.* Dilute 1.0 mL of this solution to 10.0 mL with *water R.*

Reference solution (b) Dissolve the contents of a vial of *methylergometrine for system suitability CRS* (containing impurities A, B, C, D, E, F, G, H and I) in 1.0 mL of a mixture of 30 volumes of mobile phase B and 70 volumes of *water R.*

Column:
— *size:* l = 0.10 m, Ø = 4.6 mm;
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (3.5 µm).

Mobile phase:
— *mobile phase A*: 2 g/L solution of *ammonium carbamate R*;
— *mobile phase B*: *acetonitrile R, water R* (50:50 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	85	15
2 - 7	85 → 65	15 → 35
7 - 12	65	35
12 - 17	65 → 20	35 → 80
17 - 19	20	80

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 310 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram supplied with *methylergometrine for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C, D, E, F, G, H and I.

Relative retention With reference to methylergometrine (retention time = about 12 min): impurity A = about 0.2; impurity B = about 0.5; impurity C = about 0.6; impurity D = about 0.7; impurity I = about 1.10; impurity E = about 1.14; impurity F = about 1.2; impurity G = about 1.3; impurity H = about 1.4.

System suitability Reference solution (b):
— *resolution*: minimum 3.0 between the peaks due to methylergometrine and impurity I; minimum 1.5 between the peaks due to impurities I and E.

Limits:
— *impurity I*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
— *impurity C*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *impurities A, B, D, E, F, G, H*: for each impurity, not more than 1.5 times the area of the principal peak in the

chromatogram obtained with reference solution (a) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.6 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 2.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

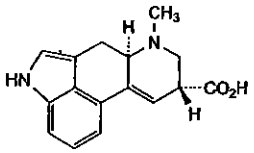
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.300 g in 60 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

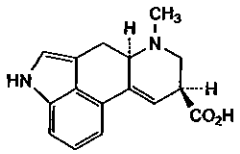
1 mL of *0.1 M perchloric acid* is equivalent to 45.55 mg of $C_{24}H_{29}N_3O_6$.

STORAGE
In an airtight container, protected from light.

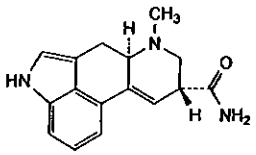
IMPURITIES
Specified impurities *A, B, C, D, E, F, G, H, I.*



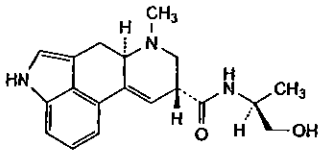
A. (6a*R*,9*R*)-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxylic acid,



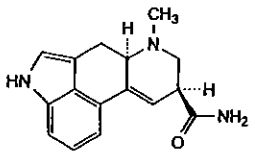
B. (6a*R*,9*S*)-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxylic acid,



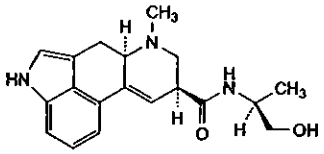
C. (6a*R*,9*R*)-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide,



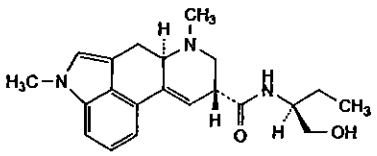
D. (6a*R*,9*R*)-*N*-[(1*S*)-2-hydroxy-1-methylethyl]-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide (ergometrine),



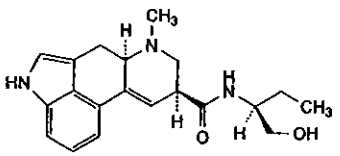
E. (6a*R*,9*S*)-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide,



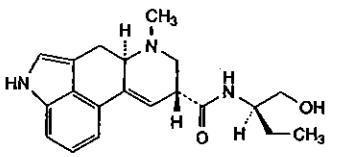
F. (6a*R*,9*S*)-*N*-[(1*S*)-2-hydroxy-1-methylethyl]-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide (ergometrinine),



G. (6a*R*,9*R*)-*N*-[(1*S*)-1-(hydroxymethyl)propyl]-4,7-dimethyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide (methysergide),



H. (6a*R*,9*S*)-*N*-[(1*S*)-1-(hydroxymethyl)propyl]-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide (methylergometrinine),

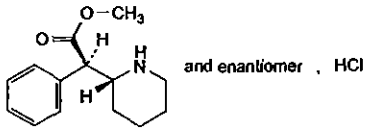


I. (6a*R*,9*R*)-*N*-[(1*R*)-1-(hydroxymethyl)propyl]-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide (1′-*epi*-methylergometrine).

Ph Eur

Methylphenidate Hydrochloride

(Ph. Eur. monograph 2235)



$C_{14}H_{20}ClNO_2$ 269.8 *298-59-9*

Action and use
Narcolepsy; hyperactivity disorder in children.

Preparations
Methylphenidate Prolonged-release Capsules
Methylphenidate Tablets

Ph Eur

DEFINITION
Methyl (2*RS*)-phenyl[(2*RS*)-piperidin-2-yl]acetate hydrochloride.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, fine, crystalline powder.

Solubility
Freely soluble in water, soluble in ethanol (96 per cent), slightly soluble in methylene chloride.

IDENTIFICATION
First identification: A, C.
Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *methylphenidate hydrochloride CRS.*

B. Thin-layer chromatography (*2.2.27*).
Test solution Dissolve 5 mg of the substance to be examined in 1.0 mL of *methanol R.*
Reference solution Dissolve 5 mg of *methylphenidate hydrochloride CRS* in 1.0 mL of *methanol R.*
Plate *TLC silica gel plate R.*
Mobile phase *concentrated ammonia R, methanol R, methylene chloride R* (1:4:95 *V/V/V*).
Application 5 µL.
Development Over 2/3 of the plate.
Drying At 60 °C for 5 min.
Detection Spray with a freshly prepared 5 g/L solution of *fast blue B salt R*; heat to 60 °C for 1 min.
Result The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot obtained with the reference solution.

C. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*
Solvent mixture Mix 20 volumes of *acetonitrile R1* and 80 volumes of a solution prepared as follows: dissolve 1.36 g of *sodium octanesulfonate R* in 950 mL of *water for chromatography R*, add 1.0 mL of *triethylamine R2*, adjust to pH 2.7 with *phosphoric acid R* and dilute to 1000 mL with *water for chromatography R.*
Test solution Dissolve 25 mg of the substance to be examined in the solvent mixture and dilute to 25.0 mL with the solvent mixture.
Reference solution (a) Dissolve 2 mg of *methylphenidate impurity C CRS* in 100.0 mL of the solvent mixture.
Reference solution (b) Dissolve the contents of a vial of *methylphenidate impurity mixture CRS* (impurities A and B) in 1.0 mL of reference solution (a).
Reference solution (c) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.
Column:
— *size: l* = 0.075 m, Ø = 4.6 mm;
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (3.5 µm);
— *temperature:* 40 °C.
Mobile phase:
— *mobile phase A:* dissolve 2.16 g of *sodium octanesulfonate R* in 950 mL of *water for chromatography R*, add 1.0 mL of *triethylamine R2*, adjust to pH 2.7 with *phosphoric acid R* and dilute to 1000 mL with *water for chromatography R;*
— *mobile phase B: acetonitrile R1;*

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	80	20
15 - 35	80 → 60	20 → 40

Flow rate 1.3 mL/min.
Detection Spectrophotometer at 215 nm.
Injection 20 µL.
Identification of impurities Use the chromatogram supplied with *methylphenidate impurity mixture CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and B; use the chromatogram obtained with reference solution (a) to identify the peak due to impurity C.
Relative retention With reference to methylphenidate (retention time = about 20 min): impurity A = about 0.35; impurity C = about 0.40; impurity B = about 0.6.
System suitability Reference solution (b):
— *resolution:* minimum 1.5 between the peaks due to impurities A and C.
Limits:
— *impurities A, B:* for each impurity, not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);
— *unspecified impurities:* for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);
— *total:* not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent);
— *disregard limit:* 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 4 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 50 mL of *ethanol (96 per cent) R* and add 5.0 mL of *0.01 M hydrochloric acid.* Carry out a potentiometric titration (*2.2.20*) using *0.1 M sodium hydroxide* and an electrode for non-aqueous acid-base titrations. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 26.98 mg of $C_{14}H_{20}ClNO_2$.

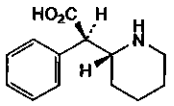
STORAGE

Protected from light.

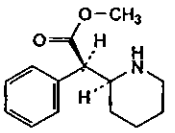
IMPURITIES

Specified impurities *A, B.*

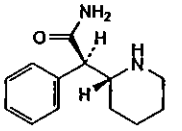
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C, D, E, F.*



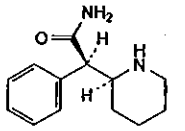
A. (2*RS*)-phenyl[(2*RS*)-piperidin-2-yl]acetic acid,



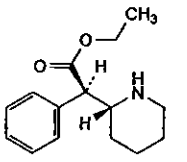
B. methyl (2*RS*)-phenyl[(2*SR*)-piperidin-2-yl]acetate,



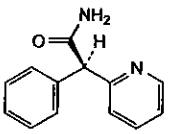
C. (2*RS*)-2-phenyl-2-[(2*RS*)-piperidin-2-yl]acetamide,



D. (2*RS*)-2-phenyl-2-[(2*SR*)-piperidin-2-yl]acetamide,



E. ethyl (2*RS*)-phenyl[(2*RS*)-piperidin-2-yl]acetate,

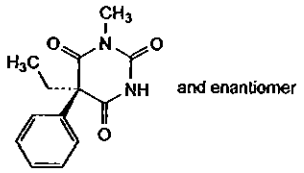


F. (2*RS*)-2-phenyl-2-(pyridin-2-yl)acetamide.

Ph Eur

Methylphenobarbital

(Ph. Eur. monograph 0189)



$C_{13}H_{14}N_2O_3$ 246.3 *115-38-8*

Action and use

Barbiturate.

Ph Eur

DEFINITION

(5*RS*)-5-Ethyl-1-methyl-5-phenylpyrimidine-2,4,6 (1*H*,3*H*,5*H*)-trione.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Practically insoluble in water, very slightly soluble in ethanol (96 per cent).

It forms water-soluble compounds with alkali hydroxides and carbonates and with ammonia.

IDENTIFICATION

First identification: A, B.

Second identification: A, C, D.

A. Determine the melting point (*2.2.14*) of the substance to be examined. Mix equal parts of the substance to be examined and *methylphenobarbital CRS* and determine the melting point of the mixture. The difference between the melting points (which are about 178 °C) is not greater than 2 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *methylphenobarbital CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *methylene chloride R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 10 mg of *methylphenobarbital CRS* in *methylene chloride R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel GF_{254} plate R.*

Mobile phase *concentrated ammonia R, ethanol (96 per cent) R, methylene chloride R* (5:15:80 *V/V/V*); use the lower layer.

Application 10 µL.

Development Over 2/3 of the plate.

Detection Examine immediately in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. To about 10 mg add 0.2 mL of *sulfuric acid R* and 0.1 mL of *nitric acid R.* Heat on a water-bath for 10 min.

Cool in iced water and add 5 mL of *water R* and 5 mL of *strong sodium hydroxide solution R*. Add 5 mL of *acetone R*, shake and allow to stand. A dark-red colour develops in the upper layer.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Dissolve 1.0 g, with gentle heating, in a mixture of 4 mL of *dilute sodium hydroxide solution R* and 6 mL of *water R*.

Acidity

Boil 1.0 g with 50 mL of *water R* for 2 min, allow to cool and filter. To 10 mL of the filtrate add 0.15 mL of *methyl red solution R*. The solution is orange-yellow. Not more than 0.1 mL of *0.1 M sodium hydroxide* is required to produce a pure yellow colour.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50 mg of the substance to be examined in 10.0 mL of *methanol R* and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 2 mg of *phenobarbital CRS* (impurity A) in 1.0 mL of *methanol R* and dilute to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the test solution.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 6.60 g of *sodium acetate R* in 900 mL of *water R*, add 3 mL of *glacial acetic acid R*, adjust to pH 4.5 with *glacial acetic acid R* and dilute to 1000 mL with *water R*. Mix 40 volumes of this solution with 60 volumes of *methanol R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL.

Run time 3.5 times the retention time of methylphenobarbital.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity A.

Relative retention With reference to methylphenobarbital (retention time = about 7 min): impurity A = about 0.7.

System suitability Reference solution (b):
- *resolution*: minimum 5.0 between the peaks due to impurity A and methylphenobarbital.

Limits:
- *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 1.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.7 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

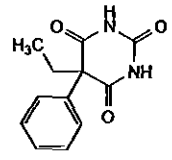
ASSAY

Dissolve 0.100 g in 70 mL of *ethanol (96 per cent) R* and add 20 mL of *water R*. Stir with a mechanical stirrer for about 30 min and sonicate to achieve complete dissolution. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M sodium hydroxide* is equivalent to 24.63 mg of $C_{13}H_{14}N_2O_3$.

IMPURITIES

Specified impurities *A*.

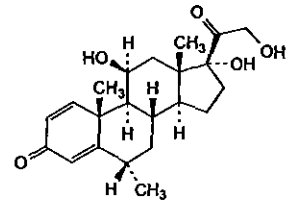


A. 5-ethyl-5-phenylpyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (phenobarbital).

Ph Eur

Methylprednisolone

(Ph. Eur. monograph 0561)



$C_{22}H_{30}O_5$ 374.5 *83-43-2*

Action and use

Glucocorticoid.

Preparation

Methylprednisolone Tablets

Ph Eur

DEFINITION

11β,17,21-Trihydroxy-6α-methylpregna-1,4-diene-3,20-dione.

Content

97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, sparingly soluble in ethanol (96 per cent), slightly soluble in acetone and in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification: A, B.

Second identification: C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *methylprednisolone CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *acetone R*, evaporate to dryness on a water-bath and record new spectra using the residues.

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (c).

C. Thin-layer chromatography (*2.2.27*).

Test solution (a) Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 2 mL with the same solvent (solution A). Dilute 1 mL of solution A to 5 mL with *methylene chloride R*.

Test solution (b) Transfer 0.4 mL of solution A to a glass tube 100 mm long and 20 mm in diameter and fitted with a ground-glass stopper or a polytetrafluoroethylene cap and evaporate the solvent with gentle heating under a stream of *nitrogen R*. Add 2 mL of a 15 per cent *V/V* solution of *glacial acetic acid R* and 50 mg of *sodium bismuthate R*. Stopper the tube and shake the suspension in a mechanical shaker protected from light for.1 h. Add 2 mL of a 15 per cent *V/V* solution of *glacial acetic acid R* and filter into a 50 mL separating funnel, washing the filter with 2 quantities, each of 5 mL, of *water R*. Shake the clear filtrate with 10 mL of *methylene chloride R*. Wash the organic layer with 5 mL of *1 M sodium hydroxide* and 2 quantities, each of 5 mL, of *water R*. Dry over *anhydrous sodium sulfate R*.

Reference solution (a) Dissolve 10 mg of *methylprednisolone CRS* in *methanol R* and dilute to 2 mL with the same solvent (solution B). Dilute 1 mL of solution B to 5 mL with *methylene chloride R*.

Reference solution (b) Transfer 0.4 mL of solution B to a glass tube 100 mm long and 20 mm in diameter and fitted with a ground-glass stopper or a polytetrafluoroethylene cap and evaporate the solvent with gentle heating under a stream of *nitrogen R*. Add 2 mL of a 15 per cent *V/V* solution of *glacial acetic acid R* and 50 mg of *sodium bismuthate R*. Stopper the tube and shake the suspension in a mechanical shaker protected from light for 1 h. Add 2 mL of a 15 per cent *V/V* solution of *glacial acetic acid R* and filter into a 50 mL separating funnel, washing the filter with 2 quantities, each of 5 mL, of *water R*. Shake the clear filtrate with 10 mL of *methylene chloride R*. Wash the organic layer with 5 mL of *1 M sodium hydroxide* and 2 quantities, each of 5 mL, of *water R*. Dry over *anhydrous sodium sulfate R*.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *butanol R* saturated with *water R*, *toluene R*, *ether R* (5:10:85 *V/V/V*).

Application 5 µL of test solution (a) and reference solution (a), 10 µL of test solution (b) and reference solution (b), applying the latter 2 in small quantities in order to obtain small spots.

Development Over 3/4 of the plate.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in each of the chromatograms obtained with the test solutions is similar in position and size to the principal spot in the chromatogram obtained with the corresponding reference solution.

Detection B Spray with *alcoholic solution of sulfuric acid R* and heat at 120 °C for 15 min. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in each of the chromatograms obtained with the test solutions is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with the corresponding reference solution. The principal spot in each of the chromatograms obtained with test solution (b) and reference solution (b) have an R_F value distinctly higher than that of the principal spot in each of the chromatograms obtained with test solution (a) and reference solution (a).

D. Add about 2 mg to 2 mL of *sulfuric acid R* and shake to dissolve. Within 5 min, an intense red colour develops. When examined in ultraviolet light at 365 nm, brownish-red fluorescence is seen. Add this solution to 10 mL of *water R* and mix. The colour fades and there is a yellowish-green fluorescence in ultraviolet light at 365 nm.

TESTS

Specific optical rotation (*2.2.7*)

+ 97.0 to + 103.0 (dried substance).

Dissolve 0.250 g in *ethanol (96 per cent) R* and dilute to 25.0 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture *phosphoric acid R, acetonitrile R, water R* (0.1:50:50 *V/V/V*).

Test solution Dissolve 30.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dissolve 3 mg of *methylprednisolone for system suitability A CRS* (containing impurities A, B, C, D, E, F, G, H and I) in the solvent mixture and dilute to 5.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture.

Reference solution (c) Dissolve 30.0 mg of *methylprednisolone CRS* in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm);

— *temperature*: 45 °C.

Mobile phase:

— *mobile phase A*: *phosphoric acid R, tetrahydrofuran R, acetonitrile R, water R* (0.1:1.5:10:90 *V/V/V/V*);

— *mobile phase B*: *phosphoric acid R, tetrahydrofuran R, acetonitrile R* (0.1:1.5:100 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 14	83	17
14 - 30	83 → 52	17 → 48

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 247 nm.

Injection 10 µL of the test solution and reference solutions (a) and (b).

Identification of impurities Use the chromatogram supplied with *methylprednisolone for system suitability A CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D, E, F, G, H and I.

Relative retention With reference to methylprednisolone (retention time = about 12 min): impurity B = about 0.85; impurity H = about 0.88; impurity A = about 0.92; impurity F = about 1.1; impurities G and I = about 1.54; impurity C = about 1.7; impurity E = about 1.9; impurity D (isomer 1) = about 2.10; impurity D (isomer 2) = about 2.2.

System suitability Reference solution (a):

- *resolution*: minimum 1.7 between the peaks due to impurity A and methylprednisolone;
- *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity F and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to methylprednisolone.

Limits:

- *impurity D*: for the sum of the areas of the 2 isomer peaks, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *impurity A*: not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
- *sum of impurities G and I*: not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
- *impurities B, H*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *impurities C, E, F*: for each impurity, not more than 0.15 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);
- *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 0.500 g by drying in an oven at 105 °C.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (c).

Calculate the percentage content of $C_{22}H_{30}O_5$ taking into account the assigned content of *methylprednisolone CRS*.

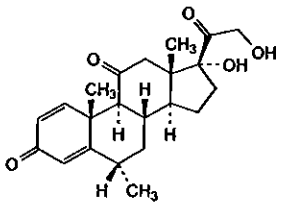
STORAGE

Protected from light, at a temperature of 2 °C to 8 °C.

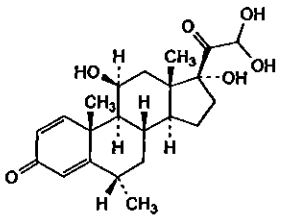
IMPURITIES

Specified impurities *A, B, C, D, E, F, G, H, I.*

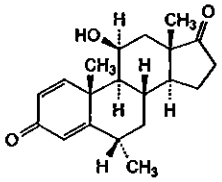
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *J, K, L.*



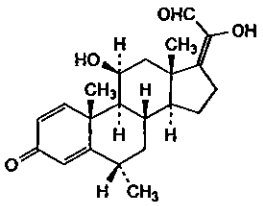
A. 17,21-dihydroxy-6α-methylpregna-1,4-diene-3,11,20-trione,



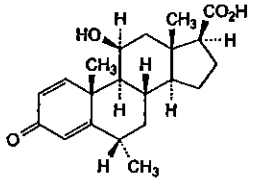
B. 11β,17,21,21-tetrahydroxy-6α-methylpregna-1,4-diene-3,20-dione,



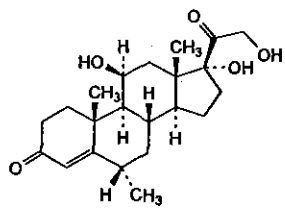
C. 11β-hydroxy-6α-methylandrosta-1,4-diene-3,17-dione,



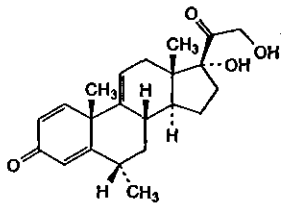
D. (*EZ*)-11β,20-dihydroxy-6α-methylpregna-1,4,17(20)-triene-3,21-dione,



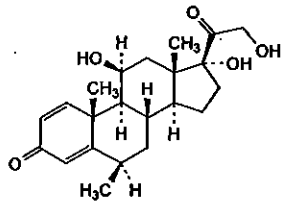
E. 11β-hydroxy-6α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid,



F. 11β,17,21-trihydroxy-6α-methylpregn-4-ene-3,20-dione,

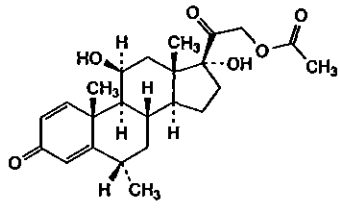


G. 17,21-dihydroxy-6α-methylpregna-1,4,9(11)-triene-3,20-dione,

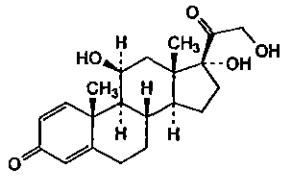


H. 11β,17,21-trihydroxy-6β-methylpregna-1,4-diene-3,20-dione,

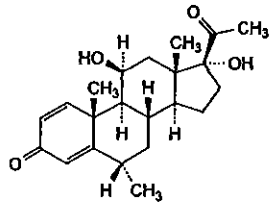
I. unknown structure,



J. 11β,17-dihydroxy-6α-methyl-3,20-dioxopregna-1,4-dien-21-yl acetate (methylprednisolone acetate),



K. 11β,17,21-trihydroxypregna-1,4-diene-3,20-dione (prednisolone),

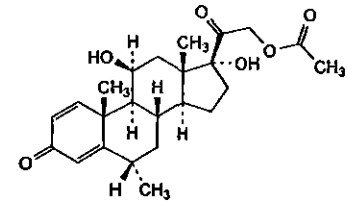


L. 11β,17-dihydroxy-6α-methylpregna-1,4-diene-3,20-dione.

Ph Eur

Methylprednisolone Acetate

(Ph. Eur. monograph 0933)



$C_{24}H_{32}O_6$ 416.5 *53-36-1*

Action and use
Glucocorticoid.

Preparation
Methylprednisolone Acetate Injection

Ph Eur

DEFINITION

11β,17-Dihydroxy-6α-methyl-3,20-dioxopregna-1,4-dien-21-yl acetate.

Content
97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, sparingly soluble in acetone and in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *methylprednisolone acetate CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *acetone R*, evaporate to dryness and record new spectra using the residues.

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (d).

TESTS

Specific optical rotation (*2.2.7*)
+ 107 to + 113 (dried substance).

Dissolve 0.250 g in *ethanol (96 per cent) R* and dilute to 25.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Buffer solution Mix 20 mL of a 103 g/L solution of *hydrochloric acid R*, 50 mL of a 68 g/L solution of *sodium acetate R* and 150 mL of a 37.3 g/L solution of *potassium chloride R* and dilute to 1.0 L with *water R*.

Solvent mixture *acetonitrile R*, buffer solution (50:50 *V/V*).

Test solution (a) Dissolve 20.0 mg of the substance to be examined in the solvent mixture and dilute to 20.0 mL with the solvent mixture.

Test solution (b) Dilute 1.0 mL of test solution (a) to 10.0 mL with the solvent mixture.

Reference solution (a) Dissolve 5 mg of *methylprednisolone acetate for system suitability CRS* (containing impurities E, G and J) in the solvent mixture and dilute to 5.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve the contents of a vial of *methylprednisolone acetate for peak identification CRS* (impurities A and K) in 1.0 mL of the solvent mixture.

Reference solution (d) Dissolve 20.0 mg of *methylprednisolone acetate CRS* in the solvent mixture and dilute to 20.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 10.0 mL with the solvent mixture.

Column:
— *size*: l = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3.5 µm);
— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: *acetonitrile R, water for chromatography R* (28:72 *V/V*);
— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	95	5
15 - 25	95 → 50	5 → 50
25 - 28	50	50

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL of test solution (a) and reference solutions (a), (b) and (c).

Identification of impurities Use the chromatogram supplied with *methylprednisolone acetate for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities E, G and J; use the chromatogram supplied with *methylprednisolone acetate for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A and K.

Relative retention With reference to methylprednisolone acetate (retention time = about 15 min):
impurity A = about 0.4; impurity E = about 0.6;
impurity J = about 0.9; impurity G = about 1.1;
impurity K = about 1.2.

System suitability Reference solution (a):
— *peak-to-valley ratio*: minimum 3.0, where H_p = height above the baseline of the peak due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to methylprednisolone acetate.

Calculation of percentage contents:
— for each impurity, use the concentration of methylprednisolone acetate in reference solution (b).

Limits:
— *impurity J*: maximum 0.3 per cent;
— *impurities A, G*: for each impurity, maximum 0.2 per cent;
— *impurities E, K*: for each impurity, maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 1.0 per cent;
— *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Mobile phase *acetonitrile R, water for chromatography R* (32:68 *V/V*).

Injection Test solution (b) and reference solution (d).

Run time 1.5 times the retention time of methylprednisolone acetate.

Retention time Methylprednisolone acetate = about 15 min.

Calculate the percentage content of $C_{24}H_{32}O_6$ taking into account the assigned content of *methylprednisolone acetate CRS*.

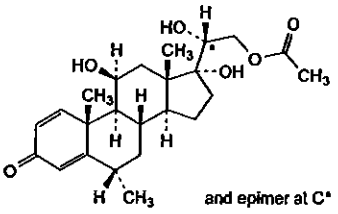
STORAGE

Protected from light.

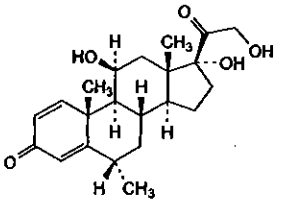
IMPURITIES

Specified impurities *A, E, G, J, K.*

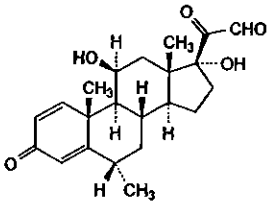
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D, F, H, I.*



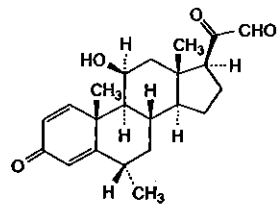
A. (20*RS*)-11β,17,20-trihydroxy-6α-methyl-3-oxopregna-1,4-dien-21-yl acetate,



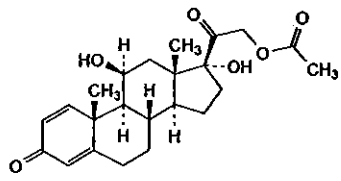
B. 11β,17,21-trihydroxy-6α-methylpregna-1,4-diene-3,20-dione (methylprednisolone),



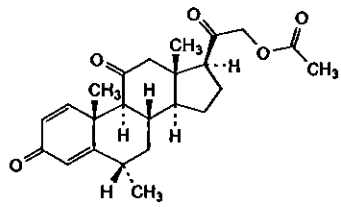
C. 11β,17-dihydroxy-6α-methylpregna-1,4-diene-3,20,21-trione,



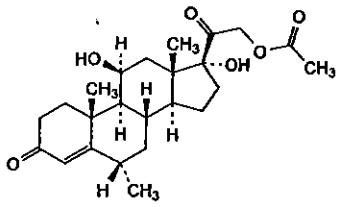
D. 11β-hydroxy-6α-methylpregna-1,4-diene-3,20,21-trione,



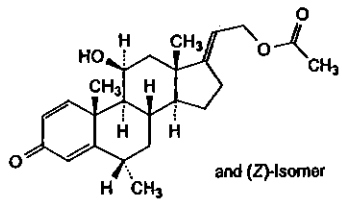
E. 11β,17-dihydroxy-3,20-dioxopregna-1,4-dien-21-yl acetate (prednisolone acetate),



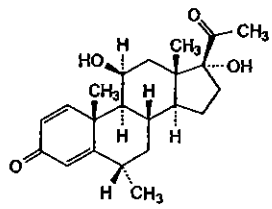
F. 6α-methyl-3,11,20-trioxopregna-1,4-dien-21-yl acetate,



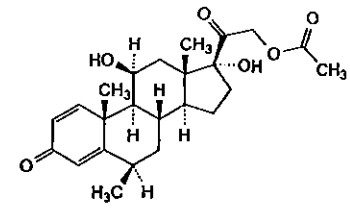
G. 11β,17-dihydroxy-6α-methyl-3,20-dioxopregn-4-en-21-yl acetate,



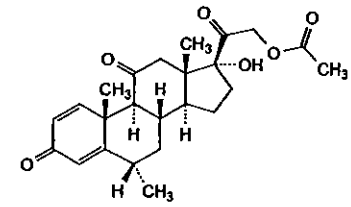
H. (*EZ*)-11β-hydroxy-6α-methyl-3-oxopregna-1,4,17(20)-trien-21-yl acetate,



I. 11β,17-dihydroxy-6α-methylpregna-1,4-diene-3,20-dione,



J. 11β,17-dihydroxy-6β-methyl-3,20-dioxopregna-1,4-dien-21-yl acetate,

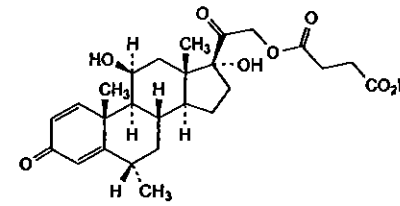


K. 17-hydroxy-6α-methyl-3,11,20-trioxopregna-1,4-dien-21-yl acetate.

Ph Eur

Methylprednisolone Hydrogen Succinate

(Ph. Eur. monograph 1131)



$C_{26}H_{34}O_8$ 474.5 *2921-57-5*

Action and use
Glucocorticoid.

Ph Eur

DEFINITION

4-[(11β,17-Dihydroxy-6α-methyl-3,20-dioxopregna-1,4-dien-21-yl)oxy]-4-oxobutanoic acid.

Content

97.5 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, hygroscopic powder.

Solubility

Practically insoluble in water, slightly soluble in acetone and in anhydrous ethanol. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *methylprednisolone hydrogen succinate CRS.*

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (e).

TESTS

Appearance of solution

The solution is clear (*2.2.1*).

Dissolve 0.100 g in 5 mL of *sodium hydrogen carbonate solution R*.

Specific optical rotation (*2.2.7*)

+ 97 to + 104 (dried substance).

Dissolve 0.250 g in *ethanol (96 per cent) R* and dilute to 25.0 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 20.0 mg of the substance to be examined in 1 mL of *tetrahydrofuran R* and dilute to 20.0 mL with the mobile phase.

Test solution (b) Dilute 1.0 mL of test solution (a) to 10.0 mL with the mobile phase.

Reference solution (a) Dissolve 5 mg of *methylprednisolone hydrogen succinate for performance test CRS* (containing impurity D) in 0.25 mL of *tetrahydrofuran R* and dilute to 5.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of test solution (a) to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (c) Dissolve 5 mg of *methylprednisolone hydrogen succinate for peak identification CRS* (containing impurity F) in 0.25 mL of *tetrahydrofuran R* and dilute to 5.0 mL with the mobile phase.

Reference solution (d) Dissolve 5 mg of *methylprednisolone R* (impurity A) in 0.25 mL of *tetrahydrofuran R* and dilute to 5.0 mL with the mobile phase.

Reference solution (e) Dissolve 20.0 mg of *methylprednisolone hydrogen succinate CRS* in 1 mL of *tetrahydrofuran R* and dilute to 20.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 10.0 mL with the mobile phase.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *glacial acetic acid R, acetonitrile R, water R* (2:31:67 *V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL of test solution (a) and reference solutions (a), (b), (c) and (d).

Run time Twice the retention time of methylprednisolone hydrogen succinate.

Identification of impurities Use the chromatogram supplied with *methylprednisolone hydrogen succinate for performance test CRS* and the chromatogram obtained with reference solution (a) to identify the peak due to impurity D; use the chromatogram supplied with *methylprednisolone hydrogen succinate for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peak due to impurity F; use the chromatogram obtained with reference solution (d) to identify the peak due to impurity A.

Relative retention With reference to methylprednisolone hydrogen succinate (retention time = about 21 min): impurity A = about 0.5; impurity F = about 0.9; impurity D = about 1.1.

System suitability Reference solution (a):
- *peak-to-valley ratio*: minimum 4, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to methylprednisolone hydrogen succinate.

Calculation of percentage contents:
- for each impurity, use the concentration of methylprednisolone hydrogen succinate in reference solution (b).

Limits:
- *impurity A*: maximum 0.3 per cent;
- *impurity F*: maximum 0.2 per cent;
- *impurity D*: maximum 0.15 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.5 per cent;
- *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (e).

Calculate the percentage content of $C_{26}H_{34}O_8$ taking into account the assigned content of *methylprednisolone hydrogen succinate CRS*.

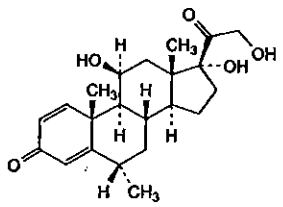
STORAGE

In an airtight container, protected from light.

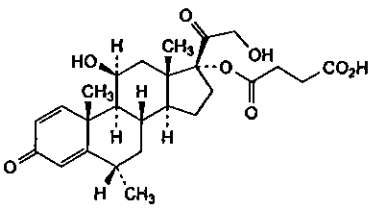
IMPURITIES

Specified impurities *A, D, F.*

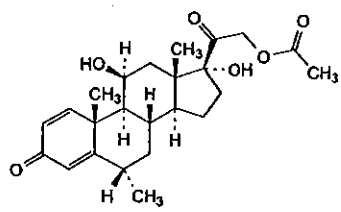
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, E, G.*



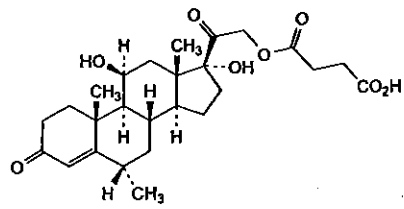
A. 11β,17,21-trihydroxy-6α-methylpregna-1,4-diene-3,20-dione (methylprednisolone),



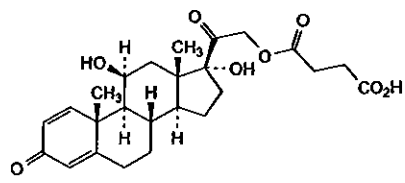
B. 4-[(11β,21-dihydroxy-6α-methyl-3,20-dioxopregna-1,4-dien-17-yl)oxy]-4-oxobutanoic acid (methylprednisolone 17-(hydrogen succinate)),



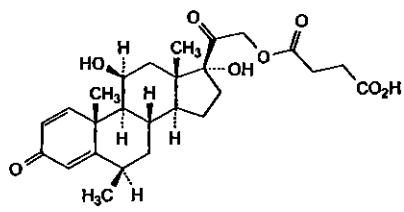
C. 11β,17-dihydroxy-6α-methyl-3,20-dioxopregna-1,4-dien-21-yl acetate (methylprednisolone acetate),



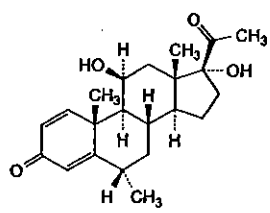
D. 4-[(11β,17-dihydroxy-6α-methyl-3,20-dioxopregn-4-en-21-yl)oxy]-4-oxobutanoic acid (methylhydrocortisone 21-(hydrogen succinate)),



E. 4-[(11β,17-dihydroxy-3,20-dioxopregna-1,4-dien-21-yl) oxy]-4-oxobutanoic acid,



F. 4-[(11β,17-dihydroxy-6β-methyl-3,20-dioxopregna-1,4-dien-21-yl)oxy]-4-oxobutanoic acid,

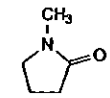


G. 11β,17-dihydroxy-6α-methylpregna-1,4-diene-3,20-dione.

Ph Eur

Methylpyrrolidone

(N-Methylpyrrolidone, Ph. Eur. monograph 1675)



C_5H_9NO 99.1 *872-50-4*

Ph Eur

DEFINITION

1-Methylpyrrolidin-2-one.

CHARACTERS

Appearance

Clear, colourless liquid.

Solubility

Miscible with water and with alcohol.

bp

About 204 °C.

Relative density

About 1.034.

Refractive index

About 1.469.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Preparation Films.

Comparison *Ph. Eur. reference spectrum of N-methylpyrrolidone.*

TESTS

Appearance

The substance to be examined is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Alkalinity

Dissolve 50 mL of the substance to be examined in 50 mL of *water R* previously adjusted with *0.02 M potassium hydroxide* or *0.02 M hydrochloric acid* until a yellow colour is obtained using 0.5 mL of *bromothymol blue solution R1* as indicator. Titrate with *0.02 M hydrochloric acid* to the initial coloration. Not more than 8.0 mL of *0.02 M hydrochloric acid* is required.

Related substances

Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution The substance to be examined.

Reference solution To 1 mL of the substance to be examined, add 1 mL of *2-pyrrolidone R* and dilute to 20 mL with *methylene chloride R*.

Column:

— *material*: fused silica,

— *size*: *l* = 30 m, Ø = 0.32 mm,

— *stationary phase*: *methylpolysiloxane R* (5 µm).

Carrier gas *nitrogen for chromatography R.*

Linear velocity 20 cm/s.

Split ratio 1:100.

Temperature:

	Time (min)	Temperature (°C)
Column	0	100
	0 - 23.3	100 → 170
	23.3 - 53	170
Injection port		280
Detector		280

Detection Flame ionisation.

Injection 1 µL.

System suitability Reference solution:
— *resolution*: minimum 2.0 between the peaks due to *N*-methylpyrrolidone and impurity G.

Limits:
— *any impurity*: maximum 0.1 per cent,
— *total*: maximum 0.3 per cent,
— *disregard limit*: 0.02 per cent.

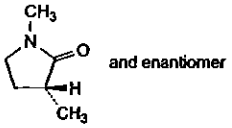
Water (*2.5.32*)
Maximum 0.1 per cent, determined on 1.000 g.

STORAGE
Protected from light.

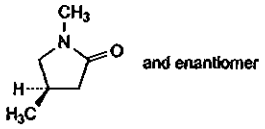
IMPURITIES

A. methanamine (methylamine),

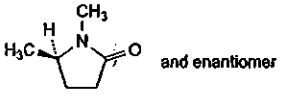
B. dihydrofuran-2(3*H*)-one (γ-butyrolactone),



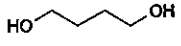
C. (3*RS*)-1,3-dimethylpyrrolidin-2-one,



D. (4*RS*)-1,4-dimethylpyrrolidin-2-one,

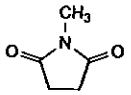


E. (5*RS*)-1,5-dimethylpyrrolidin-2-one,

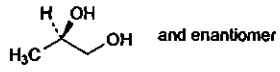


F. butane-1,4-diol,

G. pyrrolidin-2-one (2-pyrrolidone),



H. 1-methylpyrrolidine-2,5-dione (*N*-methylsuccinimide),

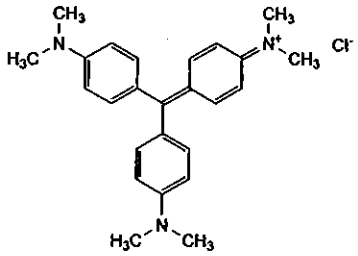


I. (*RS*)-propane-1,2-diol (propylene glycol).

Ph Eur

Methylrosanilinium Chloride

(Ph. Eur. monograph 1990)



$C_{25}H_{30}ClN_3$ 408.0 *548-62-9*

Action and use
Antiseptic dye (gentian violet).

Ph Eur

DEFINITION
4-[Bis[4-(dimethylamino)phenyl]methylidene]-*N*,*N*-dimethylcyclohexa-2,5-dien-1-iminium chloride (hexamethyl-*p*-rosanilinium chloride). It is also known as crystal violet or gentian violet.

Content
95.0 per cent to 103.0 per cent (anhydrous substance).

CHARACTERS

Appearance
Dark green, shiny powder, hygroscopic.

Solubility
Sparingly soluble in water, freely soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION
First identification: A.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *methylrosanilinium chloride CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 10 mg of *methylrosanilinium chloride CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *glacial acetic acid R, water R, butanol R* (17:17:66 *V/V/V*).

Application 2 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in daylight.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution. In the chromatogram obtained with the test solution, a secondary spot may be observed.

C. Dissolve 50 mg in *water R* and dilute to 5 mL with the same solvent; add 3 mL of *dilute sulfuric acid R*, 1 g of *zinc powder R* and heat gently. The mixture decolourises. Filter. To 3 mL of the filtrate add 0.5 mL of *silver nitrate solution R1*. A white turbidity is produced which slowly forms a dark, coagulating precipitate.

TESTS

N,N-Dimethylaniline (*2.4.26, Method A*)

Maximum 100 ppm.

Test solution Dissolve in a ground-glass-stoppered tube 0.50 g of the substance to be examined in 30.0 mL of *water R*. Add 1.0 mL of the internal standard solution. Adjust the solution to 26-28 °C. Add 1.0 mL of *strong sodium hydroxide solution R* and mix for 2 min. Add 2.0 mL of *trimethylpentane R*. Shake for 2 min and centrifuge. Use the upper layer.

Reference solution Dissolve 50.0 mg of *N,N-dimethylaniline R* in 4.0 mL of *0.1 M hydrochloric acid* and dilute to 50.0 mL with *water R*. Dilute 1.0 mL of this solution to 100.0 mL with *water R*. To 0.50 g of the substance to be examined add 5.0 mL of this solution and dilute to 30.0 mL with *water R*. Add 1.0 mL of the internal standard solution and 1.0 mL of *strong sodium hydroxide solution R*. Add 2.0 mL of *trimethylpentane R*. Shake for 2 min and centrifuge. Use the upper layer.

Limit:

— calculate the ratio (*R*) of the area of the peak due to *N,N*-dimethylaniline to the area of the peak due to the internal standard from the chromatogram obtained with the reference solution; from the chromatogram obtained with the test solution, calculate the ratio of the area of the peak due to *N,N*-dimethylaniline to the area of the peak due to the internal standard: this ratio is not greater than 0.5 *R*.

Related substances

Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution Dissolve 30.0 mg of the substance to be examined in *ethanol (96 per cent) R* and dilute to 100.0 mL with the same solvent.

Reference solution (a) Dissolve 3 mg of *methylrosanilinium for system suitability CRS* (containing impurities A and B) in *ethanol (96 per cent) R* and dilute to 10.0 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of the test solution to 20.0 mL with *ethanol (96 per cent) R*. Dilute 1.0 mL of this solution to 100.0 mL with *ethanol (96 per cent) R*.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: spherical *base-deactivated end-capped octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *glacial acetic acid R, water for chromatography R, methanol R* (10:190:800 *V/V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 589 nm.

Injection 20 µL.

Run time 2.5 times the retention time of methylrosanilinium.

Identification of impurities Use the chromatogram supplied with *methylrosanilinium for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A and B.

Relative retention With reference to methylrosanilinium (retention time = about 23 min): impurity A = about 0.71; impurity B = about 0.85.

System suitability Reference solution (a):

— *resolution*: minimum 1.5 between the peaks due to impurity B and methylrosanilinium.

Limits:

— *impurity B*: maximum 10.0 per cent;

— *impurity A*: maximum 1.0 per cent;

— *unspecified impurities*: for each impurity, maximum 0.10 per cent;

— *sum of impurities other than A and B*: maximum 1.0 per cent;

— *reporting threshold*: 0.05 per cent (peak due to methylrosanilinium in the chromatogram obtained with reference solution (b)).

Substances insoluble in ethanol (90 per cent *V/V*)

Maximum 0.5 per cent.

In a conical flask introduce 1.0 g and add 50 mL of *ethanol (90 per cent V/V) R*. Boil under a reflux condenser for 1 h. Filter the warm liquid through a weighed sintered glass filter (16) (*2.1.2*) previously dried at 100-105 °C. Wash with hot *ethanol (90 per cent V/V) R* until a colourless filtrate is obtained. Dry at 100-105 °C until constant weight.

Water (*2.5.12*)

Maximum 10.0 per cent, determined on 0.100 g.

Sulfated ash (*2.4.14*)

Maximum 1.5 per cent, determined on 1.0 g.

ASSAY

Dissolve 50.0 mg in *ethanol (96 per cent) R* and dilute to 100.0 mL with the same solvent. Dilute 2.0 mL of the solution to 250.0 mL with *ethanol (96 per cent) R*. Measure the absorbance (*2.2.25*) at the maximum at 589 nm.

Calculate the content of $C_{25}H_{30}ClN_3$ taking the specific absorbance to be 2605.

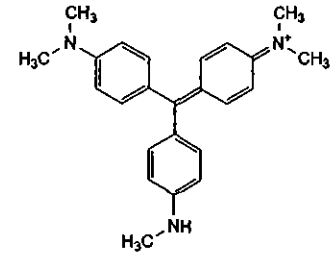
STORAGE

In an airtight container.

IMPURITIES

Specified impurities *A, B.*

A. unknown structure,

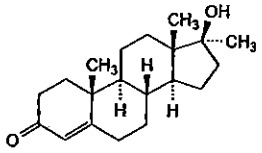


B. 4-[[4-(dimethylamino)phenyl][4-(methylamino)phenyl] methylidene]-*N,N*-dimethylcyclohexa-2,5-dien-1-iminium.

Ph Eur

Methyltestosterone

(Ph. Eur. monograph 0410)



$C_{20}H_{30}O_2$ 302.5 *58-18-4*

Action and use
Anabolic steroid.

Ph Eur

DEFINITION
17β-Hydroxy-17-methylandrost-4-en-3-one.

Content
97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS
Appearance
White or slightly yellowish-white, crystalline powder.

Solubility
Practically insoluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *methyltestosterone CRS.*

TESTS
Specific optical rotation (*2.2.7*)
+ 79 to + 85 (dried substance).

Dissolve 0.250 g in *ethanol (96 per cent) R* and dilute to 25.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 50 mg of the substance to be examined in *methanol R* and dilute to 100.0 mL with the same solvent.

Reference solution (a) Dilute 0.5 mL of the test solution to 100.0 mL with *methanol R.*

Reference solution (b) Dissolve 5 mg of *methyltestosterone for system suitability CRS* (containing impurity A) in *methanol R* and dilute to 10 mL with the same solvent.

Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A: water R;*
— *mobile phase B: methanol R;*

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	30	70
15 - 45	30 → 0	70 → 100
45 - 50	0	100

Flow rate 1.5 mL/min.
Detection Spectrophotometer at 254 nm.
Injection 20 µL.

Identification of impurities Use the chromatogram supplied with *methyltestosterone for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity A.

Relative retention With reference to methyltestosterone (retention time = about 8 min): impurity A = about 1.5.

System suitability Reference solution (b):
— *resolution*: minimum 5 between the peaks due to methyltestosterone and impurity A.

Limits:
— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

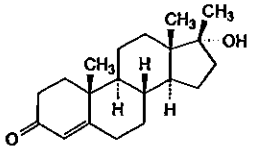
Loss on drying (*2.2.32*)
Maximum 2.0 per cent, determined on 0.500 g by drying in an oven at 105 °C for 2 h.

ASSAY
Dissolve 50.0 mg in *ethanol (96 per cent) R* and dilute to 50.0 mL with the same solvent. Dilute 10.0 mL of the solution to 100.0 mL with *ethanol (96 per cent) R.* Dilute 10.0 mL of this solution to 100.0 mL with *ethanol (96 per cent) R.* Measure the absorbance (*2.2.25*) at the absorption maximum at 241 nm.

Calculate the content of $C_{20}H_{30}O_2$ taking the specific absorbance to be 540.

STORAGE
Protected from light.

IMPURITIES
Specified impurities *A.*



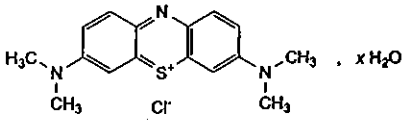
A. 17α-hydroxy-17-methylandrost-4-en-3-one.

Ph Eur

Methylthioninium Chloride Hydrate

Methylthioninium Chloride

(Ph. Eur. monograph 1132)



$C_{16}H_{18}ClN_3S,xH_2O$ 319.9 *122965-43-9*
(anhydrous substance)

Action and use
Reducing agent; antidote to methaemoglobinaemia.

Preparation
Methylthioninium Injection

Ph Eur

DEFINITION

3,7-Bis(dimethylamino)phenothiazin-5-ylium chloride (methylene blue) hydrate.

Content
93.0 per cent to 102.0 per cent (dried substance).

It contains a variable quantity of water.

CHARACTERS

Appearance
Dark blue or dark green, hygroscopic, crystalline powder with a metallic sheen.

Solubility
Slightly soluble in water and in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *methylthioninium chloride CRS.*

B. Ignite 50 mg with 0.5 g of *anhydrous sodium carbonate R.* Cool and dissolve the residue in 10 mL of *dilute nitric acid R.* Filter. The filtrate, without further addition of *dilute nitric acid R,* gives reaction (a) of chlorides (*2.3.1*).

TESTS

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R,* mobile phase A (30:70 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture. Sonicate for 5 min.

Reference solution (a) Dissolve 50.0 mg of *methylthioninium chloride CRS* in the solvent mixture and dilute to 50.0 mL with the solvent mixture. Sonicate for 5 min.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture.

Reference solution (c) Dissolve 10 mg of *methylthioninium for system suitability CRS* (containing impurity A) in the solvent mixture and dilute to 10 mL with the solvent mixture.

Reference solution (d) Dilute 1.0 mL of reference solution (b) to 10.0 mL with the solvent mixture.

Column:
— *size:* l = 0.10 m, Ø = 4.6 mm;
— *stationary phase: end-capped ethylene-bridged phenylsilyl silica gel for chromatography (hybrid material) R* (3.5 µm);
— *temperature:* 30 °C.

Mobile phase:
— *mobile phase A:* 0.1 per cent *V/V* solution of *trifluoroacetic acid R;*
— *mobile phase B: acetonitrile for chromatography R;*

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	80	20
5 - 25	80 → 30	20 → 70
25 - 32	30	70

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 246 nm.

Injection 5 µL of the test solution and reference solutions (b), (c) and (d).

Identification of impurities Use the chromatogram supplied with *methylthioninium for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peak due to impurity A.

Relative retention With reference to methylthioninium (retention time = about 11 min): impurity A = about 0.8.

System suitability Reference solution (c):
— *resolution:* minimum 3.5 between the peaks due to impurity A and methylthioninium.

Calculation of percentage contents:
— for impurity A, use the concentration of methylthioninium chloride hydrate in reference solution (b);
— for impurities other than A, use the concentration of methylthioninium chloride hydrate in reference solution (d).

Limits:
— *impurity A:* maximum 5.0 per cent;
— *unspecified impurities:* for each impurity, maximum 0.10 per cent;
— *sum of impurities other than A:* maximum 0.5 per cent;
— *reporting threshold:* 0.05 per cent.

Elemental impurities
Any method that fulfils the requirements of general chapter *2.4.20. Determination of elemental impurities* may be used.

Element	Maximum content (ppm)
Aluminium	100
Cadmium	1
Chromium	100
Copper	300
Iron	200
Lead	10
Manganese	10
Mercury	1
Molybdenum	10
Nickel	10
Tin	10
Zinc	100

Loss on drying (*2.2.32*)
8.0 per cent to 24.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 5 h.

Sulfated ash (*2.4.14*)
Maximum 0.25 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (a).

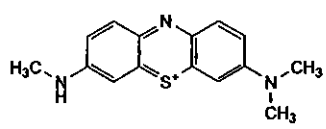
System suitability Reference solution (a):
— *symmetry factor*: maximum 3.0 for the principal peak.

Calculate the percentage content of $C_{16}H_{18}ClN_3S$ taking into account the assigned content of *methylthioninium chloride CRS*.

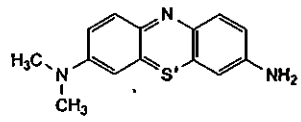
STORAGE
In an airtight container, protected from light, at a temperature not exceeding 30 °C.

IMPURITIES
Specified impurities *A.*

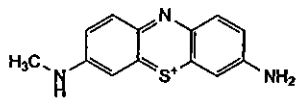
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C.*



A. 3-(dimethylamino)-7-(methylamino)phenothiazin-5-ylium (azure B),



B. 3-amino-7-(dimethylamino)phenothiazin-5-ylium (azure A),

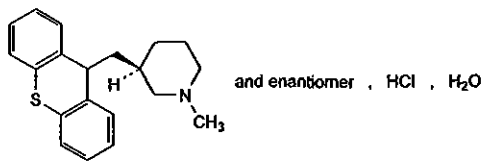


C. 3-amino-7-(methylamino)phenothiazin-5-ylium (azure C).

Ph Eur

Metixene Hydrochloride

(Ph. Eur. monograph 1347)



$C_{20}H_{24}ClNS,H_2O$ 363.9 *7081-40-5*

Action and use
Anticholinergic.

Ph Eur

DEFINITION
Metixene hydrochloride contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of (*RS*)-1-methyl-3-[(9*H*-thioxanthen-9-yl)methyl]piperidine hydrochloride, calculated with reference to the dried substance.

CHARACTERS
A white or almost white, crystalline or fine crystalline powder, soluble in water, soluble in alcohol and in methylene chloride.

IDENTIFICATION
A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *metixene hydrochloride CRS*.

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS
Appearance of solution
Dissolve 0.40 g in *methanol R* and dilute to 20.0 mL with the same solvent. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method I*).

pH (*2.2.3*)
Dissolve 0.18 g in *carbon dioxide-free water R* heating if necessary at about 50 °C, cool and dilute to 10.0 mL with the same solvent. The pH of the solution, measured immediately, is 4.4 to 5.8.

Related substances
Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel plate R. Carry out the test rapidly and protected from light.*

Test solution Dissolve 50 mg of the substance to be examined in *methylene chloride R* and dilute to 5.0 mL with the same solvent.

Reference solution (a) Dissolve 5 mg of *metixene hydrochloride CRS* in *methylene chloride R* and dilute to 100.0 mL with the same solvent.

Reference solution (b) Dissolve 20 mg of *thioxanthene CRS* in 50 mL of *methylene chloride R*. Dilute 1.0 mL of the solution to 20.0 mL with *methylene chloride R*.

Reference solution (c) Dissolve 5 mg of *thioxanthone CRS* in 50 mL of *methylene chloride R*. Dilute 1.0 mL of the solution to 20.0 mL with *methylene chloride R*.

Reference solution (d) Dilute 4 mL of reference solution (a) to 10.0 mL with *methylene chloride R*.

Apply to the plate as narrow bands 5 µL of each solution. Develop over a path of 10 cm using a mixture of 10 volumes of *glacial acetic acid R*, 10 volumes of *methanol R* and 80 volumes of *methylene chloride R*. Dry the plate in a stream of cold air. Spray with a mixture of 1 volume of *sulfuric acid R* and 9 volumes of *alcohol R* and heat at 100 °C for 10 min. Allow the plate to cool and examine in ultraviolet light at 365 nm. Thioxanthene shows orange fluorescence and thioxanthone shows greenish-blue fluorescence.
Any band corresponding to thioxanthene in the chromatogram obtained with the test solution is not more intense than the band in the chromatogram obtained with reference solution (b) (0.2 per cent); any band corresponding to thioxanthone in the chromatogram obtained with the test solution is not more intense than the band in the chromatogram obtained with reference solution (c) (0.05 per cent); any band, apart from the principal band and the bands corresponding to thioxanthene and thioxanthone, is not more intense than the band in the chromatogram obtained with reference solution (a) (0.5 per cent) and at most one such band is more intense than the band in the chromatogram obtained with reference solution (d) (0.2 per cent). The test is not valid unless the bands in the

chromatograms obtained with reference solutions (b) and (c) are clearly visible and differentiated.

Loss on drying (*2.2.32*)
Not less than 4.0 per cent and not more than 6.0 per cent, determined on 0.500 g by drying in an oven at 138-142 °C.

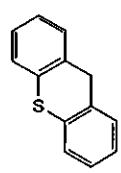
Sulfated ash (*2.4.14*)
Not more than 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.250 g in a mixture of 5.0 mL of *0.01 M hydrochloric acid* and 50 mL of *alcohol R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

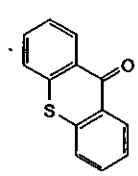
1 mL of *0.1 M sodium hydroxide* is equivalent to 34.59 mg of $C_{20}H_{24}ClNS$.

STORAGE
Store protected from light.

IMPURITIES



A. 9*H*-thioxanthene,

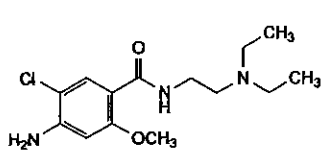


B. 9*H*-thioxanthen-9-one (thioxanthone).

Ph Eur

Metoclopramide

(Ph. Eur. monograph 1348)



$C_{14}H_{22}ClN_3O_2$ 299.8 *364-62-5*

Action and use
Dopamine receptor antagonist; antiemetic.

Ph Eur

DEFINITION
4-Amino-5-chloro-*N*-[2-(diethylamino)ethyl]-2-methoxybenzamide.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, fine powder.

Solubility
Practically insoluble in water, sparingly soluble or slightly soluble in ethanol (96 per cent), slightly soluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION
First identification: A, B.

Second identification: A, C.

A. Melting point (*2.2.14*): 145 °C to 149 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *metoclopramide CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *methanol R*, evaporate to dryness at room temperature then heat at 50 °C for about 1 h. Record new spectra using the residues.

C. Thin-layer chromatography (*2.2.27*) as described in the test for impurity E with the following modifications.

Test solution Dissolve 40 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 20 mg of *metoclopramide CRS* in *methanol R* and dilute to 5 mL with the same solvent.

Detection Examine in ultraviolet light at 254 nm then spray with *dimethylaminobenzaldehyde solution R1*, allow to dry in air and examine in daylight.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution.

TESTS
Appearance of solution
The freshly prepared solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Dissolve 2.5 g in 25 mL of a 103.0 g/L solution of *hydrochloric acid R*.

Impurity E
Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.160 g of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dissolve 20 mg of *metoclopramide CRS* and 10 mg of *sulpiride CRS* in *methanol R* and dilute to 5 mL with the same solvent.

Reference solution (b) Dissolve 20 mg of *N,N-diethylethane-1,2-diamine R* (impurity E) in *methanol R* and dilute to 50 mL with the same solvent. Dilute 2 mL of the solution to 25 mL with *methanol R*.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *concentrated ammonia R, dioxan R, methanol R, methylene chloride R* (2:10:14:90 *V/V/V/V*).

Application 10 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *dimethylaminobenzaldehyde solution R1*, allow to dry in air and examine in daylight.

Retardation factors Impurity E = about 0.2; metoclopramide = about 0.6.

System suitability Reference solution (a):
— the chromatogram shows 2 clearly separated spots.

Limit:

— *impurity E*: any spot due to impurity E in the chromatogram obtained with the test solution is not more intense than the corresponding spot in the chromatogram obtained with reference solution (b) (0.2 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture Mobile phase B, mobile phase A (5:95 *V/V*).

Test solution Dissolve 20.0 mg of the substance to be examined in the solvent mixture and dilute to 20.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *metoclopramide impurity A CRS* in the solvent mixture and dilute to 100 mL with the solvent mixture. Mix 1 mL of the solution with 0.1 mL of the test solution and dilute to 10 mL with the solvent mixture.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped ethylene-bridged octadecylsilyl silica gel for chromatography (hybrid material) R* (3.5 µm);

— *temperature*: 37 °C.

Mobile phase:

— *mobile phase A*: 5 g/L solution of *ammonium acetate R* in *water for chromatography R* adjusted to pH 7.0 with *ammonia R* or *acetic acid R*;

— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 1.5	95	5
1.5 - 16.5	95 → 42.5	5 → 57.5

Flow rate 0.9 mL/min.

Detection Spectrophotometer at 275 nm.

Injection 5 µL.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity A.

Relative retention With reference to metoclopramide (retention time = about 9 min): impurity A = about 0.9.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to impurity A and metoclopramide.

Calculation of percentage contents:

— for each impurity, use the concentration of metoclopramide in reference solution (a).

Limits:

— *unspecified impurities*: for each impurity, maximum 0.10 per cent;

— *total*: maximum 0.2 per cent;

— *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

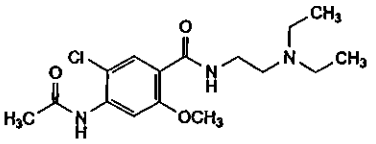
Dissolve 0.250 g in 50 mL of *anhydrous acetic acid R* and add 5 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 29.98 mg of $C_{14}H_{22}ClN_3O_2$.

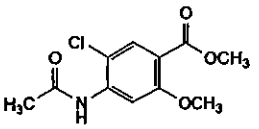
IMPURITIES

Specified impurities *E.*

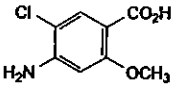
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for otherlunspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, F, G, H.*



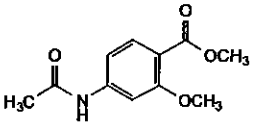
A. 4-acetamido-5-chloro-*N*-[2-(diethylamino)ethyl]-2-methoxybenzamide,



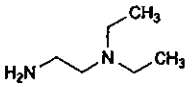
B. methyl 4-acetamido-5-chloro-2-methoxybenzoate,



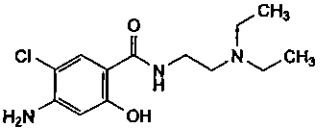
C. 4-amino-5-chloro-2-methoxybenzoic acid,



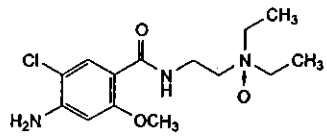
D. methyl 4-acetamido-2-methoxybenzoate,



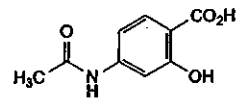
E. *N*,*N*-diethylethane-1,2-diamine,



F. 4-amino-5-chloro-*N*-[2-(diethylamino)ethyl]-2-hydroxybenzamide,



G. 2-(4-amino-5-chloro-2-methoxybenzamido)-*N,N*-diethylethan-1-amine N-oxide,



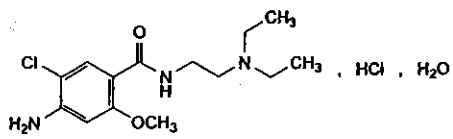
H. 4-acetamido-2-hydroxybenzoic acid.

Ph Eur

Metoclopramide Hydrochloride Monohydrate

Metoclopramide Hydrochloride

(Ph. Eur. monograph 0674)



$C_{14}H_{23}Cl_2N_3O_2,H_2O$ · 354.3 *54143-57-6*

Action and use
Dopamine receptor antagonist; antiemetic.

Preparations
Metoclopramide Injection
Metoclopramide Oral Solution
Metoclopramide Tablets

Ph Eur

DEFINITION
4-Amino-5-chloro-*N*-[2-(diethylamino)ethyl]-2-methoxybenzamide hydrochloride monohydrate.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white, crystalline powder or crystals.

Solubility
Very soluble in water, freely soluble in ethanol (96 per cent), sparingly soluble in methylene chloride.

mp
About 183 °C, with decomposition.

IDENTIFICATION
First identification: A, B, D.

Second identification: A, C, D, E.

A. pH (*2.2.3*): 4.5 to 6.0 for solution S (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *metoclopramide hydrochloride CRS.*

C. Thin-layer chromatography (*2.2.27*) as described in the test for impurity E with the following modifications.

Test solution Dissolve 40 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 20 mg of *metoclopramide hydrochloride CRS* in *methanol R* and dilute to 5 mL with the same solvent.

Detection Examine in ultraviolet light at 254 nm then spray with *dimethylaminobenzaldehyde solution R1*, allow to dry in air and examine in daylight.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dilute 1 mL of solution S to 2 mL with *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

E. Dissolve about 2 mg in 2 mL of *water R*. The solution gives the reaction of primary aromatic amines (*2.3.1*).

TESTS
Solution S
Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Impurity E
Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.40 g of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 10 mg of *N,N-diethylethane-1,2-diamine R* (impurity E) in *methanol R* and dilute to 50 mL with the same solvent.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *concentrated ammonia R, dioxan R, methanol R, methylene chloride R* (2:10:14:90 *V/V/V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *dimethylaminobenzaldehyde solution R1*, allow to dry in air and examine in daylight.

Retardation factors Impurity E = about 0.2; metoclopramide = about 0.6.

Limit:
— *impurity E*: any spot due to impurity E in the chromatogram obtained with the test solution is not more intense than the corresponding spot in the chromatogram obtained with the reference solution (0.5 per cent).

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture Mobile phase B, mobile phase A (5:95 *V/V*).

Test solution Dissolve 24.0 mg of the substance to be examined in the solvent mixture and dilute to 20.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *metoclopramide impurity A CRS* in the solvent mixture and dilute to 100 mL with the solvent mixture. Mix 1 mL of the solution with 0.1 mL of the test solution and dilute to 10 mL with the solvent mixture.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped ethylene-bridged octadecylsilyl silica gel for chromatography (hybrid material) R* (3.5 µm);
— *temperature*: 37 °C.

Mobile phase:
— *mobile phase A*: 5 g/L solution of *ammonium acetate R* in *water for chromatography R* adjusted to pH 7.0 with *ammonia R* or *acetic acid R*;
— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 1.5	95	5
1.5 - 16.5	95 → 42.5	5 → 57.5

Flow rate 0.9 mL/min.

Detection Spectrophotometer at 275 nm.

Injection 5 µL.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity A.

Relative retention With reference to metoclopramide (retention time = about 9 min): impurity A = about 0.9.

System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the peaks due to impurity A and metoclopramide.

Calculation of percentage contents:
— for each impurity, use the concentration of metoclopramide hydrochloride monohydrate in reference solution (a).

Limits:
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.2 per cent;
— *reporting threshold*: 0.05 per cent.

Water (*2.5.12*)
4.5 per cent to 5.5 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in a mixture of 5.0 mL of *0.01 M hydrochloric acid* and 50 mL of *ethanol (96 per cent) R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 33.63 mg of $C_{14}H_{23}Cl_2N_3O_2$.

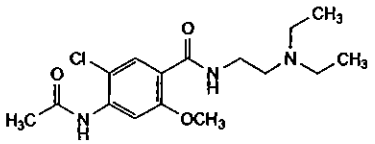
STORAGE

Protected from light.

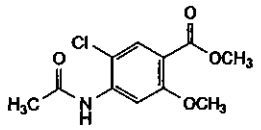
IMPURITIES

Specified impurities *E.*

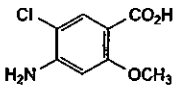
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, F, G, H.*



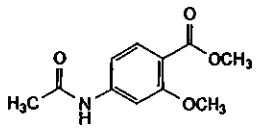
A. 4-acetamido-5-chloro-*N*-[2-(diethylamino)ethyl]-2-methoxybenzamide,



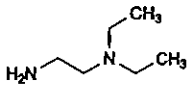
B. methyl 4-acetamido-5-chloro-2-methoxybenzoate,



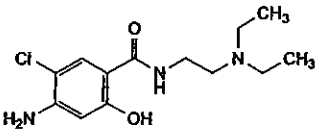
C. 4-amino-5-chloro-2-methoxybenzoic acid,



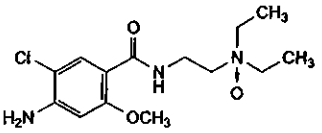
D. methyl 4-acetamido-2-methoxybenzoate,



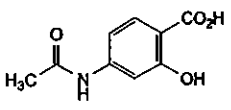
E. *N*,*N*-diethylethane-1,2-diamine,



F. 4-amino-5-chloro-*N*-[2-(diethylamino)ethyl]-2-hydroxybenzamide,



G. 2-(4-amino-5-chloro-2-methoxybenzamido)-*N*,*N*-diethylethan-1-amine *N*-oxide,

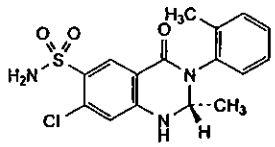


H. 4-acetamido-2-hydroxybenzoic acid.

Ph Eur

Metolazone

(Ph. Eur. monograph 1757)



and enantiomer

$C_{16}H_{16}ClN_3O_3S$ 365.8

Action and use
Thiazide-like diuretic.

Ph Eur

DEFINITION

(2*RS*)-7-Chloro-2-methyl-3-(2-methylphenyl)-4-oxo-1,2,3,4-tetrahydroquinazoline-6-sulfonamide.

Content
97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
White or slightly yellowish, crystalline powder.

Solubility
Very slightly soluble in water, sparingly soluble in methanol, slightly soluble in ethyl acetate, very slightly soluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *metolazone CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *anhydrous ethanol R*, evaporate to dryness and record new spectra using the residues.

TESTS

Related substances
Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 30.0 mg of the substance to be examined in *methanol R* and dilute to 10.0 mL with the same solvent.

Test solution (b) Dilute 2.0 mL of test solution (a) to 100.0 mL with *methanol R.*

Reference solution (a) Dissolve 3.0 mg of *metolazone for system suitability CRS* (containing impurities A, B, C, D and E) in 1 mL of *methanol R.*

Reference solution (b) Dilute 1.0 mL of test solution (a) to 100.0 mL with *methanol R.* Dilute 5.0 mL of this solution to 10.0 mL with *methanol R.*

Reference solution (c) Dissolve 30.0 mg of *metolazone CRS* in *methanol R* and dilute to 10.0 mL with the same solvent. Dilute 2.0 mL of this solution to 100.0 mL with *methanol R.*

Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature:* 30 °C.

Mobile phase:
— *mobile phase A:* 5.44 g/L solution of *potassium dihydrogen phosphate R;*
— *mobile phase B: methanol R;*

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	70	30
5 - 25	70 → 50	30 → 50
25 - 35	50	50
35 - 38	50 → 70	50 → 30
38 - 48	70	30

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 10 µL of test solution (a) and reference solutions (a) and (b).

Identification of impurities Use the chromatogram supplied with *metolazone for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D and E.

System suitability Reference solution (a):
— *resolution:* minimum 1.6 between the peaks due to impurities E and C and minimum 1.5 between the peaks due to impurities A and B.

Limits:
— *impurities A, B, C, D, E:* for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
— *unspecified impurities:* for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total:* not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
— *disregard limit:* 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (c).

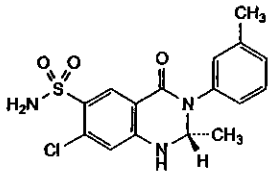
Calculate the percentage content of $C_{16}H_{16}ClN_3O_3S$ from the declared content of *metolazone CRS.*

STORAGE

Protected from light.

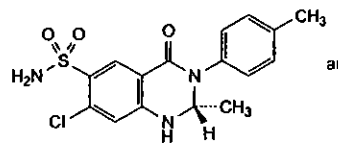
IMPURITIES

Specified impurities *A, B, C, D, E.*

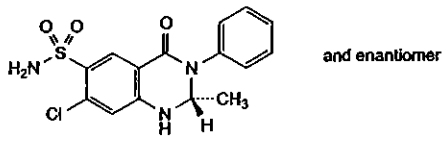


and enantiomer

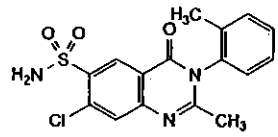
A. (2*RS*)-7-chloro-2-methyl-3-(3-methylphenyl)-4-oxo-1,2,3,4-tetrahydroquinazoline-6-sulfonamide,



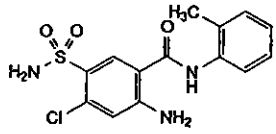
B. (2*RS*)-7-chloro-2-methyl-3-(4-methylphenyl)-4-oxo-1,2,3,4-tetrahydroquinazoline-6-sulfonamide,



C. (2*RS*)-7-chloro-2-methyl-4-oxo-3-phenyl1,2,3,4-tetrahydroquinazoline-6-sulfonamide,



D. 7-chloro-2-methyl-3-(2-methylphenyl)-4-oxo-3,4-dihydroquinazoline-6-sulfonamide,

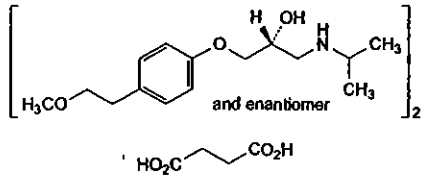


E. 2-amino-4-chloro-*N*-(2-methylphenyl)-5-sulfamoylbenzamide.

Ph Eur

Metoprolol Succinate

(Ph. Eur. monograph 1448)



$C_{34}H_{56}N_2O_{10}$ 653 *98418-47-4*

Action and use
Beta-adrenoceptor antagonist.

Ph Eur

DEFINITION
Bis[(2*RS*)-1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]propan-2-ol] butanedioate.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water, soluble in methanol, slightly soluble in ethanol (96 per cent), very slightly soluble in ethyl acetate.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of metoprolol succinate.*

TESTS
Solution S
Dissolve 0.500 g in *carbon dioxide-free water R* and dilute to 25.0 mL with the same solvent.

Appearance of solution
Solution S is not more opalescent than reference suspension II (*2.2.1*) and is colourless (*2.2.2, Method II*).

pH (*2.2.3*)
7.0 to 7.6 for solution S.

Impurities M, N, O
Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.50 g of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution Dilute 1 mL of the test solution to 50 mL with *methanol R*. Dilute 5 mL of this solution to 50 mL with *methanol R*.

Plate *TLC silica gel plate R.*

Mobile phase Place 2 beakers, each containing 30 volumes of *concentrated ammonia R*, at the bottom of a chromatographic tank containing a mixture of 20 volumes of *methanol R* and 80 volumes of *ethyl acetate R*.

Application 10 µL.

Development Over 2/3 of the plate in a tank saturated for at least 1 h.

Drying In air for at least 3 h.

Detection Expose the plate to iodine vapour for at least 15 h.

Limits:
— *any impurity*: any spot, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.2 per cent);
— *disregard* any spot on the line of application.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dissolve 1.5 mg of *metoprolol impurity A CRS* and 2.5 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.15 m, Ø = 3.9 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 3.9 g of *ammonium acetate R* in 810 mL of *water R*, add 2.0 mL of *triethylamine R*, 3.0 mL of *phosphoric acid R*, 10.0 mL of *glacial acetic acid R* and 146 mL of *acetonitrile R* and mix.

Flow rate 1 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µL.

Run time 3 times the retention time of metoprolol.

Relative retention With reference to metoprolol (retention time = about 7 min): impurity C = about 0.4; impurity A = about 0.8.

System suitability Reference solution (a):
— *resolution*: minimum 6.0 between the peaks due to impurity A and metoprolol.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity C by 0.1;
— *impurity C*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak due to succinic acid.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 40 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 32.64 mg of $C_{34}H_{56}N_2O_{10}$.

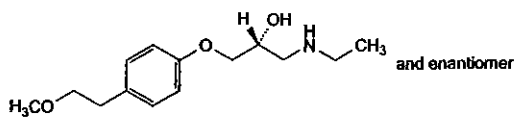
STORAGE

Protected from light.

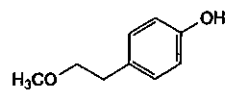
IMPURITIES

Specified impurities *C, M, N, O.*

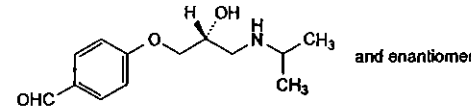
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, D, E, F, G, H, J.*



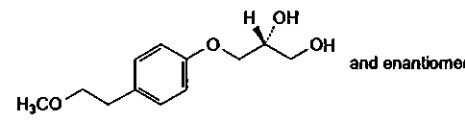
A. (2*RS*)-1-(ethylamino)-3-[4-(2-methoxyethyl) phenoxy]propan-2-ol,



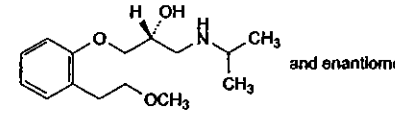
B. 4-(2-methoxyethyl)phenol,



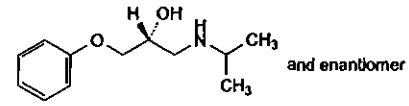
C. 4-[(2*RS*)-2-hydroxy-3-[(1-methylethyl)amino]propoxy] benzaldehyde,



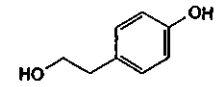
D. (2*RS*)-3-[4-(2-methoxyethyl)phenoxy]propane-1,2-diol,



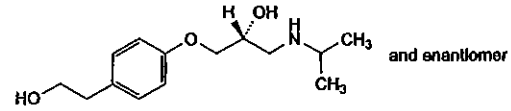
E. (2*RS*)-1-[2-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]propan-2-ol,



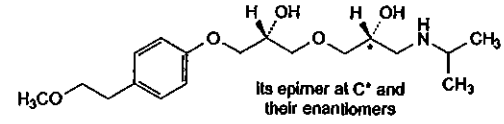
F. (2*RS*)-1-[(1-methylethyl)amino]-3-phenoxypropan-2-ol,



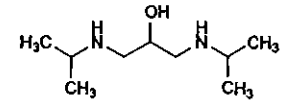
G. 2-(4-hydroxyphenyl)ethanol,



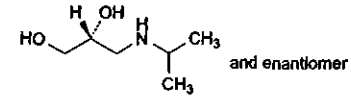
H. (2*RS*)-1-[4-(2-hydroxyethyl)phenoxy]-3-[(1-methylethyl)amino]propan-2-ol,



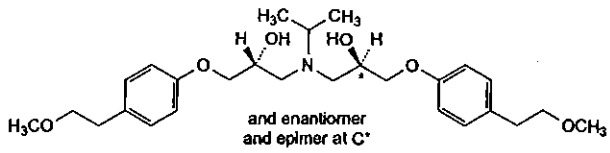
J. mixture of the 4 stereoisomers of 1-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]-3-[4-(2-methoxyethyl) phenoxy]propan-2-ol,



M. 1,3-bis[(1-methylethyl)amino]propan-2-ol,



N. (2*RS*)-3-[(1-methylethyl)amino]propane-1,2-diol,

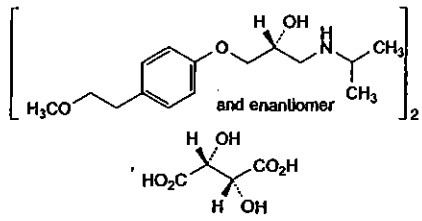


O. mixture of the 3 stereoisomers of 1,1′-[(1-methylethyl) imino]bis[3-[4-(2-methoxyethyl)phenoxy]propan-2-ol].

Ph Eur

Metoprolol Tartrate

(Ph. Eur. monograph 1028)



$C_{34}H_{56}N_2O_{12}$ 685 *56392-17-7*

Action and use
Beta-adrenoceptor antagonist.

Preparations
Metoprolol Injection
Metoprolol Oral Suspension
Metoprolol Tartrate Prolonged-release Tablets
Metoprolol Tartrate Tablets

Ph Eur

DEFINITION
Bis[(2*RS*)-1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]propan-2-ol] (2*R*,3*R*)-2,3-dihydroxybutanedioate.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder or colourless crystals.

Solubility
Very soluble in water, freely soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION
A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *metoprolol tartrate CRS.*

If the spectra obtained in the solid state show differences, record further spectra using discs prepared by placing 25 µL of a 100 g/L solution in *methylene chloride R* on a disc of *potassium bromide R* and evaporating the solvent. Examine immediately.

TESTS
Solution S
Dissolve 0.500 g in *carbon dioxide-free water R* and dilute to 25.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_8 (*2.2.2, Method II*).

pH (*2.2.3*)
6.0 to 7.0 for solution S.

Specific optical rotation (*2.2.7*)
+ 7.0 to + 10.0 (dried substance), determined on solution S.

Impurities M, N, O
Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.50 g of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dilute 1 mL of the test solution to 20 mL with *methanol R*. Dilute 5 mL of this solution to 50 mL with *methanol R*.

Reference solution (b) Dilute 4 mL of reference solution (a) to 10 mL with *methanol R*.

Plate *TLC silica gel plate R.*

Mobile phase Place 2 beakers, each containing 30 volumes of *concentrated ammonia R*, at the bottom of a chromatographic tank containing a mixture of 20 volumes of *methanol R* and 80 volumes of *ethyl acetate R*.

Application 5 µL.

Development Over 2/3 of the plate in a tank saturated for at least 1 h.

Drying In air for at least 3 h.

Detection Expose the plate to iodine vapour for at least 15 h.

Limits:
— *any impurity*: any spot, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent) and at most 1 such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent);
— *disregard* any spot on the line of application.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dissolve 1.5 mg of *metoprolol impurity A CRS* and 2.5 mg of *metoprolol tartrate CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 20.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 50.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.15 m, Ø = 3.9 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 3.9 g of *ammonium acetate R* in 810 mL of *water R*, add 2.0 mL of *triethylamine R*, 3.0 mL of *phosphoric acid R*, 10.0 mL of *glacial acetic acid R* and 146 mL of *acetonitrile R* and mix.

Flow rate 1 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µL.

Run time 3 times the retention time of metoprolol.

Relative retention With reference to metoprolol (retention time = about 7 min): impurity H = about 0.3;

impurity C = about 0.4; impurity G = about 0.45; impurity F = about 0.7; impurity A = about 0.8; impurity J = about 1.4; impurity D = about 1.6; impurity E = about 1.8; impurity B = about 2.

System suitability Reference solution (a):
— *resolution*: minimum 6.0 between the peaks due to impurity A and metoprolol.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity C by 0.1;
— *impurities A, B, C, D, E, F, G, H, J*: for each impurity, not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak due to tartaric acid.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* over *anhydrous calcium chloride R* for 4 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 30 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

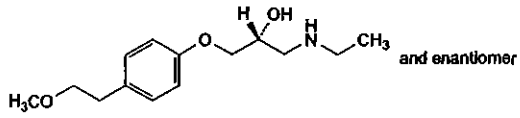
1 mL of *0.1 M perchloric acid* is equivalent to 34.24 mg of $C_{34}H_{56}N_2O_{12}$.

STORAGE

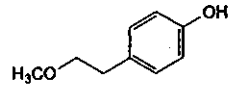
Protected from light.

IMPURITIES

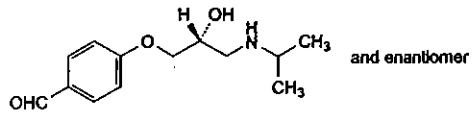
Specified impurities *A, B, C, D, E, F, G, H, J, M, N, O.*



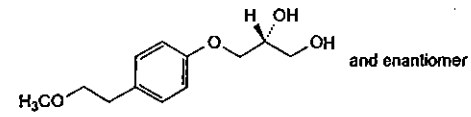
A. (2*RS*)-1-(ethylamino)-3-[4-(2-methoxyethyl) phenoxy]propan-2-ol,



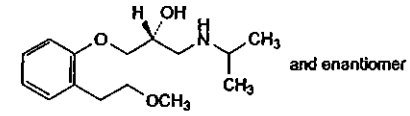
B. 4-(2-methoxyethyl)phenol,



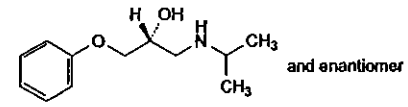
C. 4-[(2*RS*)-2-hydroxy-3-[(1-methylethyl)amino] propoxy]benzaldehyde,



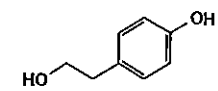
D. (2*RS*)-3-[4-(2-methoxyethyl)phenoxy]propane-1,2-diol,



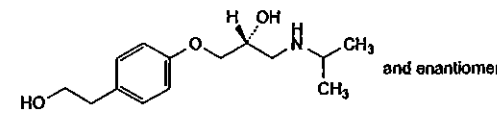
E. (2*RS*)-1-[2-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]propan-2-ol,



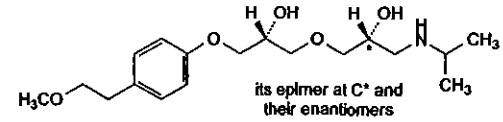
F. (2*RS*)-1-[(1-methylethyl)amino]-3-phenoxypropan-2-ol,



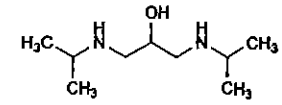
G. 2-(4-hydroxyphenyl)ethanol,



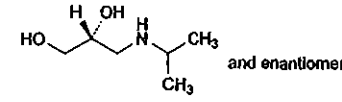
H. (2*RS*)-1-[4-(2-hydroxyethyl)phenoxy]-3-[(1-methylethyl)amino]propan-2-ol,



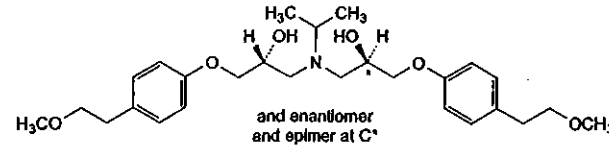
J. mixture of the 4 stereoisomers of 1-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]-3-[4-(2-methoxyethyl) phenoxy]propan-2-ol,



M. 1,3-bis[(1-methylethyl)amino]propan-2-ol,



N. (2*RS*)-3-[(1-methylethyl)amino]propane-1,2-diol,

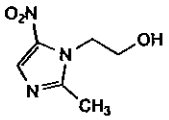


O. mixture of the 3 stereoisomers of 1,1′-[(1-methylethyl) imino]bis[3-[4-(2-methoxyethyl)phenoxy]propan-2-ol].

Ph Eur

Metronidazole

(Ph. Eur. monograph 0675)



$C_6H_9N_3O_3$ 171.2 *443-48-1*

Action and use
Imidazole antibacterial.

Preparations
Metronidazole Gel
Metronidazole Infusion
Metronidazole Suppositories
Metronidazole Tablets

Ph Eur

DEFINITION
2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethanol.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or yellowish, crystalline powder.

Solubility
Slightly soluble in water, in acetone, in alcohol and in methylene chloride.

IDENTIFICATION
First identification: C.
Second identification: A, B, D.

A. Melting point (*2.2.14*): 159 °C to 163 °C.

B. Dissolve 40.0 mg in *0.1 M hydrochloric acid* and dilute to 100.0 mL with the same acid. Dilute 5.0 mL of the solution to 100.0 mL with *0.1 M hydrochloric acid.* Examined between 230 nm and 350 nm (*2.2.25*), the solution shows an absorption maximum at 277 nm and a minimum at 240 nm. The specific absorbance at the maximum is 365 to 395.

C. Infrared absorption spectrophotometry (*2.2.24*).
Preparation Discs.
Comparison *metronidazole CRS.*

D. To about 10 mg add about 10 mg of *zinc powder R*, 1 mL of *water R* and 0.25 mL of *dilute hydrochloric acid R*. Heat on a water-bath for 5 min. Cool. The solution gives the reaction of primary aromatic amines (*2.3.1*).

TESTS

Appearance of solution
The solution is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution GY_6 (*2.2.2, Method II*).

Dissolve 1.0 g in *1 M hydrochloric acid* and dilute to 20 mL with the same acid.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions protected from light.*

Test solution Dissolve 0.05 g of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase and dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *metronidazole impurity A CRS* in the mobile phase, add 10.0 mL of the test solution and dilute to 100.0 mL with the mobile phase. Dilute 1.0 mL to 100.0 mL with the mobile phase.

Column:
— *size: l* = 0.25 m, Ø = 4.6 mm,
— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 30 volumes of *methanol R* and 70 volumes of a 1.36 g/L solution of *potassium dihydrogen phosphate R,*

Flow rate 1 mL/min.

Detection Spectrophotometer at 315 nm.

Injection 10 µL.

Run time 3 times the retention time of metronidazole.

Relative retention With reference to metronidazole (retention time = about 7 min): impurity A = about 0.7.

System suitability Reference solution (b):
— *resolution*: minimum of 2.0 between the peaks due to metronidazole and to impurity A.

Limits:
— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent),
— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.01 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

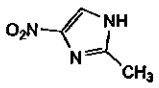
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.150 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

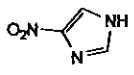
1 mL of *0.1 M perchloric acid* is equivalent to 17.12 mg of $C_6H_9N_3O_3$.

STORAGE
Protected from light.

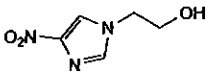
IMPURITIES



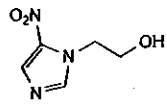
A. 2-methyl-4-nitroimidazole,



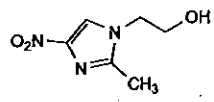
B. 4-nitroimidazole,



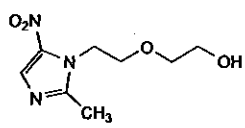
C. 2-(4-nitro-1*H*-imidazol-1-yl)ethanol,



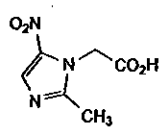
D. 2-(5-nitro-1*H*-imidazol-1-yl)ethanol,



E. 2-(2-methyl-4-nitro-1*H*-imidazol-1-yl)ethanol,



F. 2-[2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy]ethanol,

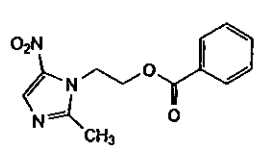


G. 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)acetic acid.

Ph Eur

Metronidazole Benzoate

(Ph. Eur. monograph 0934)



$C_{13}H_{13}N_3O_4$ 275.3 *13182-89-3*

Action and use
Imidazole antibacterial.

Preparation
Metronidazole Oral Suspension

Ph Eur

DEFINITION
2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl benzoate.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or slightly yellowish, crystalline powder or flakes.

Solubility
Practically insoluble in water, freely soluble in methylene chloride, soluble in acetone, slightly soluble in alcohol.

IDENTIFICATION
First identification: C.

Second identification: A, B, D.

A. Melting point (*2.2.14*): 99 °C to 102 °C.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 0.100 g in a 103 g/L solution of *hydrochloric acid R* and dilute to 100.0 mL with the same acid. Dilute 1.0 mL of the solution to 100.0 mL with a 103 g/L solution of *hydrochloric acid R*.

Spectral range 220-350 nm.

Absorption maxima At 232 nm and 275 nm.

Specific absorbance at the absorption maximum at 232 nm 525 to 575.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of metronidazole benzoate.*

D. To about 10 mg add about 10 mg of *zinc powder R*, 1 mL of *water R* and 0.3 mL of *hydrochloric acid R*. Heat on a water-bath for 5 min and cool. The solution gives the reaction of primary aromatic amines (*2.3.1*).

TESTS
Appearance of solution
The solution is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution GY_3 (*2.2.2, Method II*).

Dissolve 1.0 g in *dimethylformamide R* and dilute to 10 mL with the same solvent.

Acidity
Dissolve 2.0 g in a mixture of 20 mL of *dimethylformamide R* and 20 mL of *water R*, previously neutralised with *0.02 M hydrochloric acid* or *0.02 M sodium hydroxide* using 0.2 mL of *methyl red solution R*. Not more than 0.25 mL of *0.02 M sodium hydroxide* is required to change the colour of the indicator.

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture Mobile phase B, mobile phase A (45:55 *V/V*).

Test solution Dissolve 0.100 g of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 5.0 mg of *metronidazole CRS* (impurity A), 5.0 mg of *metronidazole impurity A CRS* (impurity B) and 5.0 mg of *benzoic acid CRS* (impurity C) in the solvent mixture and dilute to 50.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 10.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: spherical *di-isobutyloctadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 180 m^2/g, a pore size of 8 nm and a carbon loading of 10 per cent.

Mobile phase:
— *mobile phase A*: 1.5 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 3.2 with *phosphoric acid R*;
— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	80	20
5 - 15	80 → 55	20 → 45
15 - 40	55	45

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 235 nm.

Injection 10 µL.

Relative retention With reference to metronidazole benzoate (retention time = about 20 min): impurity B = about 0.17; impurity A = about 0.20; impurity C = about 0.7.

System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the peaks due to impurities A and B.

Limits:
— *impurities A, B, C*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.01 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 80 °C for 3 h.

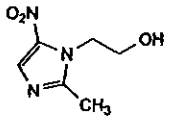
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.250 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

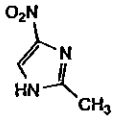
1 mL of *0.1 M perchloric acid* is equivalent to 27.53 mg of $C_{13}H_{13}N_3O_4$.

STORAGE
Protected from light.

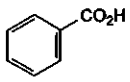
IMPURITIES
Specified impurities *A, B, C.*



A. 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethanol (metronidazole),



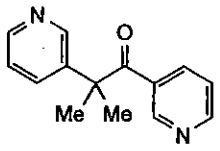
B. 2-methyl-4-nitroimidazole,



C. benzenecarboxylic acid (benzoic acid).

Ph Eur

Metyrapone



$C_{14}H_{14}N_2O$ 226.3 *54-36-4*

Action and use
11-Beta-hydroxylase inhibitor; inhibition of the formation of corticosteroids.

Preparation
Metyrapone Capsules

DEFINITION
Metyrapone is 2-methyl-1,2-di(3-pyridyl)-propan-1-one. It contains not less than 97.0% and not more than 103.0% of $C_{14}H_{14}N_2O$, calculated with reference to the dried substance.

CHARACTERISTICS
A white to light amber, crystalline powder.

Sparingly soluble in *water*; freely soluble in *ethanol (96%)*. It dissolves in dilute mineral acids.

IDENTIFICATION
A. The *infrared absorption spectrum*, Appendix II A, is concordant with *reference spectrum 1* of metyrapone *(RS 230)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.002% w/v solution in *0.1M hydrochloric acid* exhibits a maximum only at 260 nm. The *absorbance* at 260 nm is about 1.0.

C. To 5 mL of a 1% w/v solution in 1M *sulfuric acid* add 0.2 mL of *potassium tetraiodomercurate solution*. A cream precipitate is produced.

TESTS
Melting point
50° to 53°, Appendix V A.

Related substances
Carry out in subdued light the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 90 volumes of *propan-2-ol*, 5 volumes of 13.5M *ammonia* and 5 volumes of *water* as the mobile phase. Apply separately to the plate 10 µL of each of two solutions of the substance being examined in *dichloromethane* containing (1) 5.0% w/v and (2) 0.010% w/v. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Loss on drying
When dried over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 6 hours, loses not more than 0.5% of its weight. Use 1 g.

Sulfated ash
Not more than 0.1%, Appendix IX A.

ASSAY
Carry out the following procedure protected from light. Dissolve 0.1 g in sufficient *0.1M hydrochloric acid* to produce 100 mL. Dilute 5 mL to 50 mL with *0.1M hydrochloric acid* and dilute 5 mL of this solution to 50 mL with *0.1M*

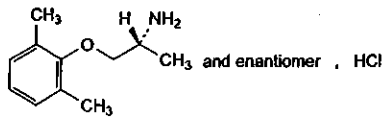
hydrochloric acid. Measure the *absorbance* of the resulting solution at the maximum at 260 nm, Appendix II B. Calculate the content of $C_{14}H_{14}N_2O$ taking 500 as the value of A(1%, 1 cm) at the maximum at 260 nm.

STORAGE

Metyrapone should be protected from light.

Mexiletine Hydrochloride

(Ph. Eur. monograph 1029)



$C_{11}H_{18}ClNO$ 215.7 *5370-01-4*

Action and use

Class I antiarrhythmic.

Preparation

Mexiletine Capsules

Ph Eur

DEFINITION

(2*RS*)-1-(2,6-Dimethylphenoxy)propan-2-amine hydrochloride.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water and in methanol, sparingly soluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *mexiletine hydrochloride CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

B. Dilute 1.5 mL of solution S (see Tests) to 15 mL with *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 20 mL with the same solvent.

Appearance of solution

The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dilute 5 mL of solution S to 10 mL with *water R*.

pH (*2.2.3*)

4.0 to 5.5 for solution S.

Impurity D Liquid chromatography (*2.2.29*)

Buffer solution To 1790 mL of *water for chromatography R* add 15 mL of *triethylamine R* and 1 mL of *glacial acetic acid R*.

Solvent mixture *acetonitrile R, water R* (20:80 *V/V*).

Test solution Dissolve 0.200 g of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (a) Dissolve 5.0 mg of *mexiletine impurity D CRS* in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 10.0 mL with the solvent mixture.

Reference solution (c) Mix 1 mL of reference solution (a) and 1 mL of the test solution and dilute to 10 mL with the solvent mixture.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped ethylene-bridged octadecylsilyl silica gel for chromatography (hybrid material) R* (5 µm);

— *temperature*: 40 °C.

Mobile phase *acetonitrile R*, buffer solution (18:82 *V/V*); adjust to pH 11.5 with *triethylamine R*.

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 262 nm.

Injection 30 µL of the test solution and reference solutions (b) and (c).

Run time Twice the retention time of mexiletine.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identifiy the peak due to impurity D.

Relative retention With reference to mexiletine (retention time = about 26 min): impurity D = about 0.9.

System suitability Reference solution (c):

— *resolution*: minimum 1.5 between the peaks due to impurity D and mexiletine.

Calculation of percentage content:

— for impurity D, use the concentration of impurity D in reference solution (b).

Limit:

— *impurity D*: maximum 0.10 per cent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.200 g of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 2.0 mg of *mexiletine impurity C CRS* in the mobile phase and transfer the solution quantitatively to a volumetric flask containing 16.0 mg of *mexiletine impurity A CRS*, then dilute to 20.0 mL with the mobile phase. Mix 1.0 mL of this solution with 2.0 mL of reference solution (a) and dilute the mixture to 100.0 mL with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 65 volumes of *methanol R2* and 35 volumes of a solution prepared as follows: dissolve 11.5 g of *anhydrous sodium acetate R* in 500 mL of *water for chromatography R*, add 3.2 mL of *glacial acetic acid R*, mix and allow to cool; adjust to pH 4.8 with *glacial acetic acid R* and dilute to 1000 mL with *water for chromatography R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 262 nm.

Injection 20 µL.

Run time 5.5 times the retention time of mexiletine.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and C.

Relative retention With reference to mexiletine (retention time = about 4 min): impurity C = about 0.7; impurity A = about 1.8.

System suitability Reference solution (b):
— *resolution*: minimum 5.0 between the peaks due to impurity C and mexiletine.

Limits:
— *impurity A*: not more than 2.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *impurity C*: not more than 20 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the peak due to mexiletine in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than 2.5 times the area of the peak due to mexiletine in the chromatogram obtained with reference solution (b) (0.5 per cent);
— *disregard limit*: 0.25 times the area of the peak due to mexiletine in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.12*)
Maximum 0.5 per cent, determined on 1.00 g.

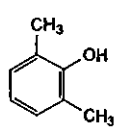
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.150 g in 50 mL of a mixture of equal volumes of *acetic anhydride R* and *anhydrous acetic acid R*. Titrate immediately with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*) and completing the titration within 2 min.

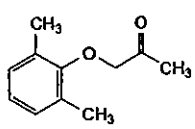
1 mL of *0.1 M perchloric acid* is equivalent to 21.57 mg of $C_{11}H_{18}ClNO$.

IMPURITIES
Specified impurities *A, C, D.*

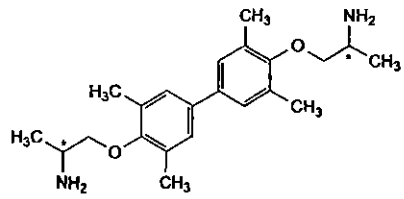
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B.*



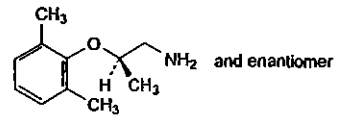
A. 2,6-dimethylphenol,



B. 1-(2,6-dimethylphenoxy)propan-2-one,



C. 1,1′-[(3,3′,5,5′-tetramethyl[1,1′-biphenyl]-4,4′-diyl)bis (oxy)]di(propan-2-amine),

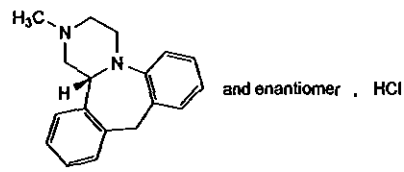


D. (2*RS*)-2-(2,6-dimethylphenoxy)propan-1-amine.

Ph Eur

Mianserin Hydrochloride

(Ph. Eur. monograph 0846)



$C_{18}H_{21}ClN_2$ 300.8 *21535-47-7*

Action and use
Monoamine reuptake inhibitor; tetracyclic antidepressant.

Preparation
Mianserin Tablets

Ph Eur

DEFINITION
(14b*RS*)-2-Methyl-1,2,3,4,10,14b-hexahydrodibenzo[*c*,*f*] pyrazino[1,2-*a*]azepine hydrochloride.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder or crystals.

Solubility
Sparingly soluble in water and in methylene chloride, slightly soluble in ethanol (96 per cent).

IDENTIFICATION
First identification: B, D.

Second identification: A, C, D.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 50.0 mg in *water R* and dilute to 50.0 mL with the same solvent. Dilute 5.0 mL of the solution to 50.0 mL with *water R*.

Spectral range 230-350 nm.

Absorption maximum At 279 nm.

Specific absorbance at the absorption maximum 64 to 72.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *mianserin hydrochloride CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *methylene chloride R* and dilute to 5 mL with the same solvent.

Reference solution (a) Dissolve 10 mg of *mianserin hydrochloride CRS* in *methylene chloride R* and dilute to 5 mL with the same solvent.

Reference solution (b) Dissolve 10 mg of *mianserin hydrochloride CRS* and 10 mg of *cyproheptadine hydrochloride CRS* in *methylene chloride R* and dilute to 5 mL with the same solvent.

Plate *TLC silica gel GF_{254} plate R.*

Mobile phase *diethylamine R, ether R, cyclohexane R* (5:20:75 *V/V/V*).

Application 2 µL.

Development Over 2/3 of the plate.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):
- the chromatogram shows 2 clearly separated principal spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

pH (*2.2.3*)
4.0 to 5.5.

Dissolve 0.10 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Buffer solution pH 3.0 Dissolve 5.0 g of *sodium octanesulfonate R* in *water R* and dilute to 350 mL with the same solvent. Stir until complete dissolution. Adjust to pH 3.0 with a mixture of 1 volume of *phosphoric acid R* and 3 volumes of *water R*. Dilute to 400 mL with *water R*.

Test solution Dissolve 25 mg of the substance to be examined in the mobile phase and dilute to 25.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve the contents of a vial of *mianserin for system suitability CRS* (containing impurities A, D and E) in 1.0 mL of the mobile phase.

Reference solution (c) Dissolve 5.0 mg of *mianserin impurity B CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 100.0 mL with the mobile phase.

Column:
- *size*: l = 0.15 m, Ø = 3.9 mm;
- *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Buffer solution pH 3.0, *methanol R* (37:63 *V/V*).

Flow rate 0.5 mL/min.

Detection Spectrophotometer at 250 nm.

Injection 10 µL.

Run time Twice the retention time of mianserin.

Identification of impurities Use the chromatogram supplied with *mianserin for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, D and E.

Relative retention With reference to mianserin (retention time = about 18 min): impurity B = about 0.2; impurity A = about 0.5; impurity D = about 0.7; impurity E = about 1.1.

System suitability Reference solution (b):
- *peak-to-valley ratio*: minimum 4.0, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to mianserin.

Limits:
- *correction factor*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 2.4; impurity D = 2.1;
- *impurity B*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent);
- *impurities A, D, E*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 65 °C at a pressure not exceeding 0.7 kPa for 3 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in a mixture of 5.0 mL of *0.01 M hydrochloric acid* and 50 mL of *ethanol (96 per cent) R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 30.08 mg of $C_{18}H_{21}ClN_2$.

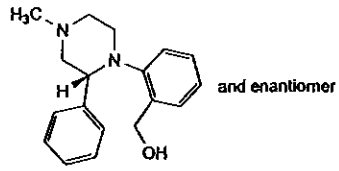
STORAGE

Protected from light.

IMPURITIES

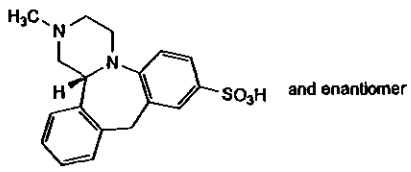
Specified impurities *A, B, D, E.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C, F.*



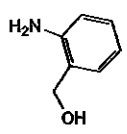
and enantiomer

A. [2-[(2*RS*)-4-methyl-2-phenylpiperazin-1-yl] phenyl]methanol,

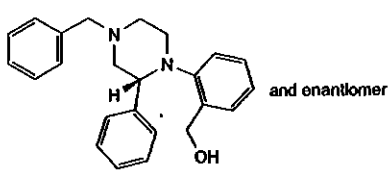


and enantiomer

B. (14b*RS*)-2-methyl-1,2,3,4,10,14b-hexahydrodibenzo[*c*,*f*] pyrazino[1,2-*a*]azepine-8-sulfonic acid,

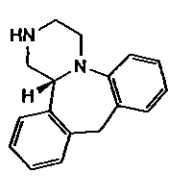


C. (2-aminophenyl)methanol,



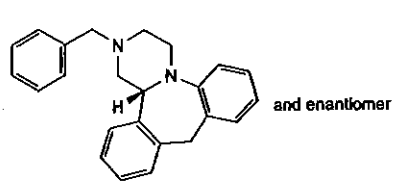
and enantiomer

D. [2-[(2*RS*)-4-benzyl-2-phenylpiperazin-1-yl]phenyl] methanol,



and enantiomer

E. (14b*RS*)-1,2,3,4,10,14b-hexahydrodibenzo[*c*,*f*]pyrazino [1,2-*a*]azepine,



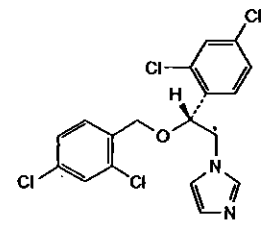
and enantiomer

F. (14b*RS*)-2-benzyl-1,2,3,4,10,14b-hexahydrodibenzo[*c*,*f*] pyrazino[1,2-*a*]azepine.

Ph Eur

Miconazole

(Ph. Eur. monograph 0935)



and enantiomer

$C_{18}H_{14}Cl_4N_2O$ 416.1 *22916-47-8*

Action and use
Antifungal azole.

Preparations
Miconazole Oromucosal Gel
Miconazole Eye Drops

Ph Eur

DEFINITION

1-[(2*RS*)-2-(2,4-Dichlorophenyl)-2-[[(2,4-dichlorophenyl) methyl]oxy]ethyl]-1*H*-imidazole.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Very slightly soluble in water, freely soluble in methanol, soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification: A, B.

Second identification: C, D.

A. Melting point (*2.2.14*): 83 °C to 87 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison miconazole CRS.

C. Melting point (*2.2.14*).

Determination A Determine the melting point of the substance to be examined.

Result A 83 °C to 87 °C.

Determination B Mix equal parts of the substance to be examined and *miconazole CRS*, and determine the melting point of the mixture.

Result B The absolute difference between the melting point of the mixture and the value obtained in determination A is not greater than 2 °C.

D. To 30 mg in a porcelain crucible add 0.3 g of *anhydrous sodium carbonate R*. Heat over an open flame for 10 min. Allow to cool. Take up the residue with 5 mL of *dilute nitric acid R* and filter. To 1 mL of the filtrate add 1 mL of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S
Dissolve 0.1 g in *methanol R* and dilute to 10 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Optical rotation (*2.2.7*)
−0.10° to + 0.10°, determined on solution S.

Related substances
Liquid chromatography (*2.2.29*).

Test solution To 0.100 g of the substance to be examined add 3.0 mL of *acetonitrile R* and 3.2 mL of *methanol R* and shake until complete dissolution. Further dilute to 10.0 mL with a 15.8 g/L solution of *ammonium acetate R*.

Reference solution (a) Dissolve 2.5 mg of *miconazole CRS* and 2.5 mg of *econazole nitrate CRS* in the mobile phase and dilute to 100 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 20.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.10 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase Dissolve 6.0 g of *ammonium acetate R* in a mixture of 300 mL of *acetonitrile for chromatography R*, 320 mL of *methanol R1* and 380 mL of *water for chromatography R*.

Flow rate 2 mL/min.

Detection Spectrophotometer at 235 nm.

Equilibration With the mobile phase for about 30 min.

Injection 10 µL.

Run time 1.2 times the retention time of miconazole.

Retention time Econazole = about 10 min; miconazole = about 20 min.

System suitability Reference solution (a):
— *resolution*: minimum 10 between the peaks due to econazole and miconazole; if necessary, adjust the composition of the mobile phase.

Limits:
— *impurities A, B, C, D, E, F, G*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 4 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

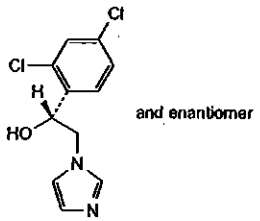
ASSAY
Dissolve 0.300 g in 50 mL of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Using 0.2 mL of *naphtholbenzein solution R* as indicator, titrate with *0.1 M perchloric acid* until the colour changes from orange-yellow to green.

1 mL of *0.1 M perchloric acid* is equivalent to 41.61 mg of $C_{18}H_{14}Cl_4N_2O$.

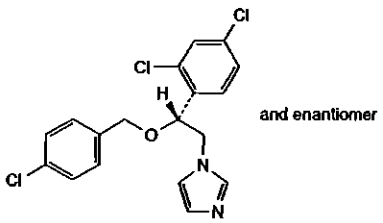
STORAGE
Protected from light.

IMPURITIES
Specified impurities *A, B, C, D, E, F, G.*

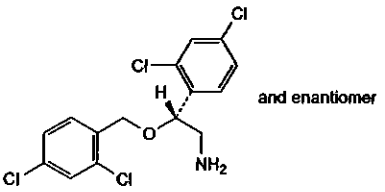
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *H, I.*



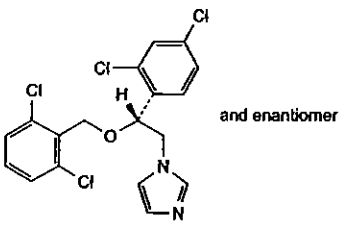
A. (1*RS*)-1-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-yl)ethan-1-ol,



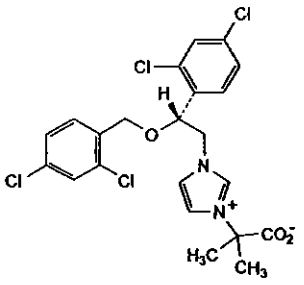
B. 1-[(2*RS*)-2-[[(4-chlorophenyl)methyl]oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole (econazole),



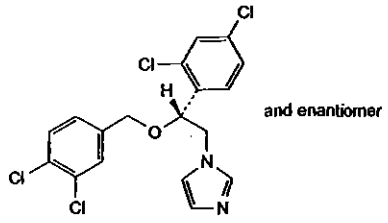
C. (2*RS*)-2-(2,4-dichlorophenyl)-2-[[(2,4-dichlorophenyl) methyl]oxy]ethan-1-amine,



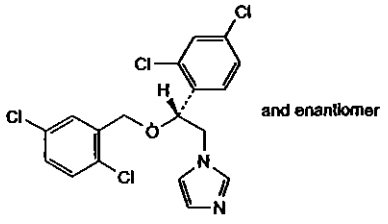
D. 1-[(2*RS*)-2-(2,4-dichlorophenyl)-2-[[(2,6-dichlorophenyl) methyl]oxy]ethyl]-1*H*-imidazole (isoconazole),



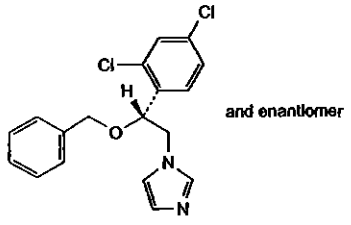
E. 2-[1-[(2*RS*)-2-(2,4-dichlorophenyl)-2-[[(2,4-dichlorophenyl)methyl]oxy]ethyl]-1*H*-imidazol-3-ium-3-yl]-2-methylpropanoate,



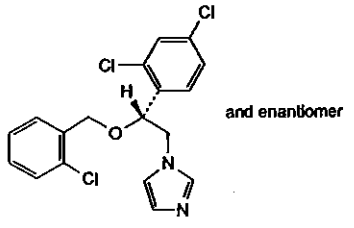
F. 1-[(2*RS*)-2-(2,4-dichlorophenyl)-2-[[(3,4-dichlorophenyl)methyl]oxy]ethyl]-1*H*-imidazole,



G. 1-[(2*RS*)-2-(2,4-dichlorophenyl)-2-[[(2,5-dichlorophenyl)methyl]oxy]ethyl]-1*H*-imidazole,



H. 1-[(2*RS*)-2-(benzyloxy)-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole,

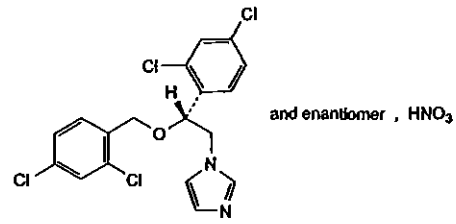


I. 1-[(2*RS*)-2-[[(2-chlorophenyl)methyl]oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole.

Ph Eur

Miconazole Nitrate

(Ph. Eur. monograph 0513)



$C_{18}H_{15}Cl_4N_3O_4$ 479.1 *22832-87-7*

Action and use
Antifungal azole.

Preparations
Miconazole Cream
Miconazole and Hydrocortisone Cream
Miconazole and Hydrocortisone Acetate Cream
Miconazole and Hydrocortisone Ointment

Ph Eur

DEFINITION

1-[(2*RS*)-2-(2,4-Dichlorophenyl)-2-[[(2,4-dichlorophenyl)methyl]oxy]ethyl]-1*H*-imidazole nitrate.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Very slightly soluble in water, sparingly soluble in methanol, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: A, B.

Second identification: C, D.

A. Melting point (*2.2.14*): 178 °C to 184 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *miconazole nitrate CRS.*

C. Melting point (*2.2.14*).

Determination A Determine the melting point of the substance to be examined.

Result A 178 °C to 184 °C.

Determination B Mix equal parts of the substance to be examined and *miconazole nitrate CRS*, and determine the melting point of the mixture.

Result B The absolute difference between the melting point of the mixture and the value obtained in determination A is not greater than 2 °C.

D. It gives the reaction of nitrates (*2.3.1*).

TESTS

Solution S
Dissolve 0.1 g in *methanol R* and dilute to 10 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

Optical rotation (*2.2.7*)
−0.10° to + 0.10°, determined on solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dissolve 2.5 mg of *miconazole nitrate CRS* and 2.5 mg of *econazole nitrate CRS* (impurity B) in the mobile phase and dilute to 100 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 20.0 mL with the mobile phase.

Column:
- *size*: *l* = 0.10 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase Dissolve 6.0 g of *ammonium acetate R* in a mixture of 300 mL of *acetonitrile for chromatography R*, 320 mL of *methanol R1* and 380 mL of *water for chromatography R*.

Flow rate 2 mL/min.

Detection Spectrophotometer at 235 nm.

Injection 10 µL.

Run time 1.2 times the retention time of miconazole.

Relative retention With reference to miconazole (retention time = about 20 min): impurity A = about 0.1; impurity E = about 0.3; impurity C = about 0.4; impurity B = about 0.6; impurity D = about 0.75; impurity F = about 0.85; impurity G = about 0.9.

System suitability Reference solution (a):
- *resolution*: minimum 10 between the peaks due to impurity B and miconazole.

Limits:
- *impurities A, B, C, D, E, F, G*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);
- *unspecified impurities*: for each impurity, not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak due to the nitrate ion.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.350 g in 75 mL of *anhydrous acetic acid R*, with slight heating if necessary. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 mL of *0.1 M perchloric acid* is equivalent to 47.91 mg of $C_{18}H_{15}Cl_4N_3O_4$.

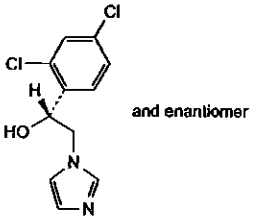
STORAGE

Protected from light.

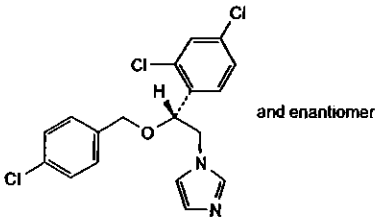
IMPURITIES

Specified impurities *A, B, C, D, E, F, G.*

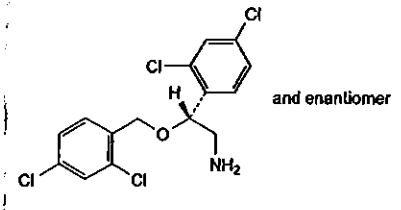
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *H, I.*



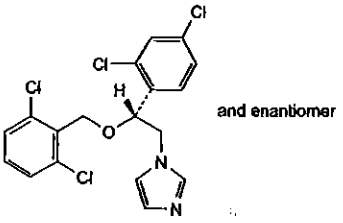
A. (1*RS*)-1-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-yl)ethan-1-ol,



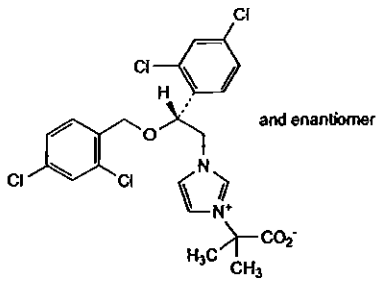
B. 1-[(2*RS*)-2-[[(4-chlorophenyl)methyl]oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole (econazole),



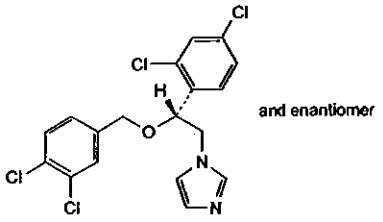
C. (2*RS*)-2-(2,4-dichlorophenyl)-2-[[(2,4-dichlorophenyl)methyl]oxy]ethan-1-amine,



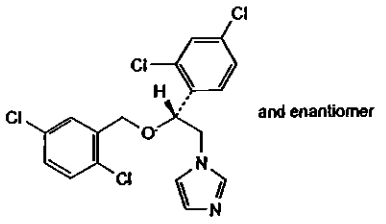
D. 1-[(2*RS*)-2-(2,4-dichlorophenyl)-2-[[(2,6-dichlorophenyl)methyl]oxy]ethyl]-1*H*-imidazole (isoconazole),



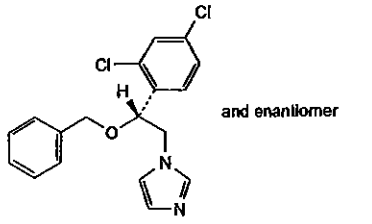
E. 2-[1-[(2*RS*)-2-(2,4-dichlorophenyl)-2-[[(2,4-dichlorophenyl)methyl]oxy]ethyl]-1*H*-imidazol-3-ium-3-yl]-2-methylpropanoate,



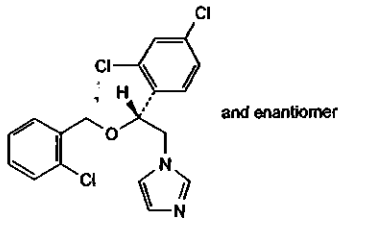
F. 1-[(2*RS*)-2-(2,4-dichlorophenyl)-2-[[(3,4-dichlorophenyl)methyl]oxy]ethyl]-1*H*-imidazole,



G. 1-[(2*RS*)-2-(2,4-dichlorophenyl)-2-[[(2,5-dichlorophenyl)methyl]oxy]ethyl]-1*H*-imidazole,



H. 1-[(2*RS*)-2-(benzyloxy)-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole,

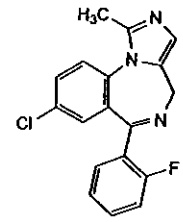


I. 1-[(2*RS*)-2-[[(2-chlorophenyl)methyl]oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole.

Ph Eur

Midazolam

(Ph. Eur. monograph 0936)



$C_{18}H_{13}ClFN_3$ 325.8 *59467-70-8*

Action and use
Benzodiazepine.

Preparations
Midazolam Injection
Midazolam Oral Solution
Midazolam Oromucosal Solution

Ph Eur

DEFINITION

8-Chloro-6-(2-fluorophenyl)-1-methyl-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine.

Content
98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance
White or yellowish, crystalline powder.

Solubility
Practically insoluble in water, freely soluble in acetone and in ethanol (96 per cent), soluble in methanol.

IDENTIFICATION

First identification: B.

Second identification: A, C, D, E.

A. Melting point (*2.2.14*): 161 °C to 164 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison midazolam CRS.

C. Examine the chromatograms obtained in the test for impurity C.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (b).

D. Mix 90 mg with 0.30 g of *anhydrous sodium carbonate R* and ignite in a crucible until an almost white residue is obtained (normally in less than 5 min). Allow to cool and dissolve the residue in 5 mL of *dilute nitric acid R*. Filter (the filtrate is also used in identification test E). Add 1.0 mL of the filtrate to a freshly prepared mixture of 0.1 mL of *alizarin S solution R* and 0.1 mL of *zirconyl nitrate solution R*. Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank prepared in the same manner. The test solution is yellow and the blank solution is red.

E. To 1 mL of the filtrate obtained in identification test D add 1 mL of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Dissolve 0.1 g in *0.1 M hydrochloric acid* and dilute to 10 mL with the same acid.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in *methanol R* and dilute to 50.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *methanol R*. Dilute 1.0 mL of this solution to 10.0 mL with *methanol R*.

Reference solution (b) Dissolve the contents of a vial of *midazolam for system suitability CRS* (containing impurities A, B, E, G and H) in 1.0 mL of *methanol R*.

Column:
- *size*: *l* = 0.25 m, Ø = 4.0 mm;
- *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Prepare a solution containing 7.7 g/L of *ammonium acetate R* and 10 mL/L of *tetrabutylammonium hydroxide solution (400 g/L) R* and adjust to pH 5.3 with *glacial acetic acid R*. Mix 44 volumes of this solution with 56 volumes of *methanol R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µL.

Run time 2.5 times the retention time of midazolam.

Identification of impurities Use the chromatogram supplied with *midazolam for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, E, G and H.

Relative retention With reference to midazolam (retention time = about 17 min): impurity E = about 0.5; impurity A = about 0.9; impurity G = about 1.2; impurity H = about 1.9; impurity B = about 2.2.

System suitability:
- *signal-to-noise ratio*: minimum 40 for the principal peak in the chromatogram obtained with reference solution (a);
- *peak-to-valley ratio*: minimum 3.0, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to midazolam in the chromatogram obtained with reference solution (b).

Limits:
- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 2.0; impurity E = 2.0; impurity H = 1.7;
- *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *impurities A, E, G, H*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Impurity C

Thin-layer chromatography (*2.2.27*).

Test solution (a) Dissolve 0.20 g of the substance to be examined in *ethanol (96 per cent) R* and dilute to 5 mL with the same solvent.

Test solution (b) Dilute 1 mL of test solution (a) to 50 mL with *ethanol (96 per cent) R*.

Reference solution (a) Dissolve the contents of a vial of *midazolam impurity C CRS* in 1.0 mL of *methanol R*. Dilute 0.5 mL of the solution to 1.0 mL with *methanol R*.

Reference solution (b) Dissolve 8 mg of *midazolam CRS* in *ethanol (96 per cent) R* and dilute to 10 mL with the same solvent.

Reference solution (c) Dissolve 40 mg of the substance to be examined in 1 mL of reference solution (a).

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *glacial acetic acid R, water R, methanol R, ethyl acetate R (2:15:20:80 V/V/V/V).*

Application 5 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (c):
- the chromatogram shows 2 clearly separated spots.

Limit:
- *impurity C*: any spot due to impurity C in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.1 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

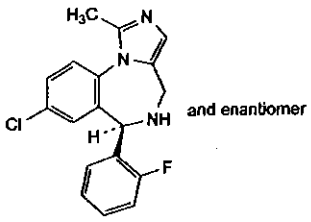
Dissolve 0.120 g in 30 mL of *anhydrous acetic acid R* and add 20 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid* to the 2nd point of inflexion, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 16.29 mg of $C_{18}H_{13}ClFN_3$.

IMPURITIES

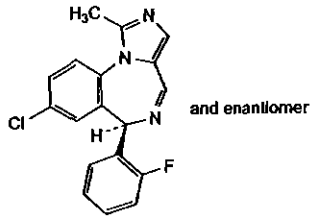
Specified impurities *A, B, C, E, G, H.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *D, F, I, J.*



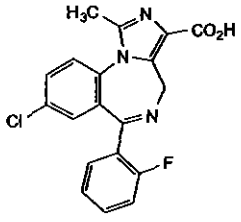
and enantiomer

A. (6*RS*)-8-chloro-6-(2-fluorophenyl)-1-methyl-5,6-dihydro-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine,

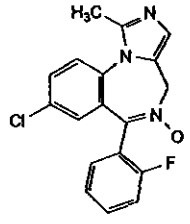


and enantiomer

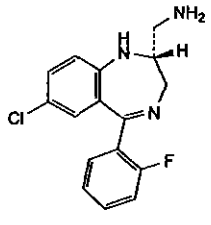
B. (6*RS*)-8-chloro-6-(2-fluorophenyl)-1-methyl-6*H*-imidazo[1,5-*a*][1,4]benzodiazepine,



C. 8-chloro-6-(2-fluorophenyl)-1-methyl-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylic acid,

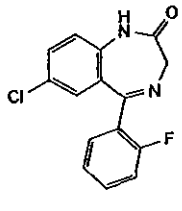


D. 8-chloro-6-(2-fluorophenyl)-1-methyl-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine 5-oxide,

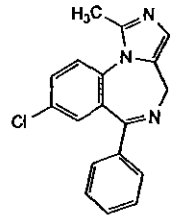


and enantiomer

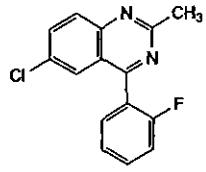
E. [(2*RS*)-7-chloro-5-(2-fluorophenyl)-2,3-dihydro-1*H*-1,4-benzodiazepin-2-yl]methanamine,



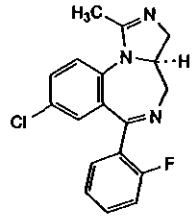
F. 7-chloro-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (1-des[(diethylamino)ethyl] flurazepam),



G. 8-chloro-1-methyl-6-phenyl-4*H*-imidazo[1,5-*a*][1,4] benzodiazepine (desfluoromidazolam),

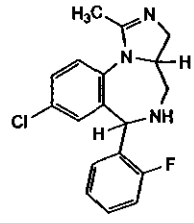


H. 6-chloro-4-(2-fluorophenyl)-2-methylquinazoline,



and enantiomer

I. (3a*RS*)-8-chloro-6-(2-fluorophenyl)-1-methyl-3a,4-dihydro-3*H*-imidazo[1,5-*a*][1,4]benzodiazepine,



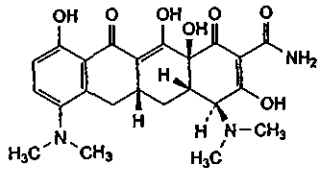
J. 8-chloro-6-(2-fluorophenyl)-1-methyl-3a,4,5,6-tetrahydro-3*H*-imidazo[1,5-*a*][1,4]benzodiazepine.

Ph Eur

Minocycline Hydrochloride Dihydrate

Minocycline Hydrochloride

(Ph. Eur. monograph 1030)



$C_{23}H_{28}ClN_3O_7,2H_2O$ 530.0 *128420-71-3*

Action and use
Tetracycline antibacterial.

Preparations
Minocycline Capsules
Minocycline Prolonged-release Capsules
Minocycline Tablets

Ph Eur

DEFINITION

(4*S*,4a*S*,5a*R*,12a*S*)-4,7-Bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide hydrochloride dihydrate.

Semi-synthetic product derived from a fermentation product.

Content

94.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Yellow, hygroscopic, crystalline powder.

Solubility

Sparingly soluble in water, slightly soluble in ethanol (96 per cent). It dissolves in solutions of alkali hydroxides and carbonates.

IDENTIFICATION

First identification: A, C.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *minocycline hydrochloride CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 5 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dissolve 5 mg of *minocycline hydrochloride CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 5 mg of *minocycline hydrochloride CRS* and 5 mg of *oxytetracycline hydrochloride CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Plate *TLC octadecylsilyl silica gel F_{254} plate R.*

Mobile phase Mix 20 volumes of *acetonitrile R*, 20 volumes of *methanol R* and 60 volumes of a 63 g/L solution of *oxalic acid R* previously adjusted to pH 2 with *concentrated ammonia R.*

Application 1 µL.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

C. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 0.200 g in *carbon dioxide-free water R* and dilute to 20.0 mL with the same solvent.

Appearance of solution

The solution is clear (*2.2.1*) and its absorbance (*2.2.25*) at 450 nm using a 1 cm cell is not greater than 0.23.

Dilute 1.0 mL of solution S to 10.0 mL with *water R.*

pH (*2.2.3*)

3.5 to 4.5 for solution S.

Light-absorbing impurities

Carry out the measurement within 1 h of preparing solution S.

The absorbance (*2.2.25*) of solution S measured at 560 nm is not greater than 0.06.

Related substances

Liquid chromatography (*2.2.29*). *Carry out the test protected from light. Store the solutions at 2-8 °C and use them within 3 h of preparation.*

Solution A Mix 18 volumes of a 3.75 g/L solution of *sodium edetate R* and 60 volumes of a 28.3 g/L solution of *ammonium oxalate R* and adjust to pH 7.2 with *dilute ammonia R2.*

Test solution Dissolve 24.0 mg of the substance to be examined in *water R* and dilute to 100.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *water R.*

Reference solution (b) Dissolve 2 mg of *minocycline for system suitability CRS* (containing impurities A, B, C, E, F, G and H) in *water R* and dilute to 5 mL with the same solvent.

Column:

— *size:* l = 0.25 m, Ø = 4.6 mm;

— *stationary phase: base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature:* 40 °C.

Mobile phase *tetrahydrofuran R, dimethylformamide R,* solution A (8:12:78 *V/V/V*).

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µL of the test solution and reference solutions (a) and (b).

Run time 3 times the retention time of minocycline.

Identification of impurities Use the chromatogram supplied with *minocycline for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C, E, F, G and H.

Relative retention With reference to minocycline (retention time = about 16 min): impurity C = about 0.52; impurity H = about 0.55; impurity B = about 0.66; impurity A = about 0.74; impurity G = about 0.79; impurity F = about 0.92; impurity E = about 2.6.

System suitability Reference solution (b):

— *resolution:* minimum 1.5 between the peaks due to impurities C and H; minimum 1.5 between the peaks due to impurities A and G; minimum 1.5 between the peaks due to impurity F and minocycline.

Calculation of percentage contents:

— *correction factors:* multiply the peak areas of the following impurities by the corresponding correction factor: impurity E = 1.6; impurity F = 1.6; impurity G = 1.4;

— for each impurity, use the concentration of minocycline hydrochloride dihydrate in reference solution (a).

Limits:

— *impurity A:* maximum 1.2 per cent;

— *impurity B:* maximum 0.8 per cent;

— *impurities C, E:* for each impurity, maximum 0.6 per cent;

— *impurities F, G:* for each impurity, maximum 0.5 per cent;

— *impurity H:* maximum 0.3 per cent;

— *any other impurity:* for each impurity, maximum 0.15 per cent;

— *total:* maximum 3.5 per cent;

— *reporting threshold:* 0.05 per cent.

Water (*2.5.12*)

5.0 per cent to 8.0 per cent, determined on 0.200 g.

Sulfated ash (*2.4.14*)

Maximum 0.5 per cent, determined on 1.0 g.

Bacterial endotoxins (*2.6.14*)
Less than 1.25 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Test solution Dissolve 30.0 mg of the substance to be examined in *water R* and dilute to 50.0 mL with the same solvent.

Reference solution Dissolve 30.0 mg of *minocycline hydrochloride CRS* in *water R* and dilute to 50.0 mL with the same solvent.

Injection Test solution and reference solution.

Calculate the percentage content of $C_{23}H_{28}ClN_3O_7$ taking into account the assigned content of *minocycline hydrochloride CRS*.

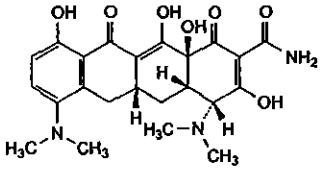
STORAGE

In an airtight container, protected from light. If the substance is sterile, the container is also sterile and tamper-evident.

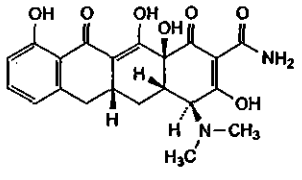
IMPURITIES

Specified impurities *A, B, C, E, F, G, H.*

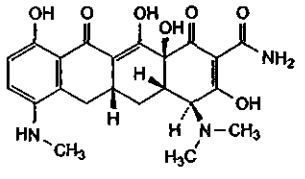
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *D, I.*



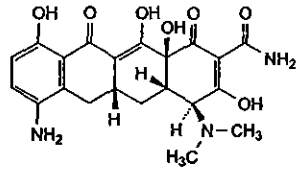
A. (4*R*,4a*S*,5a*R*,12a*S*)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epiminocycline),



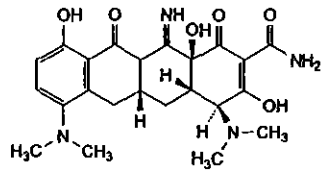
B. (4*S*,4a*S*,5a*R*,12a*S*)-4-(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (sancycline),



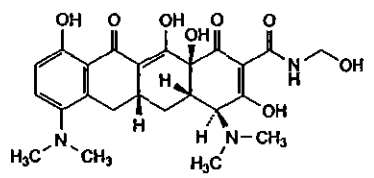
C. (4*S*,4a*S*,5a*R*,12a*S*)-4-(dimethylamino)-3,10,12,12a-tetrahydroxy-7-(methylamino)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (7-monodemethylminocycline),



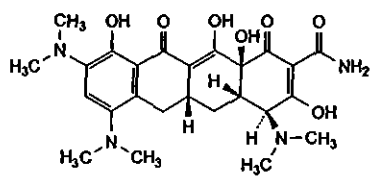
D. (4*S*,4a*S*,5a*R*,12a*S*)-7-amino-4-(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (7-aminosancycline),



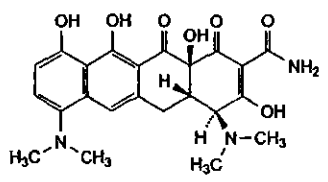
E. (4*S*,4a*S*,5a*R*,12a*S*)-4,7-bis(dimethylamino)-3,10,12a-trihydroxy-12-imino-1,11-dioxo-1,4,4a,5,5a,6,11,11a,12,12a-decahydrotetracene-2-carboxamide,



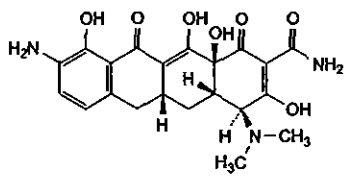
F. (4*S*,4a*S*,5a*R*,12a*S*)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-*N*-(hydroxymethyl)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide,



G. (4*S*,4a*S*,5a*R*,12a*S*)-4,7,9-tris(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide,



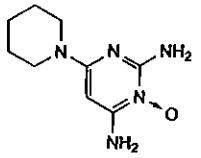
H. (4*S*,4a*S*,12a*S*)-4,7-bis(dimethylamino)-3,10,11,12a-tetrahydroxy-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide,



I. (4*S*,4a*S*,5a*R*,12a*S*)-9-amino-4-(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (9-aminosancycline).

Minoxidil

(Ph. Eur. monograph 0937)



$C_9H_{15}N_5O$ 209.3 *38304-91-5*

Action and use
Vasodilator; treatment of hypertension; male pattern baldness.

Preparation
Minoxidil Scalp Application

Ph Eur

DEFINITION
6-(Piperidin-1-yl)pyrimidine-2,4-diamine 3-oxide.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Slightly soluble in water, soluble in methanol and in propylene glycol.

IDENTIFICATION
First identification: B.

Second identification: A, C, D.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution (a) Dissolve 20.0 mg in *0.1 M hydrochloric acid* and dilute to 100.0 mL with the same acid (solution A). Dilute 2.0 mL of solution A to 100.0 mL with *0.1 M hydrochloric acid.*

Test solution (b) Dilute 2.0 mL of solution A to 100.0 mL with *0.1 M sodium hydroxide.*

Spectral range 200-350 nm.

Absorption maxima At 230 nm and 281 nm for test solution (a); at 230 nm, 262 nm and 288 nm for test solution (b).

Specific absorbances at the absorption maxima:
- — at 230 nm: 1015 to 1120 for test solution (a); 1525 to 1685 for test solution (b);
- — at 262 nm: 485 to 535 for test solution (b);
- — at 281 nm: 1060 to 1170 for test solution (a);
- — at 288 nm: 555 to 605 for test solution (b).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *minoxidil CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 10 mg of *minoxidil CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *concentrated ammonia R, methanol R* (1.5:100 *V/V*).

Application 2 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve about 10 mg in 1 mL of *methanol R.* Add 0.1 mL of *copper sulfate solution R.* A green colour develops. The solution becomes greenish-yellow on the addition of 0.1 mL of *dilute hydrochloric acid R.*

TESTS
Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 12.5 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 5 mg of *minoxidil for system suitability CRS* (containing impurities A, B and E) in the mobile phase and dilute to 20.0 mL with the mobile phase.

Column:
- — *size: l* = 0.10 m, Ø = 4.6 mm;
- — *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (2.6 µm);
- — *temperature:* 40 °C.

Mobile phase Solution containing 0.1 per cent *V/V* of *trifluoroacetic acid R* and 2 g/L of *sodium heptanesulfonate R* in a mixture of 45 volumes of *methanol R* and 55 volumes of *water for chromatography R.*

Flow rate 0.8 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 10 µL.

Run time Twice the retention time of minoxidil.

Identification of impurities Use the chromatogram supplied with *minoxidil for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B and E.

Relative retention With reference to minoxidil (retention time = about 5 min): impurity A = about 0.3; impurity B = about 0.4; impurity E = about 1.2.

System suitability Reference solution (b):
- — *resolution:* minimum 1.5 between the peaks due to impurities A and B; minimum 2.0 between the peaks due to minoxidil and impurity E.

Calculation of percentage contents:
- — for impurity B, multiply the peak area by the correction factor 1.6;
- — for each impurity, use the concentration of minoxidil in reference solution (a).

Limits:
- — *impurity E:* maximum 0.2 per cent;
- — *impurity B:* maximum 0.15 per cent;
- — *unspecified impurities:* for each impurity, maximum 0.10 per cent;
- — *total:* maximum 0.3 per cent;
- — *reporting threshold:* 0.05 per cent.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

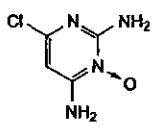
ASSAY
Dissolve 0.150 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 mL of *0.1 M perchloric acid* is equivalent to 20.93 mg of $C_9H_{15}N_5O$.

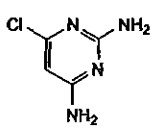
STORAGE
Protected from light.

IMPURITIES
Specified impurities *B, E.*

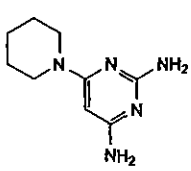
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A.*



A. 6-chloropyrimidine-2,4-diamine 3-oxide,



B. 6-chloropyrimidine-2,4-diamine,

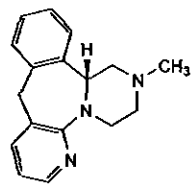


E. 6-(piperidin-1-yl)pyrimidine-2,4-diamine (deoxyminoxidil).

Ph Eur

Mirtazapine

(Ph. Eur. monograph 2338)



and enantiomer

$C_{17}H_{19}N_3$ 265.4 *85650-52-8*

Action and use
Inhibitor of 5HT and noradrenaline reuptake; antidepressant.

Preparations
Mirtazapine Tablets
Mirtazapine Oral Solution
Mirtazapine Orodispersible Tablets

Ph Eur

DEFINITION
(14b*RS*)-2-Methyl-1,2,3,4,10,14b-hexahydropyrazino[2,1-*a*]pyrido[2,3-*c*][2]benzazepine.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white powder, slightly hygroscopic to hygroscopic.

Solubility
Practically insoluble in water, freely soluble in anhydrous ethanol.

It shows polymorphism (*5.9*).

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *mirtazapine CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *anhydrous ethanol R*, evaporate to dryness and record new spectra using the residues.

TESTS
Optical rotation (*2.2.7*)
−0.10° to + 0.10° (anhydrous substance).

Dissolve 0.250 g in *anhydrous ethanol R* and dilute to 25.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R, water R* (50:50 *V/V*).

Buffer solution Dissolve 18.0 g of *tetramethylammonium hydroxide R* in 950 mL of *water R*. While stirring, adjust to pH 7.4 with *phosphoric acid R*, then dilute to 1000 mL with *water R* and mix.

Test solution Dissolve 30 mg of the substance to be examined in the solvent mixture and dilute to 20 mL with the solvent mixture.

Reference solution (a) Dissolve 3 mg of *mirtazapine for system suitability CRS* (containing impurities A, B, C, D, E and F) in 2 mL of the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase *tetrahydrofuran for chromatography R, methanol R, acetonitrile R,* buffer solution (7.5:12.5:15:65 *V/V/V/V*).

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 240 nm.

Injection 10 µL.

Run time Twice the retention time of mirtazapine.

Identification of impurities Use the chromatogram supplied with *mirtazapine for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D, E and F.

Relative retention With reference to mirtazapine (retention time = about 25 min): impurity A = about 0.2; impurity B = about 0.3; impurity C = about 0.35; impurity D = about 0.4; impurity E = about 1.3; impurity F = about 1.35.

System suitability:
- *resolution*: minimum 1.5 between the peaks due to impurities E and F in the chromatogram obtained with reference solution (a);
- *symmetry factor*: 0.8 to 2.0 for the principal peak in the chromatogram obtained with reference solution (b).

Limits:
- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.3; impurity B = 1.3; impurity F = 0.2;
- *impurities A, B, C, D, E, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.12*)
Maximum 3.5 per cent, determined on 1.00 g.

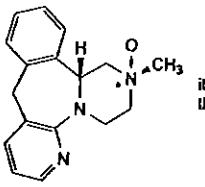
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.100 g in 35 mL of *glacial acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

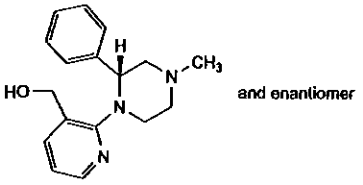
1 mL of *0.1 M perchloric acid* is equivalent to 13.27 mg of $C_{17}H_{19}N_3$.

STORAGE
In an airtight container.

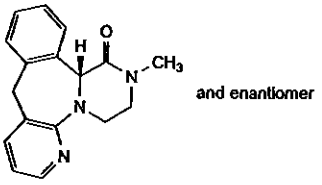
IMPURITIES
Specified impurities *A, B, C, D, E, F.*



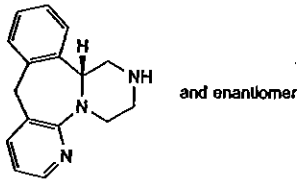
A. (14b*RS*)-2-methyl-1,2,3,4,10,14b-hexahydropyrazino[2,1-*a*]pyrido[2,3-*c*][2]benzazepine 2-oxide,



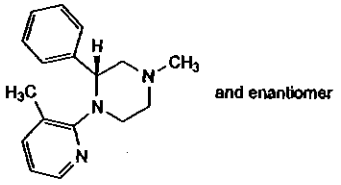
B. [2-[(2*RS*)-4-methyl-2-phenylpiperazin-1-yl]pyridin-3-yl] methanol,



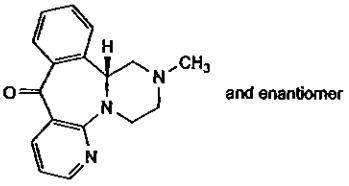
C. (14b*RS*)-2-methyl-3,4,10,14b-tetrahydropyrazino[2,1-*a*] pyrido[2,3-*c*][2]benzazepin-1(2*H*)-one,



D. (14b*RS*)-1,2,3,4,10,14b-hexahydropyrazino[2,1-*a*]pyrido [2,3-*c*][2]benzazepine,



E. (2*RS*)-4-methyl-1-(3-methylpyridin-2-yl)-2-phenylpiperazine,

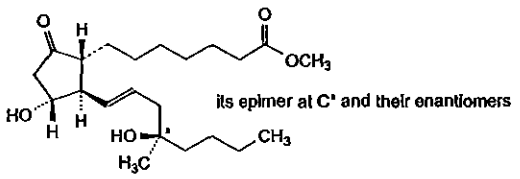


F. (14b*RS*)-2-methyl-1,3,4,14b-tetrahydropyrazino[2,1-*a*] pyrido[2,3-*c*][2]benzazepin-10(2*H*)-one.

Ph Eur

Misoprostol

(Ph. Eur. monograph 1731)



$C_{22}H_{38}O_5$ 382.5 *59122-46-2*

Action and use
Prostaglandin (PGE1) analogue.

Ph Eur

DEFINITION

Mixture of methyl 7-[(1*RS*,2*RS*,3*RS*)-3-hydroxy-2-[(1*E*,4*RS*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopentyl] heptanoate and methyl 7-[(1*RS*,2*RS*,3*RS*)-3-hydroxy-2-[(1*E*,4*SR*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopentyl] heptanoate.

The 4 stereoisomers are present in approximately equal proportions.

Content
96.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
Clear, colourless or yellowish, oily liquid, hygroscopic.

Solubility
Practically insoluble in water, soluble in ethanol (96 per cent), sparingly soluble in acetonitrile.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *misoprostol CRS.*

TESTS

Related substances
Liquid chromatography (*2.2.29*). *Use freshly prepared solutions.*

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 5.0 mL with the mobile phase.

Reference solution (a) Dissolve 25.0 mg of *misoprostol CRS* in the mobile phase and dilute to 5.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (c) Dissolve 5 mg of *misoprostol for system suitability CRS* (containing impurities A, B and C) in 1 mL of the mobile phase.

Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: silica gel for chromatography R* (5 µm).

Mobile phase Mix 5 volumes of *acetonitrile R1*, 215 volumes of *dioxan R*, 780 volumes of *heptane R* and sonicate for 10 min.

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µL of the test solution and reference solutions (b) and (c).

Run time 1.5 times the retention time of misoprostol.

Identification of impurities Use the chromatogram supplied with *misoprostol for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B and C.

Relative retention With reference to misoprostol (retention time = about 18 min): impurity C = about 0.2; impurity A = about 0.7; impurity B (1st peak) = about 0.85; impurity B (2nd peak) = about 0.91.

System suitability Reference solution (c):
— *resolution:* minimum 1.2 between the peaks due to impurity B (2nd peak) and misoprostol.

Limits:
— *correction factor:* for the calculation of content, multiply the peak area of impurity C by 0.13;
— *impurity B* (sum of 1st and 2nd peaks): not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
— *impurity A*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
— *impurity C*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than 15 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Diastereoisomers
Liquid chromatography (*2.2.29*). *Use freshly prepared solutions.*

Test solution Dissolve 10.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: silica gel for chromatography R* (5 µm);
— *temperature:* 40 °C.

Mobile phase Mix 20 volumes of *2-propanol R*, 40 volumes of *anhydrous ethanol R*, 940 volumes of *heptane R* and sonicate for 10 min.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 205 nm.

Injection 20 µL.

Run time 1.5 times the retention time of the 1st peak of misoprostol.

Retention time Misoprostol 1st peak = about 19 min; misoprostol 2nd peak = about 21 min.

System suitability Test solution:
— *resolution:* minimum 2.0 between the 1st and 2nd peaks of misoprostol.

Limit:
— *1st peak of misoprostol:* 45 per cent to 55 per cent of the sum of the areas of the 2 peaks due to misoprostol.

Water (*2.5.32*)
Maximum 1.0 per cent.

Use 1.0 mL of a 10 mg/mL solution of the substance to be examined in *methanol R*.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection 20 µL of the test solution and reference solution (a).

System suitability Reference solution (a):

— *symmetry factor*: maximum 3.7 for the peak due to misoprostol.

Calculate the percentage content of $C_{22}H_{38}O_5$ using the declared content of *misoprostol CRS*.

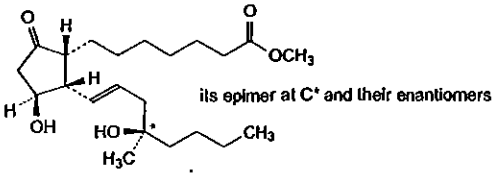
STORAGE

In an airtight container, at −20 °C.

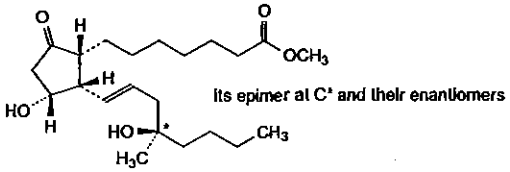
IMPURITIES

Specified impurities *A, B, C.*

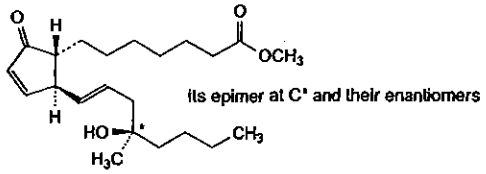
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *D, E, F.*



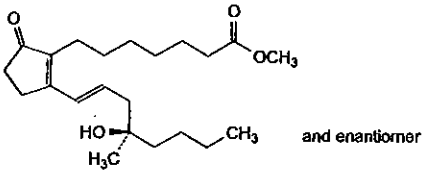
A. mixture of methyl 7-[(1*RS*,2*SR*,3*SR*)-3-hydroxy-2-[(1*E*,4*RS*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopentyl]heptanoate and methyl 7-[(1*RS*,2*SR*,3*SR*)-3-hydroxy-2-[(1E,4*SR*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopentyl]heptanoate (8-epimisoprostol),



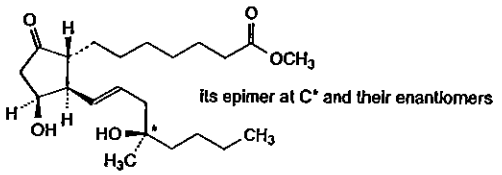
B. mixture of methyl 7-[(1*RS*,2*SR*,3*RS*)-3-hydroxy-2-[(1*E*,4*RS*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopentyl]heptanoate and methyl 7-[(1*RS*,2*SR*,3*RS*)-3-hydroxy-2-[(1*E*,4*SR*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopentyl]heptanoate (12-epimisoprostol),



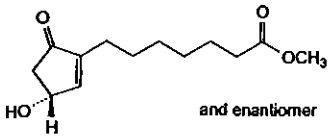
C. mixture of methyl 7-[(1*RS*,2*SR*)-2-[(1*E*,4*RS*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopent-3-enyl]heptanoate and methyl 7-[(1*RS*,2*SR*)-2-[(1*E*,4*SR*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopent-3-enyl]heptanoate (misoprostol A),



D. methyl 7-[2-[(1*E*,4*RS*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopent-1-enyl]heptanoate (misoprostol B),



E. mixture of methyl 7-[(1*RS*,2*RS*,3*SR*)-3-hydroxy-2-[(1*E*,4*RS*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopentyl]heptanoate and methyl 7-[(1*RS*,2*RS*,3*SR*)-3-hydroxy-2-[(1*E*,4*SR*)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopentyl]heptanoate (11-epi misoprostol),



F. methyl 7-[(3*RS*)-3-hydroxy-5-oxocyclopent-1-enyl] heptanoate.

Ph Eur

Mites for Allergen Products

(Ph. Eur. monograph 2625)

Ph Eur

DEFINITION

Mites for allergen products are inactivated mite cultures that may consist of faecal particles, mite bodies, mite parts, eggs, larvae and residues from the growth medium. They contain soluble substances, including proteins, whose functional activity is unrelated to any antigenic and allergenic properties they may have.

PRODUCTION

Mites for allergen products are obtained from cultivation techniques. The method of mite cultivation is described and must ensure the quality, homogeneity and traceability of the mites. Critical cultivation parameters such as temperature and humidity are controlled and monitored. Unless otherwise justified, the culture medium is selected to avoid the presence of materials with potential allergenicity. Appropriate measures are taken to avoid contamination by foreign mite cultures. Mite cultures are macroscopically free of moulds.

After cultivation, the mite culture is inactivated by methods whose capability to maintain allergenic properties are qualified.

Certain mite fractions (e.g. mite bodies or mite faeces) may be purified or enriched before the initiation of the active substance manufacture. In these cases, the process must be performed using qualified methods.

Where major changes to the production of the mites take place (e.g. when a new process or supplier of mites is introduced), such changes are qualified.

Microbial contamination of the mite culture may be unavoidable and should be monitored on a representative number of batches of mites according to a justified sampling plan, and each time a new supplier and/or a new process for the mites production is introduced; if a determination of microbial contamination is not applicable, this must be justified. Microbial contamination values and potential increases in microbial contamination are monitored during stability studies, in order to assess this aspect along with the mites for allergen products characteristics upon storage.

Control methods and acceptance criteria relating to identity and purity of the mites are established. The acceptance criteria must ensure the consistency of the mite source material from a qualitative and quantitative point of view. The mite source material is stored under controlled conditions justified by stability data. The collection and production, as well as the handling of the source material, are such that consistent composition is ensured from batch to batch.

MITES FOR ALLERGEN PRODUCTS REFERENCE BATCH

An appropriate reference batch is established for each species. The nature of the reference batch depends on the testing approach to verify batch-to-batch consistency and to establish acceptable quality. The reference batch may be, for example, an internal reference preparation (if available), a source material extract or a sample of a production batch. Its characterisation must be described. The extent of characterisation of the reference batch depends on the mite source material, knowledge of the allergenic components and availability of suitable reagents. The reference batch is stored under controlled conditions ensuring its stability.

BATCH-TO-BATCH CONSISTENCY

To establish batch-to-batch consistency, one or more of the following tests are performed on each batch. The choice of tests must be justified.

Total protein (*2.5.33*)

Protein profile

Determined by using suitable electrophoresis methods (*2.2.31*, *2.2.54*).

Allergen profile

Relevant allergenic components are identified by means of suitable techniques using allergen-specific antibodies.

Major allergen content

Determined by using suitable immunochemical methods (*2.7.1*) such as enzyme-linked immunosorbent assay (ELISA).

Total allergenic activity

Determined by testing inhibition of the binding capacity of specific immunoglobulin E antibodies or by a suitable equivalent *in vitro* method.

CHARACTERS

Mites for allergen products are supplied as yellow to brown powders, which may contain one or more of the following: mite bodies, parts, eggs, larvae and faecal particles. The mite source material may contain residual amounts of medium components.

IDENTIFICATION

The identity of the mite species is confirmed by their relevant macroscopic (e.g. colour, appearance) and microscopic (e.g. shape of the mites, size of their legs, number and distribution of hairs and if necessary the geometry of dorsal striations) morphological characteristics in comparison to those of a reference batch or reference documents. Identity may also be confirmed using protein analysis methods (e.g. protein profile, individual allergen test) or by genetic identification, if performed by generally accepted methods.

TESTS

Foreign matter

Foreign matter is defined as any particles that are not part of the mite culture, i.e. any particulate matter not arising from the medium or the mites. Foreign matter is typically detected using a suitable microscopic method; a limit for the amount of foreign matter has to be established based on historical data.

Water (*2.5.12* or *2.5.32*) **or loss on drying** (*2.2.32*)

The water content of dried material is determined; specification limits must be supported by batch analysis and stability data.

Purity

When the source material is a purified fraction of the mite culture (e.g. mite bodies), the purity of the fraction needs to be evaluated and results are within a predefined limit.

STORAGE

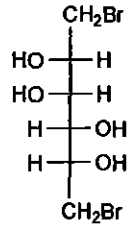
The mite source material is stored under controlled conditions justified by stability data.

LABELLING

The label states:
— the species of the mite;
— the fraction of mite culture, where applicable.

Ph Eur

Mitobronitol



$C_6H_{12}Br_2O_4$ 308.0 *488-41-5*

Action and use

Cytotoxic.

Preparation

Mitobronitol Tablets

DEFINITION

Mitobronitol is 1,6-dibromo-1,6-dideoxy-D-mannitol.
It contains not less than 98.5% and not more than 101.0% of $C_6H_{12}Br_2O_4$ calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white, crystalline solid.

Slightly soluble in *water*, in *acetone* and in *ethanol (96%)*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of mitobronitol *(RS 236)*.

B. Dissolve 0.1 g in 10 mL of 1M *sodium hydroxide*, boil, cool, acidify with 2M *nitric acid* and add 1 mL of *silver nitrate solution*. A pale yellow, curdy precipitate is produced.

C. Dissolve 20 mg in 2 mL of a mixture of 1 volume of *periodic acid solution* and 24 volumes of *water*. Add 1 mL of 0.25M *barium chloride* and shake well. A white, flocculent precipitate is produced.

TESTS

Acidity

Shake 2 g with 50 mL of *carbon dioxide-free water* for 15 minutes and filter. 40 mL of the filtrate requires not more than 0.3 mL of *0.02M sodium hydroxide VS* for neutralisation using *phenolphthalein solution R1* as indicator.

Clarity and colour of solution

A 4.0% w/v solution in *dimethylformamide* is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method I.

Ionic halide

Shake 0.20 g with 30 mL of *water* for 5 minutes and filter. 15 mL of the filtrate complies with the *limit test for chlorides*, Appendix VII (500 ppm, calculated as Cl).

Loss on drying

When dried at 105° for 2 hours, loses not more than 1.0% of its weight. Use 1 g.

Sulfated ash

Not more than 0.2%, Appendix IX A.

ASSAY

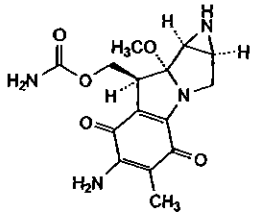
Dissolve 0.2 g in 20 mL of 1M *sodium hydroxide* by heating gently, cool, add 25 mL of *0.1M silver nitrate VS* and acidify with 5 mL of 5M *nitric acid*. Add 2 mL of *ammonium iron(III) sulfate solution R2* and titrate the excess of silver nitrate with *0.1M ammonium thiocyanate VS*. Each mL of *0.1M silver nitrate VS* is equivalent to 15.40 mg of $C_6H_{12}Br_2O_4$.

STORAGE

Mitobronitol should be protected from light.

Mitomycin

(Ph. Eur. monograph 1655)



$C_{15}H_{18}N_4O_5$ 334.3 *50-07-7*

Action and use

Antibacterial; cytotoxic.

Ph Eur

DEFINITION

[(1a*S*,8*S*,8a*R*,8b*S*)-6-Amino-8a-methoxy-5-methyl-4,7-dioxo-1,1a,2,4,7,8,8a,8b-octahydroazirino[2′,3′:3,4]pyrrolo[1,2-*a*]indol-8-yl]methyl carbamate (mitomycin C).

Substance produced by a strain of *Streptomyces caespitosus*.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Blue-violet crystals or crystalline powder.

Solubility

Slightly soluble in water, freely soluble in dimethylacetamide, sparingly soluble in methanol, slightly soluble in acetone.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *mitomycin CRS*.

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

pH *(2.2.3)*

5.5 to 7.5.

Dissolve 10 mg in 10 mL of *carbon dioxide-free water R*.

Related substances

Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use.*

Test solution Dissolve 50.0 mg of the substance to be examined in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *methanol R*. Dilute 5.0 mL of this solution to 10.0 mL with *methanol R*.

Reference solution (b) Dissolve 10 mg of *cinnamamide R* (impurity A) in *methanol R* and dilute to 10 mL with the same solvent. Mix 2 mL of this solution and 1 mL of the test solution and dilute to 10 mL with *methanol R*.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: spherical *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 30 °C.

Mobile phase:
- *mobile phase A*: *methanol R*, 0.77 g/L solution of *ammonium acetate R* (20:80 *V/V*);
- *mobile phase B*: *methanol R*, 0.77 g/L solution of *ammonium acetate R* (50:50 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	100	0
10 - 30	100 → 0	0 → 100
30 - 45	0	100
45 - 50	0 → 100	100 → 0

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µL.

Relative retention With reference to mitomycin (retention time = about 21 min): impurity D = about 0.6; impurity C = about 1.2; impurity A = about 1.3; impurity B = about 1.6.

System suitability Reference solution (b):
- *resolution*: minimum 15.0 between the peaks due to mitomycin and impurity A.

Limits:

- *correction factor:* for the calculation of content, multiply the peak area of impurity A by 0.35;
- *impurities A, B, C, D:* for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *any other impurity:* for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *total:* not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent);
- *disregard limit:* 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)

Maximum 2.5 per cent, determined on 0.30 g.

Bacterial endotoxins (*2.6.14, Method B*)

Less than 10 IU/ mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in *dimethylacetamide R* and dilute to 50.0 mL with the same solvent.

Reference solution (a) Dissolve 25.0 mg of *mitomycin CRS* in *dimethylacetamide R* and dilute to 50.0 mL with the same solvent.

Reference solution (b) Dissolve 10 mg of *cinnamamide R* (impurity A) in *methanol R* and dilute to 20 mL with the same solvent. Mix 2 mL of this solution with 2 mL of reference solution (a).

Column:

- *size:* l = 0.30 m, Ø = 3.9 mm;
- *stationary phase: end-capped phenylsilyl silica gel for chromatography R* (10 µm) with a specific surface area of 330 m^2/g, a carbon loading of 8 per cent and a pore size of 12.5 nm.

Mobile phase Mix 23 volumes of *methanol R*, 77 volumes of a solution containing 2.05 g/L of *ammonium acetate R* and 2.8 mL/L of *dilute acetic acid R*.

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 365 nm and 254 nm.

Injection 20 µL.

Run time Twice the retention time of mitomycin.

Relative retention With reference to mitomycin (retention time = about 8 min): impurity A = about 1.2.

System suitability:

- *resolution:* minimum 1.8 between the peaks due to mitomycin and impurity A in the chromatogram obtained with reference solution (b) at 254 nm,
- *symmetry factor:* maximum 1.3 for the principal peak in the chromatogram obtained with reference solution (a) at 365 nm.

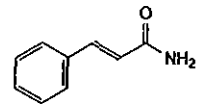
Calculate the percentage content of $C_{15}H_{18}N_4O_5$, using the chromatograms obtained with the test solution and reference solution (a) at 365 nm and taking into account the assigned content of *mitomycin CRS*.

STORAGE

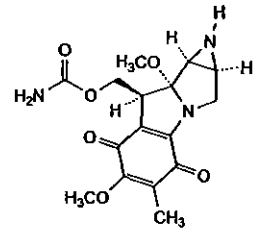
Protected from light.

IMPURITIES

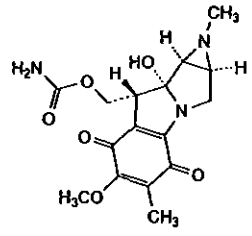
Specified impurities *A, B, C, D.*



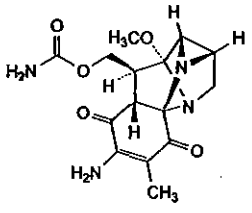
A. (*E*)-3-phenylprop-2-enamide (cinnamamide),



B. [(1a*S*,8*S*,8a*R*,8b*S*)-6,8a-dimethoxy-5-methyl-4,7-dioxo-1,1a,2,4,7,8,8a,8b-octahydroazirino[2′,3′:3,4]pyrrolo[1,2-*a*]indol-8-yl]methyl carbamate (mitomycin A),



C. [(1a*S*,8*R*,8a*R*,8b*S*)-8a-hydroxy-6-methoxy-1,5-dimethyl-4,7-dioxo-1,1a,2,4,7,8,8a,8b-octahydroazirino[2′,3′:3,4]pyrrolo[1,2-*a*]indol-8-yl]methyl carbamate (mitomycin B),

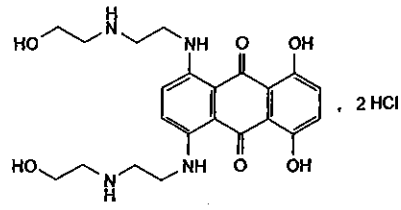


D. [(1*S*,2*S*,4*S*,5*R*,6*S*,6a*R*,10a*S*,11*S*)-8-amino-5-methoxy-9-methyl-7,10-dioxo-2,3,6,6a,7,10-hexahydro-1,2,5-metheno-1*H*,5*H*-imidazo[2,1-*i*]indol-6-yl]methyl carbamate (albomitomycin C).

Ph Eur

Mitoxantrone Hydrochloride

(Ph. Eur. monograph 1243)



$C_{22}H_{30}Cl_2N_4O_6$ 517.4 *70476-82-3*

Action and use
Cytotoxic.

Preparation
Mitoxantrone Infusion

Ph Eur

DEFINITION

1,4-Dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino] ethyl]amino]anthracene-9,10-dione dihydrochloride.

Content
97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
Dark blue, electrostatic, hygroscopic powder.

Solubility
Sparingly soluble in water, slightly soluble in methanol, practically insoluble in acetone.

CAUTION: mitoxantrone hydrochloride and impurity A are electrostatic; the use of an antistatic gun or other suitable method to discharge the solids before weighing or transfer is recommended.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Dissolve 2-3 mg in 1 mL of *methanol R* by warming in a water-bath at 40-50 °C. Evaporate to dryness under a stream of dry nitrogen, warming gently if necessary. Examine the residue.

Comparison *mitoxantrone hydrochloride CRS.*

B. It gives reaction (b) of chlorides (*2.3.1*).

TESTS

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in about 40 mL of the mobile phase, sonicating if necessary, and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 20.0 mg of *mitoxantrone hydrochloride CRS* in about 40 mL of the mobile phase, sonicating if necessary, and dilute to 50.0 mL with the mobile phase.

Reference solution (b) Dilute 1 mL of the test solution to 100 mL with the mobile phase.

Reference solution (c) Dissolve 2.0 mg of *mitoxantrone impurity A CRS* in 1.0 mL of reference solution (a).

Reference solution (d) Dilute 1 mL of reference solution (b) to 10 mL with the mobile phase.

Column:
— *size: l* = 0.30 m, Ø = 3.0 mm;
— *stationary phase: phenylsilyl silica gel for chromatography R* (10 µm).

Mobile phase Mix 750 volumes of *water R*, 250 volumes of *acetonitrile R* and 25 volumes of a solution prepared as follows: dissolve 22.0 g of *sodium heptanesulfonate R* in about 150 mL of *water R* and filter through a 0.45 µm filter; wash the filter with *water R* and combine the filtrate and washings; add 32.0 mL of *glacial acetic acid R* and dilute to 250 mL with *water R*.

Flow rate 3 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 50 µL of the test solution and reference solutions (b), (c) and (d).

Run time 3 times the retention time of mitoxantrone.

System suitability Reference solution (c):
— *resolution*: minimum 3.0 between the peaks due to mitoxantrone and impurity A.

Limits:
— *impurities A, B, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent);
— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (d) (0.1 per cent).

Ethanol
Gas chromatography (*2.2.28*).

Internal standard solution Dilute 2.0 mL of *propanol R* to 100 mL with *water R*. Dilute 5.0 mL of this solution to 100 mL with *water R*.

Test solution Mix 0.100 g of the substance to be examined with 2.0 mL of the internal standard solution and dilute to 5.0 mL with *water R*. Place the flask in an ultrasonic bath for 2 min, then shake the flask for 2 min. If necessary, repeat the sonication and shaking until dissolution is complete.

Reference solution Dilute 2.0 mL of *anhydrous ethanol R* to 100.0 mL with *water R*. Dilute 5.0 mL of the solution to 100.0 mL with *water R*. Dilute 10.0 mL of this solution and 10.0 mL of the internal standard solution to 25.0 mL with *water R*.

Column:
— *size: l* = 2 m, Ø = 3 mm;
— *stationary phase: ethylvinylbenzene-divinylbenzene copolymer R.*

Carrier gas *helium for chromatography R.*

Flow rate 19 mL/min.

Temperature:
— *column*: 120 °C;
— *injection port*: 175 °C;
— *detector*: 210 °C.

Detection Flame ionisation.

Injection 1 µL.

Retention time Ethanol = about 1 min; propanol = about 2 min.

System suitability Reference solution:
— *resolution*: minimum 6 between the peaks due to ethanol and propanol.

Calculate the content of ethanol, taking its density (*2.2.5*) to be 0.790 g/mL at 20 °C.

Limit:
— *ethanol*: maximum 1.6 per cent *m/m*.

Water (*2.5.12*)
Maximum 6.0 per cent, determined on 0.300 g.

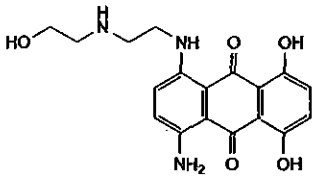
ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

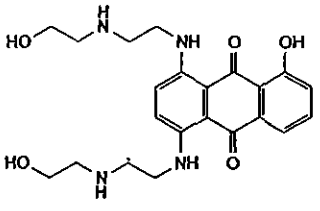
Calculate the percentage content of $C_{22}H_{30}Cl_2N_4O_6$ from the declared content of *mitoxantrone hydrochloride CRS*.

STORAGE
In an airtight container.

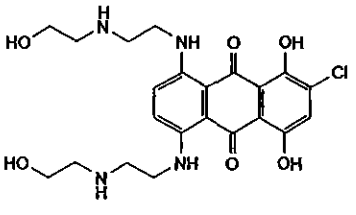
IMPURITIES
Specified impurities *A, B, C, D.*



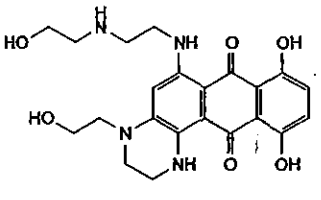
A. 1-amino-5,8-dihydroxy-4-[[2-[(2-hydroxyethyl) amino]ethyl]amino]anthracene-9,10-dione,



B. 5-hydroxy-1,4-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino] anthracene-9,10-dione,



C. 2-chloro-1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino] ethyl]amino]anthracene-9,10-dione,

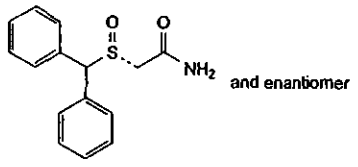


D. 8,11-dihydroxy-4-(2-hydroxyethyl)-6-[[2-[(2-hydroxyethyl)amino]ethyl]amino]-1,2,3,4-tetrahydronaphtho[2,3-*f*]quinoxaline-7,12-dione.

Ph Eur

Modafinil

(Ph. Eur. monograph 2307)



$C_{15}H_{15}NO_2S$ 273.4 *68693-11-8*

Action and use
Narcolepsy and sleep disorders.

Preparation
Modafinil Tablets

Ph Eur

DEFINITION
2-[(*RS*)-(Diphenylmethyl)sulfinyl]acetamide.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Very slightly soluble or practically insoluble in water, sparingly soluble in methanol, slightly soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *modafinil CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

TESTS
Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R1*, *water R* (35:65 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in 35 mL of *acetonitrile R1* and dilute to 50.0 mL with *water R*. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (a) Dissolve 50.0 mg of *modafinil CRS* in 35 mL of *acetonitrile R1* and dilute to 50.0 mL with *water R*. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 2.0 mL of this solution to 20.0 mL with the solvent mixture.

Reference solution (c) Add 2.0 mL of the solvent mixture to a vial of *modafinil for system suitability CRS* (containing impurities A, B and C) and sonicate for 10 min.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 40 °C.

Mobile phase Mix 35 volumes of *acetonitrile R1* and 65 volumes of a 6.8 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 2.3 with *phosphoric acid R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µL of the test solution and reference solutions (b) and (c).

Run time 4 times the retention time of modafinil.

Identification of impurities Use the chromatogram supplied with *modafinil for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B and C.

Relative retention With reference to modafinil (retention time = about 4 min): impurity A = about 1.3; impurity B = about 1.8; impurity C = about 3.0.

System suitability Reference solution (c):

— *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to modafinil.

Limits:

— *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *impurities B, C*: for each impurity, not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

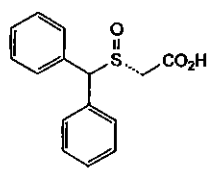
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

Calculate the percentage content of modafinil from the declared content of $C_{15}H_{15}NO_2S$ in *modafinil CRS*.

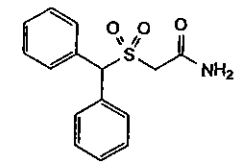
IMPURITIES

Specified impurities *A, B, C.*

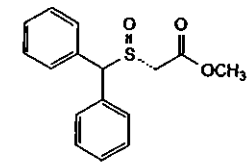


and enantiomer

A. [(*RS*)-(diphenylmethyl)sulfinyl]acetic acid,



B. 2-[(diphenylmethyl)sulfonyl]acetamide,



and enantiomer

C. methyl [(*RS*)-(diphenylmethyl)sulfinyl]acetate.

Ph Eur

Molgramostim Concentrated Solution

(Ph. Eur. monograph 1641)

APARSPSPST QPWEHVNAIQ EARRLLNLSR
DTAAEMNETV EVISEMFDLQ EPTCLQTRLE
LYKQGLRGSL TKLKGPLTMM ASHYKQHCPP
TPETSCATQI ITFESFKENL KDFLLVIPFD
CWEPVQE

$C_{639}H_{1007}N_{171}O_{196}S_8$ 14 477

Action and use
Recombinant granulocyte macrophage colony-stimulating factor.

Ph Eur

DEFINITION

Solution of a protein having the structure of the granulocyte macrophage colony stimulating factor which is produced and secreted by various human blood cell types. The protein stimulates the differentiation and proliferation of leucocyte stem cells into mature granulocytes and macrophages.

Content
Minimum 2.0 mg of protein per millilitre.

Potency
Minimum 0.7×10^7 IU per milligram of protein.

PRODUCTION

Molgramostim concentrated solution is produced by a method based on recombinant DNA (rDNA) technology, using bacteria as host cells. It is produced under conditions designed to minimise microbial contamination of the product.

Prior to release, the following tests are carried out on each batch of molgramostim concentrated solution, unless exemption has been granted by the competent authority.

Host-cell derived proteins
The limit is approved by the competent authority.

Host-cell or vector derived DNA
The limit is approved by the competent authority.

CHARACTERS

Appearance

Clear, colourless liquid.

IDENTIFICATION

A. It shows the expected biological activity (see Assay).

B. Isoelectric focusing (*2.2.54*).

Test solution Dilute the preparation to be examined with *water R* to obtain a concentration of 0.25 mg/mL.

Reference solution (a) Dilute *molgramostim CRS* with *water R* to obtain a concentration of 0.25 mg/mL.

Reference solution (b) Use an isoelectric point (pI) calibration solution, in the pI range of 2.5-6.5, prepared according to the manufacturer's instructions.

Focusing:
- *pH gradient*: 4.0-6.5;
- *catholyte*: 8.91 g/L (0.1 M) solution of *3-aminopropionic acid R*;
- *anolyte*: 14.7 g/L (0.1 M) solution of *glutamic acid R* in a 50 per cent *V/V* solution of *dilute phosphoric acid R* (0.5 M);
- *application*: 20 µL.

Detection Immerse the gel in a suitable volume of a solution containing 115 g/L of *trichloroacetic acid R* and 34.5 g/L of *sulfosalicylic acid R* and shake the container gently for 30 min. Transfer the gel to a mixture of 32 volumes of *glacial acetic acid R*, 100 volumes of *anhydrous ethanol R* and 268 volumes of *water R* (mixture A) and rinse for 5 min. Immerse the gel for 10 min in a staining solution prewarmed to 60 °C and prepared by adding *acid blue 83 R* at a concentration of 1.2 g/L to mixture A. Wash the gel in several containers with mixture A and keep the gel in this mixture until the background is clear (12-24 h). After adequate destaining, soak the gel for 1 h in a 10 per cent *V/V* solution of *glycerol R* in mixture A.

System suitability:
- in the electropherogram obtained with reference solution (b), the relevant isoelectric point markers are distributed along the entire length of the gel;
- in the electropherogram obtained with reference solution (a), the pI of the principal band is 4.9 to 5.4.

Results The principal band in the electropherogram obtained with the test solution corresponds in position to the principal band in the electropherogram obtained with reference solution (a). Plot the migration distances of the relevant pI markers versus their pI and determine the isoelectric points of the principal component of each of the test solution and reference solution (a). They do not differ by more than 0.2 pI units.

C. Examine the electropherograms obtained under reducing conditions in the test for impurities with molecular masses differing from that of molgramostim. The principal band in the electropherogram obtained with test solution (a) is similar in position to the principal band in the electropherogram obtained with reference solution (a).

D. Peptide mapping (*2.2.55*).

Test solution Introduce 50 µL of *tris-hydrochloride buffer solution pH 8.0 R* and 50 µL of the preparation to be examined at a concentration of 2 mg/mL into a polypropylene tube of 0.5 mL capacity. Add 4 µL of a 1 mg/mL solution of *trypsin for peptide mapping R* in a 0.01 per cent *V/V* solution of *trifluoroacetic acid R*, cap tightly and mix well. Incubate at about 37 °C for 18 h. Add 125 µL of a 764 g/L (8 M) solution of *guanidine hydrochloride R* and mix well. Add 10 µL of a 154.2 g/L (1 M) solution of *dithiothreitol R* and mix well. Place the capped tube in boiling water for 1 min. Cool to room temperature.

Reference solution Prepare at the same time and in the same manner as for the test solution but use *molgramostim CRS* instead of the preparation to be examined.

Examine the 2 tryptic digests by liquid chromatography (*2.2.29*).

Column:
- *size*: l = 0.10 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm) with a pore size of 30 nm.

Mobile phase:
- *mobile phase A*: dilute 1 mL of *trifluoroacetic acid R* in 1000 mL of *water for chromatography R*;
- *mobile phase B*: dilute 1 mL of *trifluoroacetic acid R* in 100 mL of *water for chromatography R*; add 900 mL of *acetonitrile R1* and mix;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 35.0	100 → 65	0 → 35
35.0 - 105.0	65 → 35	35 → 65
105.0 - 107.5	35 → 100	65 → 0
107.5 - 120.0	100	0

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 214 nm.

Equilibration At initial conditions for at least 12 min.

Injection 200 µL.

System suitability The chromatogram obtained with the reference solution is qualitatively similar to the *Ph. Eur. reference chromatogram of molgramostim digest.*

Results The profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.

E. *N*-Terminal sequence analysis.

Perform the Edman degradation using an automated solid-phase sequencer, operated in accordance with the manufacturer's instructions.

Load about 1 nmol of the test preparation to a sequencing cartridge using the protocol provided by the manufacturer. Run 16 sequencing cycles, noting, if appropriate, the presence of proline at positions 2, 6, 8 and 12.

Identify the phenylthiohydantoin (PTH)-amino acids released at each sequencing cycle by reverse-phase liquid chromatography. The procedure may be carried out using the column and reagents recommended by the manufacturer of the sequencing equipment for the separation of PTH-amino acids.

The separation procedure is calibrated using:
- the mixture of PTH-amino acids provided by the manufacturer of the sequencer, with the gradient conditions adjusted as indicated to achieve optimum resolution of all amino acids;
- a sample obtained from a blank sequencing cycle obtained as recommended by the equipment manufacturer.

Results The first 16 amino acids are: Ala-Pro-Ala-Arg-Ser-Pro-Ser-Pro-Ser-Thr-Gln-Pro-Trp-Glu-His-Val.

TESTS

Impurities with molecular masses differing from that of molgramostim

Polyacrylamide gel electrophoresis (*2.2.31*) under both reducing and non-reducing conditions.

Gel dimensions 0.75 mm thick.

Resolving gel 14 per cent acrylamide.

Sample buffer A Mix equal volumes of *water R* and *concentrated SDS-PAGE sample buffer R.*

Sample buffer B (reducing conditions) Mix equal volumes of *water R* and *concentrated SDS-PAGE sample buffer for reducing conditions R.*

Test solution (a) Dilute the preparation to be examined in *water R* to obtain a concentration of 1.0 mg/mL.
To 1 volume of this solution add 1 volume of *concentrated SDS-PAGE sample buffer R.*

Test solution (b) (2 per cent control). Dilute 0.020 mL of test solution (a) to 1.0 mL with sample buffer A.

Test solution (c) (1 per cent control). To 0.20 mL of test solution (b) add 0.20 mL of sample buffer A.

Test solution (d) (0.5 per cent control). To 0.20 mL of test solution (c) add 0.20 mL of sample buffer A.

Test solution (e) (0.25 per cent control). To 0.20 mL of test solution (d) add 0.20 mL of sample buffer A.

Test solution (f) (0.1 per cent control). To 0.20 mL of test solution (e) add 0.30 mL of sample buffer A.

Test solution (g) (0.05 per cent control). To 0.20 mL of test solution (f) add 0.20 mL of sample buffer A.

Test solution (h) (0.025 per cent control). To 0.20 mL of test solution (g) add 0.20 mL of sample buffer A.

Test solution (i) Prepare as for test solution (a), but using *concentrated SDS-PAGE sample buffer for reducing conditions R.*

Test solutions (j)-(p) Prepare as for test solutions (b)-(h), but using sample buffer B.

Reference solution (a) Dilute *molgramostim CRS* in *water R* to obtain a concentration of 0.02 mg/mL. Mix 1 volume of this solution with 1 volume of *concentrated SDS-PAGE sample buffer R.*

Reference solution (b) Prepare as for reference solution (a), but using *concentrated SDS-PAGE sample buffer for reducing conditions R.*

Reference solution (c) Use a solution of molecular mass markers suitable for calibrating SDS-PAGE gels in the range of 14 400-94 000. Dissolve in sample buffer or sample buffer (reducing conditions), as appropriate.

Sample treatment Boil for 3 min.

Application 50 µL; apply reduced and non-reduced solutions to separate gels.

Detection Silver staining as described below.

Immerse the gel overnight in a mixture of 10 volumes of *acetic acid R*, 40 volumes of *water R* and 50 volumes of *methanol R*. Transfer the gel to a 100 g/L solution of *glutaraldehyde R* and shake for about 30 min. Replace the glutaraldehyde solution with *water R*, and keep the gel in *water R* for 20 min. Repeat this washing-step twice. Transfer the gel to a mixture containing 0.75 g/L of *sodium hydroxide R*, 14 g/L of *concentrated ammonia R* and 8 g/L of *silver nitrate R*. This solution is prepared immediately before use. Place the gel on a shaker in the dark for 5 min. Wash the gel for 30 s in each of 3 containers with *water R* and shake the gel in a mixture consisting of 0.05 g/L of *citric acid monohydrate R*, 0.05 per cent *V/V* of *formaldehyde R* and 0.005 per cent *V/V* of *methanol R* in *water R*. Protein bands become visible during this step. Keep the gel in the solution until sufficiently stained and then rinse the gel repeatedly with *water R* in a shaking water bath. Soak gels in a solution consisting of 10 per cent *V/V* of *acetic acid R* and 1 per cent *V/V* of *glycerol R*.

System suitability:
- the validation criteria are met (*2.2.31*);
- a band is seen in the electropherogram obtained with test solution (h);
- a gradation of intensity of staining is seen in the electropherograms obtained with test solutions (a)-(h) and (i)-(p);
- the molecular mass of the principal band in the electropherogram obtained with reference solution (a) or (b) is within the range of 15 100 to 17 100.

Limits Compare the staining intensity of each non-molgramostim band observed in the electropherogram obtained with test solution (a) to the staining intensity of the principal band in the electropherograms obtained with test solutions (b)-(h). Proceed similarly with the electropherograms obtained with test solutions (i)-(p).
The impurity level is estimated as the dilution, in percentage, of the solution giving the electropherogram with the closest intensity of staining.

Reducing conditions:
- *impurity with an apparent molecular mass of 20 000*: maximum 1 per cent;
- *impurity with an apparent molecular mass of 25 000*: maximum 0.1 per cent;
- *impurity with an apparent molecular mass of 30 000*: maximum 0.3 per cent;
- *total*: maximum 2 per cent.

Non-reducing conditions:
- *total of all impurities of molecular masses higher than 30 000*: maximum 1 per cent.

Related proteins

Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution (a) Dilute the preparation to be examined with *0.05 M phosphate buffer solution pH 7.0 R* to obtain a concentration of 0.5 mg/mL.

Test solution (b) Mix 1 volume of test solution (a) with 4 volumes of a 0.125 mg/mL solution of *human albumin R* or *bovine albumin R* in *0.05 M phosphate buffer solution pH 7.0 R.*

Reference solution (a) Dilute *molgramostim CRS* with *0.05 M phosphate buffer solution pH 7.0 R* to obtain a concentration of 0.5 mg/mL.

Reference solution (b) Mix 1 volume of reference solution (a) with 4 volumes of a 0.125 mg/mL solution of *human albumin R* or *bovine albumin R* in *0.05 M phosphate buffer solution pH 7.0 R.*

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped butylsilyl silica gel for chromatography R* (5 µm) with a pore size of 30 nm.

Mobile phase:
- *mobile phase A*: to about 800 mL of *water for chromatography R* add 1.0 mL of *trifluoroacetic acid R* and dilute to 1000 mL with *water for chromatography R;*
- *mobile phase B*: to 100 mL of *water for chromatography R* add 1.0 mL of *trifluoroacetic acid R* and 900 mL of *acetonitrile R1;*

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	64 → 44	36 → 56
30 - 35	44 → 0	56 → 100
35 - 45	0	100
45 - 50	0 → 64	100 → 36
50 - 60	64	36

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 214 nm.

Injection 100 µL of test solution (a), reference solutions (a) and (b).

System suitability Reference solution (b):
- *retention time*: molgramostin = about 22 min,
- *repeatability*: maximum relative standard deviation of 5.0 per cent after 4 injections,
- *resolution*: minimum 2 between the peaks due to albumin and molgramostim.

Limits:
- *any impurity*: for each impurity, maximum 1.5 per cent,
- *total of impurities eluting between 5 min and 30 min*: maximum 4 per cent.

Bacterial endotoxins (*2.6.14*)
Less than 5 IU in the volume that contains 1.0 mg of protein.

ASSAY

Protein
Liquid chromatography (*2.2.29*) as described in the test for related proteins.

Injection 150 µL of test solution (b) and reference solution (b).

Calculate the content of molgramostim using the declared content of molgramostim in *molgramostim CRS*.

Potency
Determination of the biological activity of molgramostim concentrated solution based on the stimulation of proliferation of TF-1 cells by molgramostim.

The following method uses the conversion of tetrazolium bromide (MTT) as a staining method. Validated alternative stains such as Almar blue have also been found suitable.

TF-1 cells are incubated with varying dilutions of test and reference preparations of molgramostim. They are then incubated with a solution of MTT. This cytochemical stain is converted by cellular dehydrogenases to a purple formazan product. The formazan is then measured spectrophotometrically. The potency of the preparation to be examined is determined by comparison of the dilutions of the test preparation with the dilutions of the appropriate International Standard of molgramostim or with a reference preparation calibrated in International Units, which yield the same response (50 per cent maximal stimulation).

The International Unit is the activity contained in a stated amount of the appropriate International Standard.
The equivalence in International Units of the International Standard is stated by the World Health Organization.

Add 50 µL of dilution medium to all wells of a 96-well microtitre plate. Add an additional 50 µL of this solution to the wells designed for the blanks. Add 50 µL of each solution to be tested in triplicate (test preparation and reference preparation at a concentration of about 65 IU/mL, plus a series of 10 twofold dilutions to obtain a standard curve). Then add to each well 50 µL of a TF-1 cell suspension containing 3×10^5 cells per millilitre, maintaining the cells in a uniform suspension during addition.

Incubate the plate at 36.0-38.0 °C for a minimum of 24 h in a humidified incubator using 6 ± 1 per cent CO_2.
Add 25 µL of a 5.0 g/L sterile solution of *tetrazolium bromide R* to each well. Reincubate for 5 h. Remove the plates from the incubator and add to each well 100 µL of a 240 g/L solution of *sodium dodecyl sulfate R* previously adjusted to pH 2.7 with hydrochloric acid. Reincubate overnight.

Determine the relative quantity of purple formazan product formed in each well by measuring the absorbance (*2.2.25*) using a 96-well microtitre plate reader. Read each plate at 570 nm and at 690 nm. Subtract the reading at 690 nm from the reading at 570 nm. Analyse the data by fitting a sigmoidal dose-response curve to the data obtained and by using a suitable statistical method, for example the 4-parameter model (see *5.3*).

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency.
The confidence limits ($P = 0.95$) of the estimated potency are not less than 74 per cent and not more than 136 per cent of the stated potency.

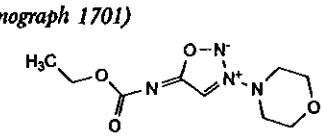
STORAGE
In an airtight container, protected from light, at a temperature below −65 °C.

LABELLING
The label states:
- the content, in milligrams of protein per millilitre,
- the potency, in International Units per milligram of protein.

Ph Eur

Molsidomine

(Ph. Eur. monograph 1701)



$C_9H_{14}N_4O_4$ 242.2 *25717-80-0*

Action and use
Nitric oxide donor; treatment of angina pectoris.

Ph Eur

DEFINITION
N-(Ethoxycarbonyl)-3-(morpholin-4-yl)sydnonimine.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Sparingly soluble in water, soluble in anhydrous ethanol and in methylene chloride.

mp
About 142 °C.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *molsidomine CRS*.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution B_7 (*2.2.2, Method II*).

Dissolve 1.0 g in *anhydrous ethanol R* by heating at about 50 °C for about 5 min and dilute to 20.0 mL with the same solvent.

pH (*2.2.3*)

5.5 to 7.5.

Dissolve 0.50 g in *carbon dioxide-free water R* and dilute to 50.0 mL with the same solvent.

Impurity B

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Detection Spectrophotometer at 240 nm.

Injection 20 µL of test solution (a) and reference solution (b).

Relative retention With reference to molsidomine (retention time = about 9 min): impurity B = about 0.43.

System suitability Reference solution (b):
— *signal-to-noise ratio*: minimum 20 for the principal peak.

Limit:
— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (3 ppm).

Impurity E

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.200 g of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *morpholine for chromatography R* in 500.0 mL of *water for chromatography R*. Dilute 20.0 mL of the solution to 500.0 mL with *water for chromatography R*. Dilute 5.0 mL of this solution to 100.0 mL with *water for chromatography R*.

Reference solution (b) Mix 10.0 mL of the test solution with 10.0 mL of reference solution (a).

Column:
— *size*: *l* = 0.25 m, Ø = 4.0 mm;
— *stationary phase*: *resin for reversed-phase ion chromatography R*;
— *temperature*: 25 °C.

Mobile phase Mix 3.0 mL of *methanesulfonic acid R* and 75 mL of *acetonitrile R* in *water for chromatography R* and dilute to 5000 mL with *water for chromatography R*.

Suppressor regenerant *water for chromatography R*.

Flow rate 1.0 mL/min.

Expected background conductivity Less than 0.5 µS.

Detection Conductivity detector at 10 µS.

Injection 50 µL.

Run time 20 min.

Relative retention With reference to molsidomine (retention time = about 3 min): impurity E = about 2.4.

System suitability Reference solution (b):
— *signal-to-noise ratio*: minimum 6 for the peak due to impurity E.

Limit:
— *impurity E*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.01 per cent).

Related substances

Liquid chromatography (*2.2.29*). *Protect the solutions from light.*

Solvent mixture *methanol R*, mobile phase A (10:90 *V/V*).

Test solution (a) Dissolve 0.200 g of the substance to be examined in 2.5 mL of *methanol R* and dilute to 5.0 mL with mobile phase A.

Test solution (b) Dilute 1.0 mL of test solution (a) to 20.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of test solution (b) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 2.4 mg of *molsidomine impurity B CRS* in 80 mL of *methanol R* and dilute to 100.0 mL with *methanol R*. Dilute 2.0 mL of the solution to 100.0 mL with the solvent mixture. Dilute 5.0 mL of this solution to 20.0 mL with the solvent mixture.

Reference solution (c) Dissolve 10 mg of *linsidomine hydrochloride R* (impurity A) and 5 mg of *molsidomine impurity D CRS* in 10 mL of *methanol R* and dilute to 50.0 mL with the solvent mixture. Dilute 5.0 mL of this solution to 50.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 30 °C.

Mobile phase:
— *mobile phase A*: dissolve 4.0 g of *potassium dihydrogen phosphate R* in *water for chromatography R* and dilute to 1000 mL with the same solvent;
— *mobile phase B*: *methanol R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	90	10
3 - 10	90 → 20	10 → 80
10 - 13	20	80

Flow rate 1.3 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µL of test solution (b) and reference solutions (a) and (c).

Relative retention With reference to molsidomine (retention time = about 9 min): impurity A = about 0.2; impurity D = about 0.3.

System suitability Reference solution (c):
— *resolution*: minimum 3.5 between the peaks due to impurities A and D.

Limits:
— *unspecified impurities*: for each impurity, not more than the area of the peak due to molsidomine in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 3 times the area of the peak due to molsidomine in the chromatogram obtained with reference solution (a) (0.3 per cent);
— *disregard limit*: 0.5 times the area of the peak due to molsidomine in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

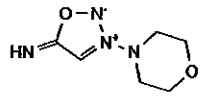
ASSAY
Dissolve 0.200 g in a mixture of 5 mL of *acetic anhydride R* and 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 24.22 mg of $C_9H_{14}N_4O_4$.

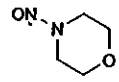
STORAGE
Protected from light.

IMPURITIES
Specified impurities *B, E.*

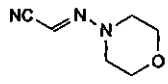
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, C, D.*



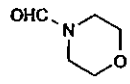
A. 3-(morpholin-4-yl)sydnonimine (linsidomine),



B. 4-nitrosomorpholine,



C. (2*E*)-(morpholin-4-ylimino)acetonitrile,



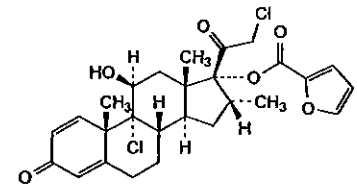
D. morpholine-4-carbaldehyde,

E. morpholine.

Ph Eur

Mometasone Furoate

(Ph. Eur. monograph 1449)



$C_{27}H_{30}Cl_2O_6$ 521.4 *83919-23-7*

Action and use
Glucocorticoid.

Preparations
Mometasone Aqueous Nasal Spray
Mometasone Cream
Mometasone Inhalation Powder, Pre-metered
Mometasone Ointment
Mometasone Scalp Application

Ph Eur

DEFINITION
9,21-Dichloro-11β-hydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-17-yl furan-2-carboxylate.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white powder.

Solubility
Practically insoluble in water, soluble in acetone and in methylene chloride, slightly soluble in ethanol (96 per cent).

IDENTIFICATION
First identification: A, D, E.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *mometasone furoate CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution Dissolve 10 mg of *mometasone furoate CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *methanol R, methylene chloride R* (10:90 *V/V*).

Application 10 µL; the volume may be adapted based on the type of plate used.

Development Over 3/4 of the plate.

Drying In air.

Detection Spray with a solution prepared as follows: dissolve 0.25 g of *2,4-dihydroxybenzaldehyde R* in *glacial acetic acid R*, dilute to 50 mL with the same solvent and add a mixture of 12.5 mL of *sulfuric acid R* and 37.5 mL of *glacial acetic acid R*; heat the plate at 90 °C for 35 min or until the spots appear and allow to cool; examine in daylight and in ultraviolet light at 365 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to

the principal spot in the chromatogram obtained with the reference solution.

C. Mix 80 mg with 0.30 g of *anhydrous sodium carbonate R* and ignite in a crucible until an almost white residue is obtained. Allow to cool and dissolve the residue in 5 mL of *dilute nitric acid R*; filter. To 1 mL of the filtrate add 1 mL of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

D. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (c).

E. Loss on drying (see Tests).

TESTS

Specific optical rotation (*2.2.7*)

+ 50 to + 55 (dried substance).

Dissolve 50.0 mg in *ethanol (96 per cent) R* and dilute to 10.0 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Solvent mixture Mix 200 mL of *acetonitrile R* and 200 mL of *water R*, then add 0.4 mL of *acetic acid R*.

Test solution (a) Dissolve 25.0 mg of the substance to be examined in 15 mL of *acetonitrile R* and dilute to 50.0 mL with the solvent mixture.

Test solution (b) Dilute 5.0 mL of test solution (a) to 25.0 mL with the solvent mixture.

Reference solution (a) Dissolve 5 mg of *mometasone furoate for system suitability CRS* (containing impurities C and J) in 3 mL of *acetonitrile R* and dilute to 10 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 25.0 mg of *mometasone furoate monohydrate CRS* in 15 mL of *acetonitrile R* and dilute to 50.0 mL with the solvent mixture. Dilute 5.0 mL of the solution to 25.0 mL with the solvent mixture.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R, water for chromatography R* (50:50 *V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL of test solution (a) and reference solutions (a) and (b).

Run time 3.5 times the retention time of mometasone furoate.

Identification of impurities Use the chromatogram supplied with *mometasone furoate for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities C and J.

Relative retention With reference to mometasone furoate (retention time = about 24 min): impurity C = about 0.9; impurity J = about 1.5.

System suitability Reference solution (a):

— *resolution*: minimum 2.5 between the peaks due to impurity C and mometasone furoate.

Limits:

— *impurity J*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

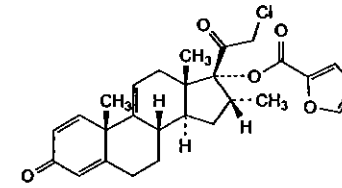
Injection Test solution (b) and reference solution (c).

Calculate the percentage content of $C_{27}H_{30}Cl_2O_6$ taking into account the assigned content of *mometasone furoate monohydrate CRS*.

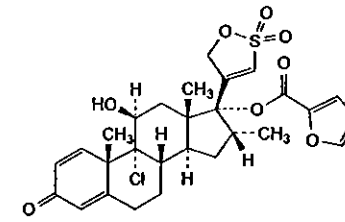
IMPURITIES

Specified impurities *J*.

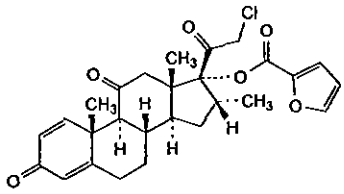
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, E, F, G, H, I, K, L, M, N, O, P, Q, R, S, T, U.*



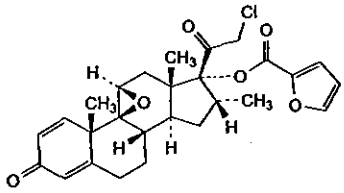
A. 21-chloro-16α-methyl-3,20-dioxopregna-1,4,9(11)-trien-17-yl furan-2-carboxylate,



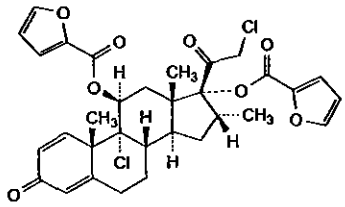
B. 9-chloro-17β-(2,2-dioxo-2,5-dihydro-1,2λ⁶-oxathiol-4-yl)-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-dien-17α-yl furan-2-carboxylate,



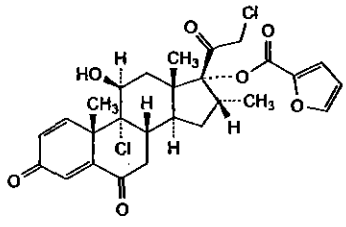
C. 21-chloro-16α-methyl-3,11,20-trioxopregna-1,4-dien-17-yl furan-2-carboxylate,



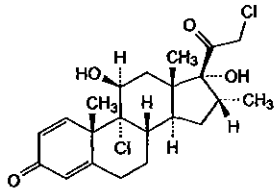
D. 21-chloro-9,11β-epoxy-16α-methyl-3,20-dioxo-9β-pregna-1,4-dien-17-yl furan-2-carboxylate,



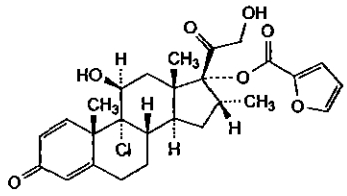
E. 9,21-dichloro-16α-methyl-3,20-dioxopregna-1,4-diene-11β,17-diyl di(furan-2-carboxylate),



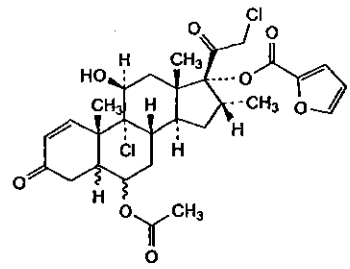
F. 9,21-dichloro-11β-hydroxy-16α-methyl-3,6,20-trioxopregna-1,4-dien-17-yl furan-2-carboxylate,



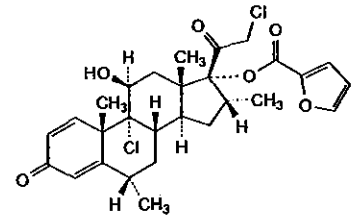
G. 9,21-dichloro-11β,17-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione (mometasone),



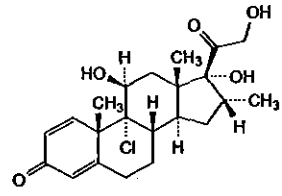
H. 9-chloro-11β,21-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-17-yl furan-2-carboxylate,



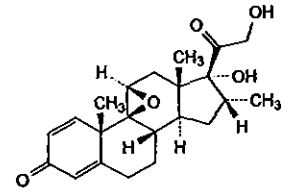
I. 6ξ-(acetyloxy)-9,21-dichloro-11β-hydroxy-16α-methyl-3,20-dioxo-5ξ-pregn-1-en-17-yl furan-2-carboxylate,



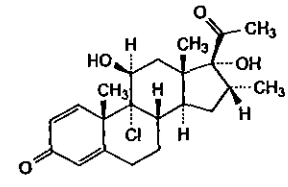
J. 9,21-dichloro-11β-hydroxy-6α,16α-dimethyl-3,20-dioxopregna-1,4-dien-17-yl furan-2-carboxylate,



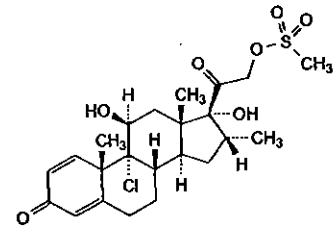
K. 9-chloro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione,



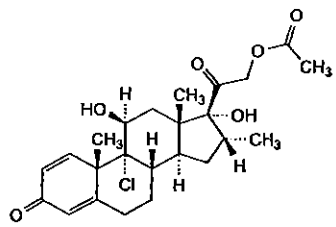
L. 9,11β-epoxy-17,21-dihydroxy-16α-methyl-9β-pregna-1,4-diene-3,20-dione,



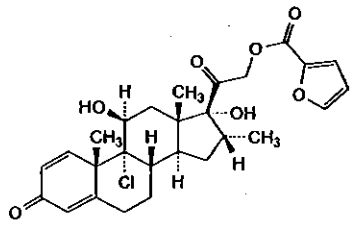
M. 9-chloro-11β,17-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione,



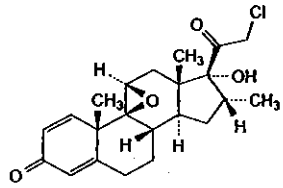
N. 9-chloro-11β,17-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl methanesulfonate,



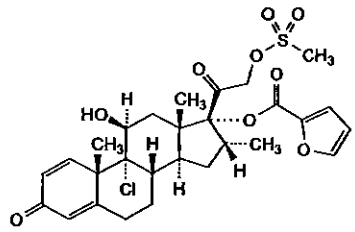
O. 9-chloro-11β,17-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl acetate,



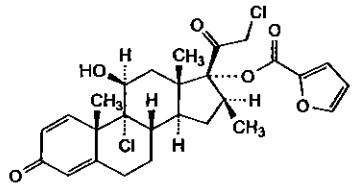
P. 9-chloro-11β,17-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl furan-2-carboxylate,



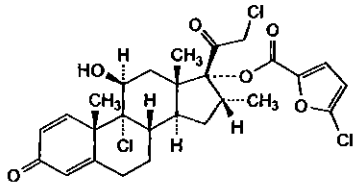
Q. 21-chloro-9,11β-epoxy-17-hydroxy-16α-methyl-9β-pregna-1,4-diene-3,20-dione,



R. 9-chloro-11β-hydroxy-21-[(methanesulfonyl)oxy]-16α-methyl-3,20-dioxopregna-1,4-dien-17-yl furan-2-carboxylate,



S. 9,21-dichloro-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-17-yl furan-2-carboxylate,



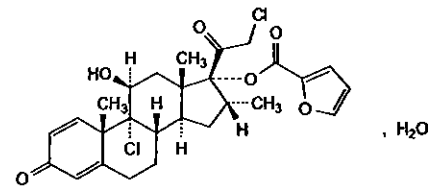
T. 9,21-dichloro-11β-hydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-17-yl 5-chlorofuran-2-carboxylate,

U. unknown structure.

Ph Eur

Mometasone Furoate Monohydrate

(Ph. Eur. monograph 2858)



$C_{27}H_{30}Cl_2O_6,H_2O$ 539.4 *141646-00-6*

Action and use
Glucocorticoid.

Ph Eur

DEFINITION

9,21-Dichloro-11β-hydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-17-yl furan-2-carboxylate monohydrate.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Practically insoluble in water, soluble in acetone and in methylene chloride, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *mometasone furoate monohydrate CRS.*

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (c).

C. Water (see Tests).

TESTS

Specific optical rotation (*2.2.7*)
+ 50 to + 55 (anhydrous substance).

Dissolve 50.0 mg in *ethanol (96 per cent) R* and dilute to 10.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Solvent mixture Mix 200 mL of *acetonitrile R* and 200 mL of *water R*, then add 0.4 mL of *acetic acid R*.

Test solution (a) Dissolve 25.0 mg of the substance to be examined in 15 mL of *acetonitrile R* and dilute to 50.0 mL with the solvent mixture.

Test solution (b) Dilute 5.0 mL of test solution (a) to 25.0 mL with the solvent mixture.

Reference solution (a) Dissolve 5 mg of *mometasone furoate for system suitability CRS* (containing impurity C) in 3 mL of *acetonitrile R* and dilute to 10.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 25.0 mg of *mometasone furoate monohydrate CRS* in 15 mL of *acetonitrile R* and dilute to

50.0 mL with the solvent mixture. Dilute 5.0 mL of the solution to 25.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R, water for chromatography R* (50:50 *V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL of test solution (a) and reference solutions (a) and (b).

Run time 3.5 times the retention time of mometasone furoate.

Identification of impurities Use the chromatogram supplied with *mometasone furoate for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peak due to impurity C.

Relative retention With reference to mometasone furoate (retention time = about 24 min): impurity C = about 0.9.

System suitability Reference solution (a):
— *resolution*: minimum 2.5 between the peaks due to impurity C and mometasone furoate.

Limits:
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.12*)
2.5 per cent to 4.0 per cent, determined on 0.200 g.

ASSAY

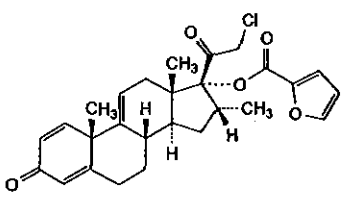
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (c).

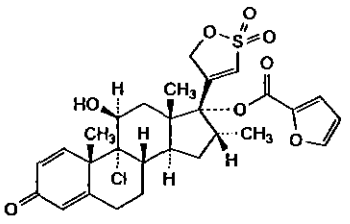
Calculate the percentage content of $C_{27}H_{30}Cl_2O_6$ taking into account the assigned content of *mometasone furoate monohydrate CRS*.

IMPURITIES

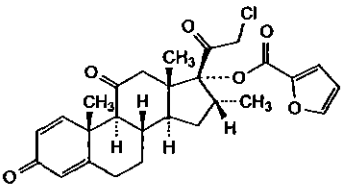
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U.*



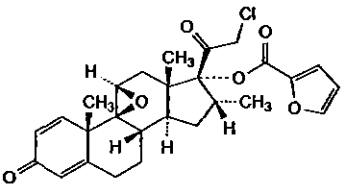
A. 21-chloro-16α-methyl-3,20-dioxopregna-1,4,9(11)-trien-17-yl furan-2-carboxylate,



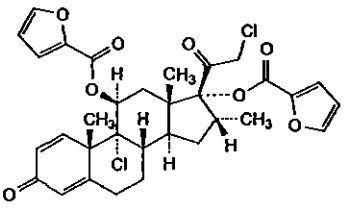
B. 9-chloro-17β-(2,2-dioxo-2,5-dihydro-1,2λ^6-oxathiol-4-yl)-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-dien-17α-yl furan-2-carboxylate,



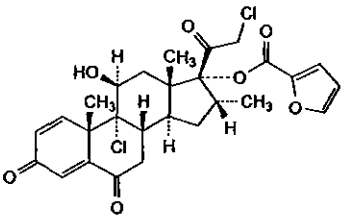
C. 21-chloro-16α-methyl-3,11,20-trioxopregna-1,4-dien-17-yl furan-2-carboxylate,



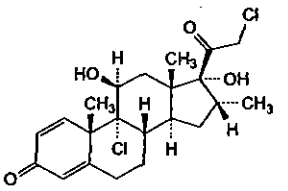
D. 21-chloro-9,11β-epoxy-16α-methyl-3,20-dioxo-9β-pregna-1,4-dien-17-yl furan-2-carboxylate,



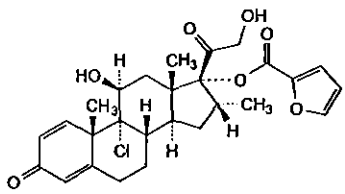
E. 9,21-dichloro-16α-methyl-3,20-dioxopregna-1,4-diene-11β,17-diyl di(furan-2-carboxylate),



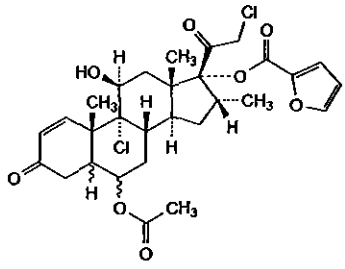
F. 9,21-dichloro-11β-hydroxy-16α-methyl-3,6,20-trioxopregna-1,4-dien-17-yl furan-2-carboxylate,



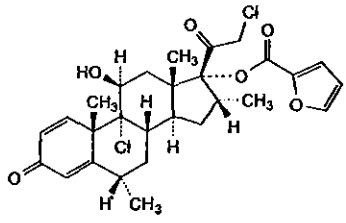
G. 9,21-dichloro-11β,17-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione (mometasone),



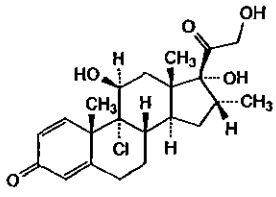
H. 9-chloro-11β,21-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-17-yl furan-2-carboxylate,



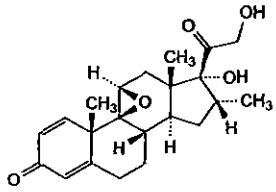
I. 6ξ-acetoxy-9,21-dichloro-11β-hydroxy-16α-methyl-3,20-dioxo-5ξ-pregn-1-en-17-yl furan-2-carboxylate,



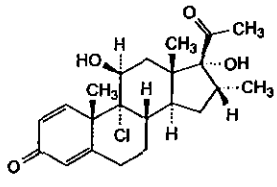
J. 9,21-dichloro-11β-hydroxy-6α,16α-dimethyl-3,20-dioxopregna-1,4-dien-17-yl furan-2-carboxylate,



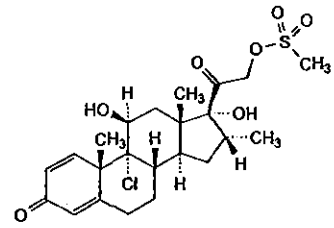
K. 9-chloro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione,



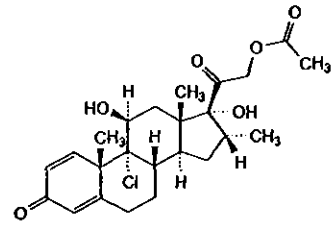
L. 9,11β-epoxy-17,21-dihydroxy-16α-methyl-9β-pregna-1,4-diene-3,20-dione,



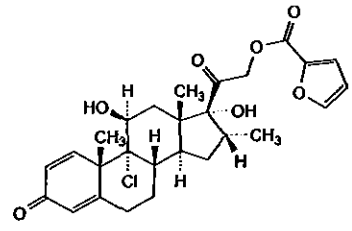
M. 9-chloro-11β,17-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione,



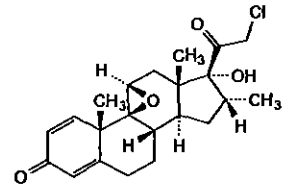
N. 9-chloro-11β,17-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl methanesulfonate,



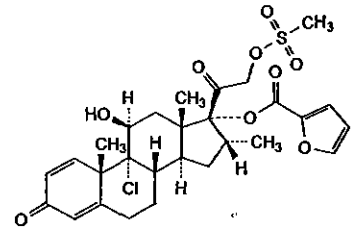
O. 9-chloro-11β,17-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl acetate,



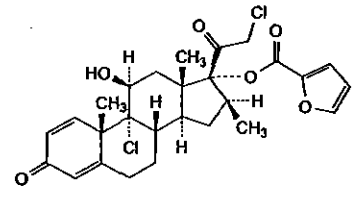
P. 9-chloro-11β,17-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl furan-2-carboxylate,



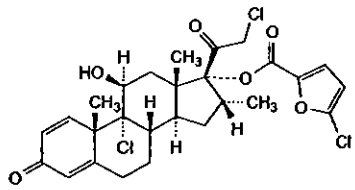
Q. 21-chloro-9,11β-epoxy-17-hydroxy-16α-methyl-9β-pregna-1,4-diene-3,20-dione,



R. 9-chloro-11β-hydroxy-16α-methyl-21-[(methylsulfonyl) oxy]-3,20-dioxopregna-1,4-dien-17-yl furan-2-carboxylate,



S. 9,21-dichloro-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-17-yl furan-2-carboxylate,



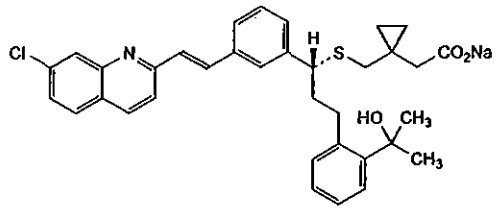
T. 9,21-dichloro-11β-hydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-17-yl 5-chlorofuran-2-carboxylate,

U. unknown structure.

Ph Eur

Montelukast Sodium

(Ph. Eur. monograph 2583)



$C_{35}H_{35}ClNNaO_3S$ 608 *151767-02-1*

Action and use
Leukotriene $CysLT_1$ receptor antagonist; treatment of asthma.

Preparations
Montelukast Chewable Tablets
Montelukast Granules
Montelukast Tablets

Ph Eur

DEFINITION
Sodium [1-[[[(1*R*)-1-[3-[(*E*)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfanyl]methyl]cyclopropyl]acetate.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white, hygroscopic powder.

Solubility
Freely soluble in water and in methylene chloride, freely soluble to very soluble in ethanol (96 per cent).

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *montelukast sodium CRS.*

B. Enantiomeric purity (see Tests).

C. Ignite 0.1 g in a crucible until an almost white residue is obtained. Take up the residue in 2 mL of *water R* and filter. The filtrate gives reaction (a) of sodium (*2.3.1*).

TESTS
Enantiomeric purity
Liquid chromatography (*2.2.29*). *Carry out the test protected from light. Prepare the solutions in amber flasks.*

Solvent mixture *acetonitrile R, water R* (50:50 *V/V*).

Test solution Dissolve 50 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve the contents of a vial of *montelukast racemate CRS* in 1.0 mL of the solvent mixture.

Column:
— *size:* l = 0.15 m, Ø = 4.0 mm;
— *stationary phase: silica gel AGP for chiral chromatography R* (5 µm);
— *temperature:* 30 °C.

Mobile phase:
— *mobile phase A:* 2.3 g/L solution of *ammonium acetate R* adjusted to pH 5.7 with *glacial acetic acid R;*
— *mobile phase B: acetonitrile R, methanol R* (40:60 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	70 → 60	30 → 40
30 - 35	60	40

Flow rate 0.9 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 10 µL.

Relative retention With reference to montelukast (retention time = about 25 min): impurity A = about 0.7.

System suitability:
— *resolution:* minimum 2.9 between the peaks due to impurity A and montelukast in the chromatogram obtained with reference solution (b);
— *signal-to-noise ratio:* minimum 10 for the principal peak in the chromatogram obtained with reference solution (a).

Calculate the percentage content of impurity A using the following expression:

$$100\left(\frac{r_1}{r_2}\right)$$

r_1 = area of the peak due to impurity A in the chromatogram obtained with the test solution;
r_2 = sum of the areas of the peaks due to montelukast and impurity A in the chromatogram obtained with the test solution.

Limit:
— *impurity A:* maximum 0.2 per cent.

Related substances
Liquid chromatography (*2.2.29*): use the normalisation procedure. *Carry out the test protected from light. Prepare the solutions in amber flasks.*

Solvent mixture *water R, methanol R* (10:90 *V/V*).

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Test solution (b) Dilute 10.0 mL of test solution (a) to 100.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 20.0 mL with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *montelukast for peak identification CRS* (containing impurities B, C, D, E and F) in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (c) In order to prepare impurity G *in situ*, transfer 1 mL of reference solution (b) to a colourless glass vial and expose to ambient light for about 20 min.

Reference solution (d) Dissolve 65.0 mg of *montelukast dicyclohexylamine CRS* in the solvent mixture and dilute to 50.0 mL with the solvent mixture. Dilute 10.0 mL of the solution to 100.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.05 m, Ø = 4.6 mm;
— *stationary phase*: *phenylsilyl silica gel for chromatography R* (1.8 µm);
— *temperature*: 30 °C.

Mobile phase:
— *mobile phase A*: mix 1.5 mL of *trifluoroacetic acid R* and 1000 mL of *water R*;
— *mobile phase B*: mix 1.5 mL of *trifluoroacetic acid R* and 1000 mL of *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	60	40
3 - 16	60 → 49	40 → 51

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 238 nm.

Injection 10 µL of test solution (a) and reference solutions (a) and (c); 20 µL of reference solution (b).

Identification of impurities Use the chromatogram supplied with *montelukast for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B, C, D, E and F; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity G.

Relative retention With reference to montelukast (retention time = about 7 min): impurity C = about 0.4; impurity G = about 0.8; impurities D and E = about 0.9; impurity F = about 1.2; impurity B = about 1.9.

System suitability Reference solution (c):
— *resolution*: minimum 2.5 between the peaks due to impurity G and montelukast; minimum 1.5 between the peaks due to montelukast and impurity F.

Limits:
— *impurity B*: maximum 0.3 per cent;
— *impurity C*: maximum 0.2 per cent;
— *impurities F, G*: for each impurity, maximum 0.15 per cent;
— *sum of impurities D and E*: maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.6 per cent;
— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)
Maximum 4.0 per cent, determined on 0.300 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution (b) and reference solution (d).

Calculate the percentage content of $C_{35}H_{35}ClNNaO_3S$ using the following expression:

$$\frac{A_1 \times m_2 \times 79.24 \times p}{A_2 \times m_1 \times (100 - a)}$$

A_1 = area of the principal peak in the chromatogram obtained with test solution (b);
A_2 = area of the principal peak in the chromatogram obtained with reference solution (d);
m_1 = mass of the substance to be examined used to prepare test solution (a), in milligrams;
m_2 = mass of *montelukast dicyclohexylamine CRS* used to prepare reference solution (d), in milligrams;
p = declared percentage content of *montelukast dicyclohexylamine CRS*;
a = percentage content of water in the substance to be examined.

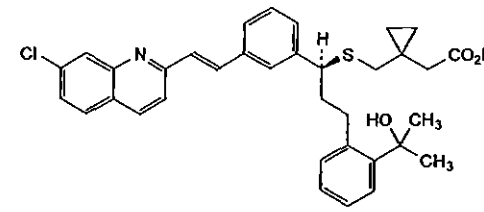
STORAGE

In an airtight container, protected from light.

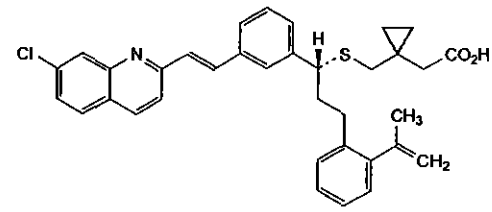
IMPURITIES

Specified impurities *A, B, C, D, E, F, G.*

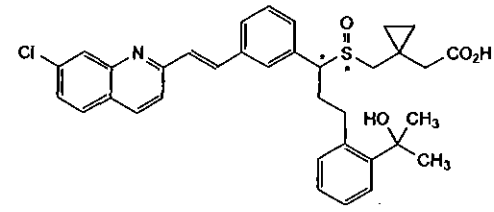
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *H, I.*



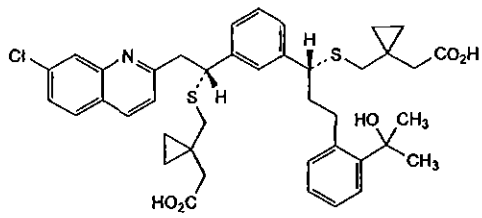
A. [1-[[[(1*S*)-1-[3-[(*E*)-2-(7-chloroquinolin-2-yl) ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl] propyl]sulfanyl]methyl]cyclopropyl]acetic acid,



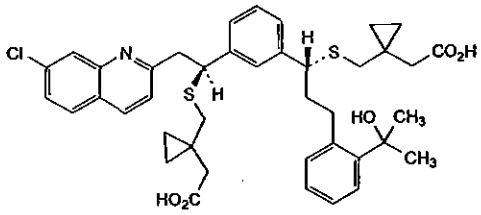
B. [1-[[[(1*R*)-1-[3-[(*E*)-2-(7-chloroquinolin-2-yl) ethenyl]phenyl]-3-[2-(1-methylethenyl)phenyl]propyl] sulfanyl]methyl]cyclopropyl]acetic acid,



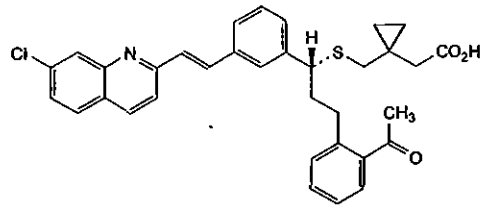
C. [1-[[[1-[3-[(*E*)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfinyl] methyl]cyclopropyl]acetic acid,



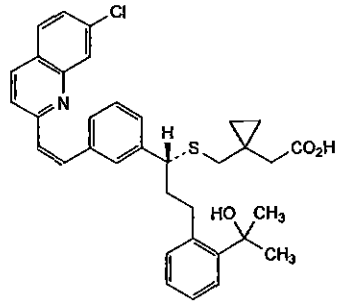
D. 1-[[[(1*R*)-1-[3-[(1*R*)-1-[[[1-(carboxymethyl) cyclopropyl]methyl]sulfanyl]-2-(7-chloroquinolin-2-yl) ethyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl] propyl]sulfanyl]methyl]cyclopropyl]acetic acid,



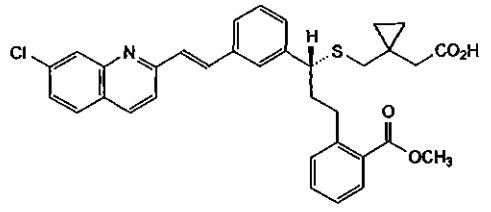
E. 1-[[[(1*R*)-1-[3-[(1*S*)-1-[[[1-(carboxymethyl) cyclopropyl]methyl]sulfanyl]-2-(7-chloroquinolin-2-yl) ethyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl] propyl]sulfanyl]methyl]cyclopropyl]acetic acid,



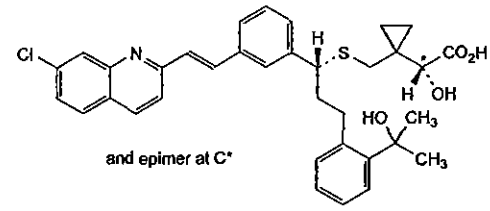
F. [1-[[[(1*R*)-3-(2-acetylphenyl)-1-[3-[(*E*)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]propyl]sulfanyl] methyl]cyclopropyl]acetic acid,



G. [1-[[[(1*R*)-1-[3-[(*Z*)-2-(7-chloroquinolin-2-yl) ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl] propyl]sulfanyl]methyl]cyclopropyl]acetic acid,



H. [1-[[[(1*R*)-1-[3-[(*E*)-2-(7-chloroquinolin-2-yl) ethenyl]phenyl]-3-[2-(methoxycarbonyl)phenyl]propyl] sulfanyl]methyl]cyclopropyl]acetic acid,

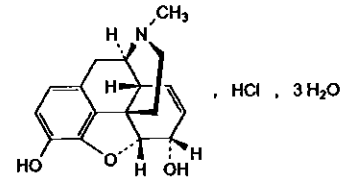


I. (2*RS*)-[1-[[[(1*R*)-1-[3-[(*E*)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl) phenyl]propyl]sulfanyl]methyl]cyclopropyl](hydroxy)acetic acid.

Ph Eur

Morphine Hydrochloride

(Ph. Eur. monograph 0097)



$C_{17}H_{20}ClNO_3,3H_2O$ 375.8 *6055-06-7*

Action and use
Opioid receptor agonist; analgesic.

Preparation
Morphine Suppositories

Ph Eur

DEFINITION

7,8-Didehydro-4,5α-epoxy-17-methylmorphinan-3,6α-diol hydrochloride trihydrate.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder or colourless, silky needles or cubical masses, efflorescent in a dry atmosphere.

Solubility
Soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in toluene.

IDENTIFICATION

First identification: A, E.

Second identification: B, C, D, E.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *morphine hydrochloride trihydrate CRS.*

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Solution A Dissolve 25.0 mg in *water R* and dilute to 25.0 mL with the same solvent.

Test solution (a) Dilute 10.0 mL of solution A to 100.0 mL with *water R.*

Test solution (b) Dilute 10.0 mL of solution A to 100.0 mL with *0.1 M sodium hydroxide.*

Spectral range 250-350 nm for test solutions (a) and (b).

Absorption maximum At 285 nm for test solution (a); at 298 nm for test solution (b).

Specific absorbance at the absorption maximum 37 to 43 for test solution (a); 64 to 72 for test solution (b).

C. To about 1 mg of powdered substance in a porcelain dish add 0.5 mL of *sulfuric acid-formaldehyde reagent R*. A purple colour develops and becomes violet.

D. It gives the reaction of alkaloids (*2.3.1*).

E. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 0.500 g in *carbon dioxide-free water R* and dilute to 25.0 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 or BY_6 (*2.2.2, Method II*).

Acidity or alkalinity

To 10 mL of solution S add 0.05 mL of *methyl red solution R*. Not more than 0.2 mL of *0.02 M sodium hydroxide* or *0.02 M hydrochloric acid* is required to change the colour of the indicator.

Specific optical rotation (*2.2.7*)

−110 to −115 (anhydrous substance), determined on solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.125 g of the substance to be examined in a 1 per cent *V/V* solution of *acetic acid R* and dilute to 50 mL with the same solution.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with a 1 per cent *V/V* solution of *acetic acid R*. Dilute 2.0 mL of this solution to 10.0 mL with a 1 per cent *V/V* solution of *acetic acid R*.

Reference solution (b) Dissolve 5 mg of *morphine for system suitability CRS* (containing impurities B, C, E and F) in a 1 per cent *V/V* solution of *acetic acid R* and dilute to 2 mL with the same solution.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 35 °C.

Mobile phase:
- *mobile phase A*: 1.01 g/L solution of *sodium heptanesulfonate R* adjusted to pH 2.6 with a 50 per cent *V/V* solution of *phosphoric acid R*;
- *mobile phase B*: *methanol R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	85	15
2 - 35	85 → 50	15 → 50
35 - 40	50	50

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 10 µL.

Identification of impurities Use the chromatogram supplied with *morphine for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B, C, E and F.

Relative retention With reference to morphine (retention time = about 12.5 min): impurity F = about 0.95; impurity E = about 1.1; impurity C = about 1.6; impurity B = about 1.9.

System suitability Reference solution (b):
- *peak-to-valley ratio*: minimum 2, where H_p = height above the baseline of the peak due to impurity F and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to morphine.

Limits:
- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.25; impurity C = 0.4; impurity E = 0.5;
- *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);
- *impurities C, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
- *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Water (*2.5.12*)

12.5 per cent to 15.5 per cent, determined on 0.10 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in a mixture of 5 mL of *0.01 M hydrochloric acid* and 30 mL of *ethanol (96 per cent) R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 32.18 mg of $C_{17}H_{20}ClNO_3$.

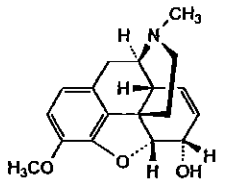
STORAGE

Protected from light.

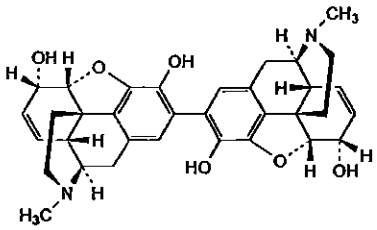
IMPURITIES

Specified impurities *B, C, E.*

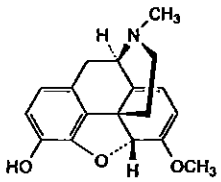
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, D, F.*



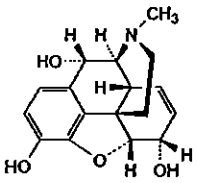
A. 7,8-didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol (codeine),



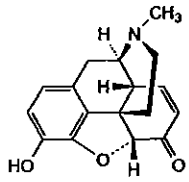
B. 7,7′,8,8′-tetradehydro-4,5α:4′,5′α-diepoxy-17,17′-dimethyl-2,2′-bimorphinanyl-3,3′,6α,6′α-tetrol (2,2′-bimorphine),



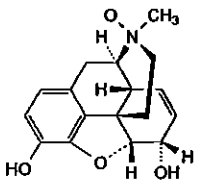
C. 6,7,8,14-tetradehydro-4,5α-epoxy-6-methoxy-17-methylmorphinan-3-ol (oripavine),



D. 7,8-didehydro-4,5α-epoxy-17-methylmorphinan-3,6α,10α-triol (10*S*-hydroxymorphine),



E. 7,8-didehydro-4,5α-epoxy-3-hydroxy-17-methylmorphinan-6-one (morphinone),



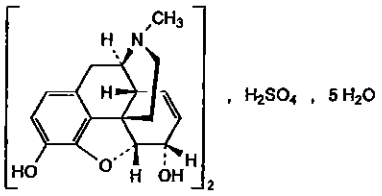
F. (17*S*)-7,8-didehydro-4,5α-epoxy-17-methylmorphinan-3,6α-diol 17-oxide (morphine *N*-oxide).

Ph Eur

Morphine Sulfate

Morphine Sulphate

(Ph. Eur. monograph 1244)



$C_{34}H_{40}N_2O_{10}S,5H_2O$ 759 *6211-15-0*

Action and use
Opioid receptor agonist; analgesic.

Preparations
Morphine Granules for Oral Suspension
Morphine Sulfate Injection
Morphine Sulfate Oral Solution
Morphine Suppositories
Morphine Tablets
Morphine Prolonged-release Capsules
Morphine Prolonged-release Tablets

Ph Eur

DEFINITION

Di(7,8-didehydro-4,5α-epoxy-17-methylmorphinan-3,6α-diol) sulfate pentahydrate.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Soluble in water, very slightly soluble in ethanol (96 per cent), practically insoluble in toluene.

IDENTIFICATION

First identification: A, E.

Second identification: B, C, D, E.

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Dissolve 20 mg in 1 mL of *water R*, add 0.05 mL of *1 M sodium hydroxide* and shake. A precipitate is formed. Filter, wash with 2 quantities, each of 0.5 mL, of *water R* and dry the precipitate at 145 °C for 1 h. Prepare discs using the dried precipitate.

Comparison Repeat the operations using 20 mg of *morphine sulfate CRS*.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Solution A Dissolve 25.0 mg in *water R* and dilute to 25.0 mL with the same solvent.

Test solution (a) Dilute 10.0 mL of solution A to 100.0 mL with *water R*.

Test solution (b) Dilute 10.0 mL of solution A to 100.0 mL with *0.1 M sodium hydroxide*.

Spectral range 250-350 nm for test solutions (a) and (b).

Absorption maximum At 285 nm for test solution (a); at 298 nm for test solution (b).

Specific absorbance at the absorption maximum 37 to 43 for test solution (a); 64 to 72 for test solution (b).

C. To about 1 mg of powdered substance in a porcelain dish add 0.5 mL of *sulfuric acid-formaldehyde reagent R*. A purple colour develops and becomes violet.

D. It gives the reaction of alkaloids (*2.3.1*).

E. It gives the reactions of sulfates (*2.3.1*).

TESTS

Solution S

Dissolve 0.500 g in *carbon dioxide-free water R* and dilute to 25.0 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 or BY_6 (*2.2.2, Method II*).

Acidity or alkalinity

To 10 mL of solution S add 0.05 mL of *methyl red solution R*. Not more than 0.2 mL of *0.02 M sodium hydroxide* or *0.02 M hydrochloric acid* is required to change the colour of the indicator.

Specific optical rotation (*2.2.7*)

−107 to −110 (anhydrous substance), determined on solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.125 g of the substance to be examined in a 1 per cent *V/V* solution of *acetic acid R* and dilute to 50 mL with the same solution.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with a 1 per cent *V/V* solution of *acetic acid R*. Dilute 2.0 mL of this solution to 10.0 mL with a 1 per cent *V/V* solution of *acetic acid R*.

Reference solution (b) Dissolve 5 mg of *morphine for system suitability CRS* (containing impurities B, C, E and F) in a 1 per cent *V/V* solution of *acetic acid R* and dilute to 2 mL with the same solution.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 35 °C.

Mobile phase:
- *mobile phase A*: 1.01 g/L solution of *sodium heptanesulfonate R* adjusted to pH 2.6 with a 50 per cent *V/V* solution of *phosphoric acid R*;
- *mobile phase B*: *methanol R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	85	15
2 - 35	85 → 50	15 → 50
35 - 40	50	50

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 10 µL.

Identification of impurities Use the chromatogram supplied with *morphine for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B, C, E and F.

Relative retention With reference to morphine (retention time = about 12.5 min): impurity F = about 0.95; impurity E = about 1.1; impurity C = about 1.6; impurity B = about 1.9.

System suitability Reference solution (b):
- *peak-to-valley ratio*: minimum 2, where H_p = height above the baseline of the peak due to impurity F and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to morphine.

Limits:
- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.25; impurity C = 0.4; impurity E = 0.5;
- *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);
- *impurities C, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
- *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Iron (*2.4.9*)

Maximum 5 ppm.

Dissolve the residue from the test for sulfated ash in *water R* and dilute to 10.0 mL with the same solvent.

Water (*2.5.12*)

10.4 per cent to 13.4 per cent, determined on 0.10 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 2.0 g.

ASSAY

Dissolve 0.500 g in 120 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 66.88 mg of $C_{34}H_{40}N_2O_{10}S$.

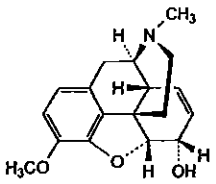
STORAGE

Protected from light.

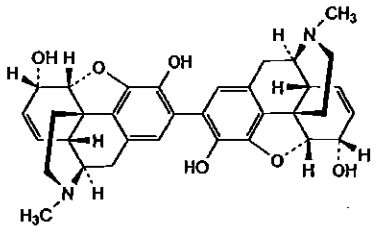
IMPURITIES

Specified impurities *B, C, E.*

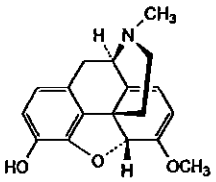
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, D, F.*



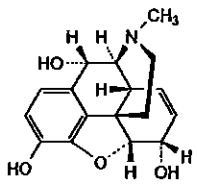
A. 7,8-didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol (codeine),



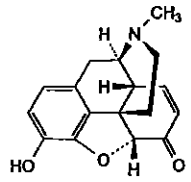
B. 7,7′,8,8′-tetradehydro-4,5α:4′,5′α-diepoxy-17,17′-dimethyl-2,2′-bimorphinanyl-3,3′,6α,6′α-tetrol (2,2′-bimorphine),



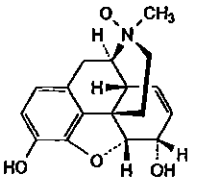
C. 6,7,8,14-tetradehydro-4,5α-epoxy-6-methoxy-17-methylmorphinan-3-ol (oripavine),



D. 7,8-didehydro-4,5α-epoxy-17-methylmorphinan-3,6α,10α-triol (10*S*-hydroxymorphine),



E. 7,8-didehydro-4,5α-epoxy-3-hydroxy-17-methylmorphinan-6-one (morphinone),



F. (17*S*)-7,8-didehydro-4,5α-epoxy-17-methylmorphinan-3,6α-diol 17-oxide (morphine *N*-oxide).

Ph Eur

Moulds for Allergen Products

(Ph. Eur. monograph 2626)

Ph Eur

DEFINITION

Moulds for allergen products primarily consist of inactivated mould cultures, which may contain mycelia and/or spores, and may also contain allergenic constituents released into the culture medium. Moulds are microscopic filamentous fungi belonging mainly to the genera *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium*. This monograph also applies to yeast cells, e.g. *Candida*.

PRODUCTION

The method of cultivation, inactivation, harvesting and post-harvesting is described and must ensure the quality, homogeneity and traceability of the mould or yeast. The type of source material harvested (mycelium and/or spores or yeast cells) is specified. Appropriate measures are taken to avoid contamination by foreign species.

Critical cultivation parameters such as temperature and humidity are controlled and monitored. Unless otherwise justified, the culture medium is selected to avoid the presence of materials with potential allergenicity.

Where major changes to the production of the source material take place (e.g. when a new process or supplier of source materials is introduced), such changes are qualified for each species.

Microbial contamination of the mould or yeast should be monitored on a representative number of batches of source material according to a justified sampling plan and each time a new supplier and/or a new process for the source material production is introduced; if a determination of microbial contamination is not applicable, this must be justified. Microbial contamination values and potential increases in microbial contamination are monitored during stability studies, in order to assess this aspect along with the source material characteristics upon storage.

Control methods and acceptance criteria relating to identity and purity of the mould or yeast are established. The acceptance criteria must ensure the consistency of the source material from a qualitative and quantitative point of view. The source material is stored under controlled conditions justified by stability data. The production, as well as the handling of the source material, are such that consistent composition is ensured from batch to batch.

SOURCE MATERIAL REFERENCE BATCH

An appropriate reference batch is established for each species. The nature of the reference batch depends on the testing approach to verify batch-to-batch consistency and to establish acceptable quality. The reference batch may be, for example, an internal reference preparation (if available), a source material extract or a sample of a production batch. Its characterisation must be described. The extent of characterisation of the reference batch depends on the species, knowledge of the allergenic components and availability of suitable reagents. The reference batch is stored under controlled conditions ensuring its stability.

BATCH-TO-BATCH CONSISTENCY

To establish batch-to-batch consistency, one or more of the following tests are performed on each batch. The choice of tests must be justified.

Total protein (*2.5.33*)

Protein profile
Determined by using suitable electrophoresis methods (*2.2.31, 2.2.54*).

Allergen profile
Relevant allergenic components are identified by means of suitable techniques using allergen-specific antibodies.

Major allergen content
Determined by using suitable immunochemical methods (*2.7.1*) such as enzyme-linked immunosorbent assay (ELISA).

Total allergenic activity
Determined by testing inhibition of the binding capacity of specific immunoglobulin E antibodies or by a suitable equivalent *in vitro* method.

CHARACTERS
Moulds or yeasts for allergen products are supplied as coloured powders or liquid suspensions.

IDENTIFICATION
The identity of the mould or yeast is confirmed by its relevant macroscopic (e.g. colour, appearance) and microscopic (e.g. appearance of the mycelium, spores, specialised cells) morphological characteristics specific to each species, in comparison to those of a reference batch or reference documents. Identity may also be confirmed using protein analysis methods (e.g. protein profile, individual allergen test) or genetic identification, if performed by generally accepted methods.

TESTS
Foreign species
The absence of foreign species is determined by macroscopic and microscopic examination.

Water (*2.5.12* or *2.5.32*) **or loss on drying** (*2.2.32*)
The water content of dried material is determined; specification limits must be supported by batch analysis and stability data.

Mycotoxins
For moulds, the content of mycotoxin is determined, unless otherwise justified.

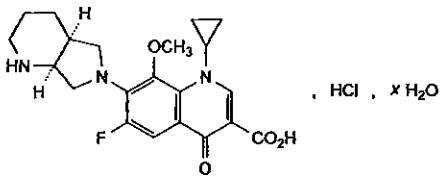
STORAGE
Moulds or yeasts for allergen products are stored under controlled conditions justified by stability data.

LABELLING
The label states the species of the mould or yeast.

Ph Eur

Moxifloxacin Hydrochloride

(Ph. Eur. monograph 2254)



$C_{21}H_{25}ClFN_3O_4,xH_2O$ 437.9 (anhydrous substance)

Anhydrous moxifloxacin hydrochloride *186826-86-8*

Action and use
Fluoroquinolone antibacterial.

Preparation
Moxifloxacin Intracameral Injection

Ph Eur

DEFINITION
1-Cyclopropyl-6-fluoro-8-methoxy-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).
It may be anhydrous or contain a variable quantity of water.

CHARACTERS
Appearance
Light yellow or yellow powder or crystals.

Solubility
Sparingly soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in acetone.
It shows polymorphism (*5.9*).

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison moxifloxacin hydrochloride CRS.
If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *anhydrous ethanol R*, evaporate to dryness and record new spectra using the residues.

B. Enantiomeric purity (see Tests).

C. Dissolve 50 mg in 5 mL of *water R*, add 1 mL of *dilute nitric acid R*, mix, allow to stand for 5 min and filter. The filtrate gives reaction (a) of chlorides (*2.3.1*).

TESTS
Appearance of solution
The solution is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution GY_2 (*2.2.2, Method II*). If intended for use in the manufacture of parenteral preparations, the solution is clear (*2.2.1*) and not more intensely coloured than reference solution GY_2 (*2.2.2, Method II*).
Dissolve 1.0 g in 20 mL of *dilute sodium hydroxide solution R*.

pH (*2.2.3*)
3.9 to 4.6.
Dissolve 0.10 g in 50 mL of *carbon dioxide-free water R*.

Enantiomeric purity
Liquid chromatography (*2.2.29*).

Buffer solution Dissolve 2.49 g of *anhydrous copper sulfate R* and 2.6 g of *isoleucine R* in 1000 mL of *water for chromatography R*.

Test solution Dissolve 5.0 mg of the substance to be examined in *water R* and dilute to 5.0 mL with the same solvent.

Reference solution (a) Dilute 3.0 mL of the test solution to 200.0 mL with *water R*. Dilute 1.0 mL of this solution to 10.0 mL with *water R*.

Reference solution (b) Dissolve 5 mg of *moxifloxacin for system suitability CRS* (containing impurity G) in 5 mL of *water R*.

Column:
— *size*: l = 0.25 m, Ø = 4.0 mm;
— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *methanol R*, buffer solution (25:75 *V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 293 nm.

Injection 20 µL.

Run time 1.5 times the retention time of moxifloxacin.

Identification of impurities Use the chromatogram supplied with *moxifloxacin for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity G.

Relative retention With reference to moxifloxacin (retention time = about 16 min): impurity G = about 0.9.

System suitability Reference solution (b):
— *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to moxifloxacin.

Calculation of percentage content:
— for impurity G, use the concentration of moxifloxacin hydrochloride in reference solution (a).

Limit:
— *impurity G*: maximum 0.15 per cent.

Related substances

Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Solution A Dissolve 0.50 g of *tetrabutylammonium hydrogen sulfate R* and 1.0 g of *potassium dihydrogen phosphate R* in about 500 mL of *water R*. Add 2 mL of *phosphoric acid R* and 0.050 g of *anhydrous sodium sulfite R* and dilute to 1000 mL with *water R*.

Test solution (a) Dissolve 50.0 mg of the substance to be examined in solution A and dilute to 50.0 mL with solution A.

Test solution (b) Dilute 1.0 mL of test solution (a) to 10.0 mL with solution A.

Reference solution (a) Dilute 1.0 mL of test solution (a) to 100.0 mL with solution A. Dilute 1.0 mL of this solution to 10.0 mL with solution A.

Reference solution (b) Dissolve 50.0 mg of *moxifloxacin hydrochloride CRS* in solution A and dilute to 50.0 mL with solution A. Dilute 1.0 mL of the solution to 10.0 mL with solution A.

Reference solution (c) Dissolve 5 mg of *moxifloxacin for peak identification A CRS* (containing impurities A, B and E) in 5 mL of solution A.

Reference solution (d) Dissolve 2 mg of *moxifloxacin for peak identification B CRS* (containing impurity F) in 2 mL of solution A.

Column:
— *size*: l = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated end-capped phenylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 45 °C.

Mobile phase Mix 28 volumes of *methanol R* and 72 volumes of a solution containing 0.5 g/L of *tetrabutylammonium hydrogen sulfate R*, 1.0 g/L of *potassium dihydrogen phosphate R* and 3.4 g/L of *phosphoric acid R*.

Flow rate 1.3 mL/min.

Detection Spectrophotometer at 293 nm.

Injection 10 µL of test solution (a) and reference solutions (a), (c) and (d).

Run time 2.5 times the retention time of moxifloxacin.

Identification of impurities Use the chromatogram supplied with *moxifloxacin for peak identification A CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B and E; use the chromatogram supplied with *moxifloxacin for peak identification B CRS* and the chromatogram obtained with reference solution (d) to identify the peak due to impurity F.

Relative retention With reference to moxifloxacin (retention time = about 11 min): impurity F = about 0.9; impurity A = about 1.1; impurity B = about 1.3; impurity E = about 1.7.

System suitability Reference solution (c):
— *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to moxifloxacin.

Limits:
— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 1.4; impurity E = 3.5;
— *impurities B, E, F*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)

Maximum 4.5 per cent, determined on 0.200 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (b).

Calculate the percentage content of $C_{21}H_{25}ClFN_3O_4$ taking into account the assigned content of *moxifloxacin hydrochloride CRS*.

STORAGE

In an airtight container, protected from light.

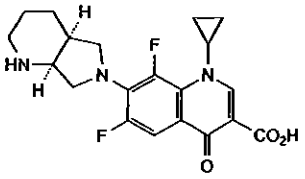
LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

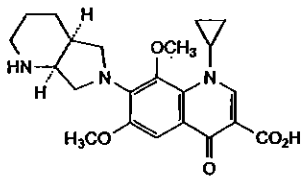
IMPURITIES

Specified impurities *B, E, F, G.*

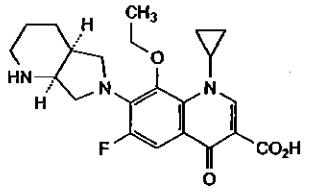
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, C, D, H.*



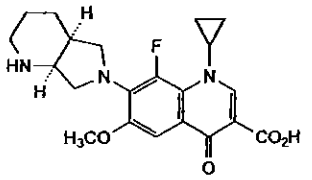
A. 1-cyclopropyl-6,8-difluoro-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid,



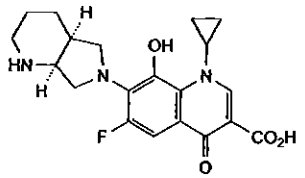
B. 1-cyclopropyl-6,8-dimethoxy-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid,



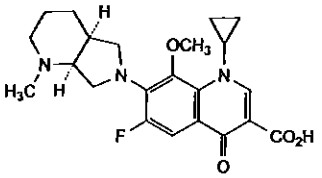
C. 1-cyclopropyl-8-ethoxy-6-fluoro-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid,



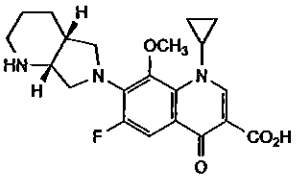
D. 1-cyclopropyl-8-fluoro-6-methoxy-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid,



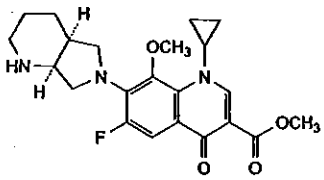
E. 1-cyclopropyl-6-fluoro-8-hydroxy-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid,



F. 1-cyclopropyl-6-fluoro-8-methoxy-7-[(4a*S*,7a*S*)-1-methyloctahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid,



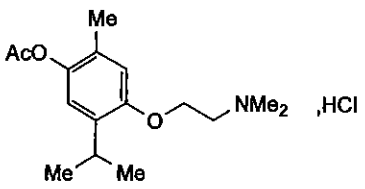
G. 1-cyclopropyl-6-fluoro-8-methoxy-7-[(4a*R*,7a*R*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid,



H. methyl 1-cyclopropyl-6-fluoro-8-methoxy-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylate.

Ph Eur

Moxisylyte Hydrochloride



$C_{16}H_{25}NO_3,HCl$ 315.8 *964-52-3*

Action and use

Alpha-adrenoceptor antagonist.

Preparation

Moxisylyte Tablets

DEFINITION

Moxisylyte Hydrochloride is 4-(2-dimethylaminoethoxy)-5-isopropyl-2-methylphenyl acetate hydrochloride. It contains

not less than 99.0% and not more than 101.0% of $C_{16}H_{25}NO_3,HCl$, calculated with reference to the dried substance.

CHARACTERISTICS

A white, crystalline powder.

Freely soluble in *water* and in *chloroform*; soluble in *ethanol (96%)*; practically insoluble in *ether* and in petroleum spirit.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of moxisylyte hydrochloride *(RS 238)*.

B. Yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH of a 5% w/v solution, 4.5 to 5.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using solutions in the mobile phase containing (1) 0.0010% w/v of *2-(6-hydroxythymoxy) ethyldimethylamine hydrochloride BPCRS* and 0.00050% w/v of *2-thymoxyethyldimethylamine hydrochloride BPCRS* and (2) 0.2% w/v of the substance being examined. For solution (3) dilute 1 volume of solution (2) to 100 volumes. For solution (4) dilute 1 volume of solution (3) to 10 volumes.

The chromatographic procedure may be carried out using (a) a stainless steel column (30 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (µBondapak C18 is suitable), (b) 0.005M *sodium hexanesulfonate* in a mixture of 2 volumes of *glacial acetic acid*, 185 volumes of *water* and 315 volumes of *methanol* as the mobile phase with a flow rate of 1.0 mL per minute and (c) a detection wavelength of 276 nm.

The peaks in the chromatogram obtained with solution (1) are due to (a) 2-(6-hydroxythymoxy)ethyldimethylamine hydrochloride and (b) 2-thymoxyethyldimethylamine hydrochloride in order of their elution. In the chromatogram obtained with solution (2) the areas of any peaks corresponding to (a) and (b) are not greater than the areas of the corresponding peaks in the chromatogram obtained with solution (1) (0.5% of 2-(6-hydroxy-thymoxy) ethyldimethylamine hydrochloride and 0.25% of 2-thymoxyethyldimethylamine hydrochloride respectively), the area of any peak with a retention time relative to moxisylyte of 2.3 is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1% of 2-(6-chlorothymoxy)ethyldimethyl-amine hydrochloride) and the area of any peak with a retention time relative to moxisylyte of 0.9 is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.1% of 2-(6-acetylthymoxy)ethyldimethylamine).

Loss on drying

When dried to constant weight at 105°, loses not more than 0.5% of its weight. Use 1 g.

Sulfated ash

Not more than 0.1%, Appendix IX A.

ASSAY

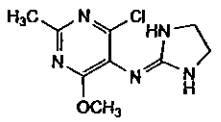
Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.5 g and *1-naphtholbenzein solution* as indicator. Each mL of *0.1M perchloric acid VS* is equivalent to 31.58 mg of $C_{16}H_{25}NO_3,HCl$.

STORAGE

Moxisylyte Hydrochloride should be protected from light.

Moxonidine

(Ph. Eur. monograph 1758)



$C_9H_{12}ClN_5O$ 241.7 *75438-57-2*

Action and use

Imidazoline I_1 receptor agonist; treatment of hypertension.

Preparation

Moxonidine Tablets

Ph Eur

DEFINITION

4-Chloro-*N*-(imidazolidin-2-ylidene)-6-methoxy-2-methylpyrimidin-5-amine.

Content

97.5 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Very slightly soluble in water, sparingly soluble in methanol, slightly soluble in methylene chloride, very slightly soluble in acetonitrile.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Discs.

Comparison *moxonidine CRS*.

TESTS

Related substances

Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.100 g of the substance to be examined in a mixture of equal volumes of *methanol R* and *water R* and dilute to 100.0 mL with the same mixture of solvents.

Reference solution (a) Dissolve 10.0 mg of *moxonidine CRS* in a mixture of equal volumes of *methanol R* and *water R* and dilute to 10.0 mL with the same mixture of solvents.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 100.0 mL with a mixture of equal volumes of *methanol R* and *water R*. Dilute 2.0 mL of this solution to 20.0 mL with a mixture of equal volumes of *methanol R* and *water R*.

Reference solution (c) Dissolve 5.0 mg of *moxonidine impurity A CRS* in a mixture of equal volumes of *methanol R* and *water R* and dilute to 100.0 mL with the same mixture of solvents.

Reference solution (d) Dilute 6.0 mL of reference solution (c) to 100.0 mL with a mixture of equal volumes of *methanol R* and *water R*.

Reference solution (e) Dilute 2.5 mL of reference solution (a) to 50.0 mL with reference solution (c).

Column:

— *size*: *l* = 0.25 m, Ø = 4 mm;

— *stationary phase*: *base-deactivated octylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase Mix 136 volumes of *acetonitrile R* with 1000 volumes of a 3.48 g/L solution of *sodium pentanesulfonate R* previously adjusted to pH 3.5 with *dilute sulfuric acid R*.

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µL of the test solution and reference solutions (b), (d) and (e).

Run time Twice the retention time of moxonidine.

Relative retentions With reference to moxonidine (retention time = about 11.6 min): impurity A = about 0.9; impurity B = about 1.7.

System suitability Reference solution (e):
— *resolution*: minimum of 2 between the peaks due to impurity A and moxonidine.

Limits:
— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.3 per cent);
— *impurity B*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

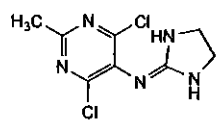
ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

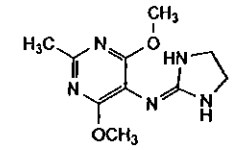
Calculate the percentage content of $C_9H_{12}ClN_5O$ from the areas of the peaks and the declared content of *moxonidine CRS*.

IMPURITIES
Specified impurities *A, B.*

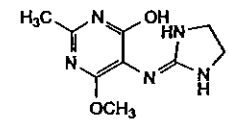
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C, D.*



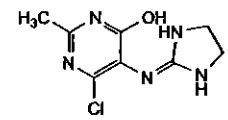
A. 4,6-dichloro-*N*-(imidazolidin-2-ylidene)-2-methylpyrimidin-5-amine (6-chloromoxonidine),



B. *N*-(imidazolidin-2-ylidene)-4,6-dimethoxy-2-methylpyrimidin-5-amine (4-methoxymoxonidine),



C. 5-[(imidazolidin-2-ylidene)amino]-6-methoxy-2-methylpyrimidin-4-ol (4-hydroxymoxonidine),

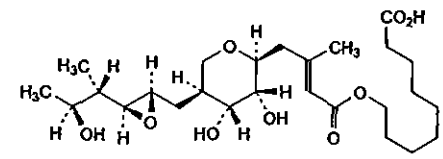


D. 6-chloro-5-[(imidazolidin-2-ylidene)amino]-2-methylpyrimidin-4-ol (6-desmethylmoxonidine).

Ph Eur

Mupirocin

(Ph. Eur. monograph 1450)



$C_{26}H_{44}O_9$ 500.6 *12650-69-0*

Action and use
Antibacterial.

Preparation
Mupirocin Ointment

Ph Eur

DEFINITION
9-[[(2*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-Dihydroxy-5-[[(2*S*,3*S*)-3-[(1*S*,2*S*)-2-hydroxy-1-methylpropyl]oxiranyl]methyl]tetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyl]oxy]nonanoic acid.

Substance produced by the growth of certain strains of *Pseudomonas fluorescens* or obtained by any other means.

Content
93.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white powder.

Solubility
Slightly soluble in water, freely soluble in acetone, in anhydrous ethanol and in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of mupirocin.*

TESTS

pH (*2.2.3*)

3.5 to 4.0 for a freshly prepared saturated solution (about 10 g/L) in *carbon dioxide-free water R.*

Specific optical rotation (*2.2.7*)

−21 to −17 (anhydrous substance).

Dissolve 0.50 g in *methanol R* and dilute to 10.0 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture Mix 50 volumes of *methanol R* and 50 volumes of a 13.6 g/L solution of *sodium acetate R* adjusted to pH 4.0 with *acetic acid R.*

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 50.0 mL with the solvent mixture.

Reference solution (b) Adjust 10 mL of reference solution (a) to pH 2.0 with *hydrochloric acid R* and allow to stand for 20 h.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 20 volumes of *water for chromatography R*, 30 volumes of *tetrahydrofuran R* and 50 volumes of a 10.5 g/L solution of *ammonium acetate R* adjusted to pH 5.7 with *acetic acid R.*

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 240 nm.

Injection 20 µL.

Run time 3.5 times the retention time of mupirocin.

Relative retention With reference to mupirocin: impurity C = about 0.75.

System suitability Reference solution (b):

— *resolution*: minimum 7.0 between the 2nd of the 2 peaks due to hydrolysis products and the peak due to mupirocin.

Limits:

— *impurity C*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (4 per cent);

— *any other impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (6 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Water (*2.5.12*)

Maximum 1.0 per cent, determined on 0.500 g.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in 5 mL of *methanol R* and dilute to 200.0 mL with a 7.5 g/L solution of *ammonium acetate R* adjusted to pH 5.7 with *acetic acid R.*

Reference solution (a) Dissolve 25.0 mg of *mupirocin lithium CRS* in 5 mL of *methanol R* and dilute to 200.0 mL with a 7.5 g/L solution of *ammonium acetate R* adjusted to pH 5.7 with *acetic acid R.*

Reference solution (b) Adjust 10 mL of the test solution to pH 2.0 with *hydrochloric acid R* and allow to stand for 20 h.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 19 volumes of *water for chromatography R*, 32 volumes of *tetrahydrofuran R* and 49 volumes of a 10.5 g/L solution of *ammonium acetate R* adjusted to pH 5.7 with *acetic acid R.*

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µL.

System suitability:

— *resolution*: minimum 7.0 between the 2nd of the 2 peaks due to hydrolysis products and the peak due to mupirocin in the chromatogram obtained with reference solution (b);

— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections of reference solution (a).

Calculate the percentage content of $C_{26}H_{44}O_9$ using the chromatogram obtained with reference solution (a), taking into account the assigned content of *mupirocin lithium CRS* and a conversion factor of 0.988.

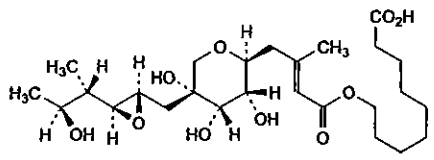
STORAGE

Protected from light.

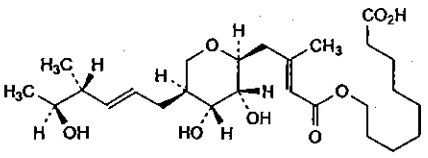
IMPURITIES

Specified impurities C.

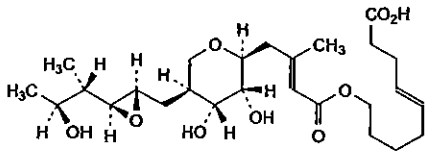
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) A, B, D, E, F.



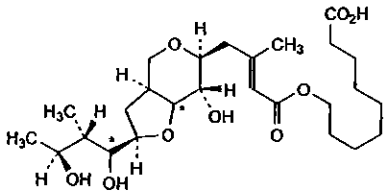
A. 9-[[(2*E*)-4-[(2*S*,3*R*,4*S*,5*R*)-3,4,5-trihydroxy-5-[[(2*S*,3*S*)3-[(1*S*,2*S*)-2-hydroxy-1-methylpropyl]oxiranyl]methyl]tetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyl]oxy]nonanoic acid (pseudomonic acid B),



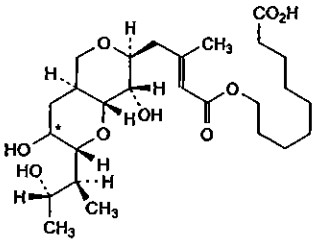
B. 9-[[(2*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5-[(2*E*,4*R*,5*S*)-5-hydroxy-4-methylhex-2-enyl]tetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyl]oxy]nonanoic acid (pseudomonic acid C),



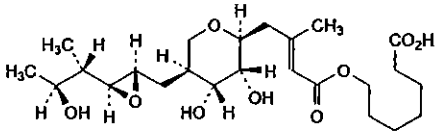
C. (4*E*)-9-[[(2*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5-[[(2*S*,3*S*)3-[(1*S*,2*S*)-2-hydroxy-1-methylpropyl]oxiranyl]methyl]tetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyl]oxy]non-4-enoic acid (pseudomonic acid D),



D. 9-[[(2*E*)-4-[(2*R*,3a*S*,6*S*,7*S*)-2-[(2*S*,3*S*)-1,3-dihydroxy-2-methylbutyl]-7-hydroxyhexahydro-4*H*-furo[3,2-*c*]pyran-6-yl]-3-methylbut-2-enoyl]oxy]nonanoic acid,



E. 9-[[(2*E*)-4-[(2*R*,3*RS*,4a*S*,7*S*,8*S*,8a*R*)-3,8-dihydroxy-2-[(1*S*,2*S*)-2-hydroxy-1-methylpropyl]hexahydro-2*H*,5*H*-pyrano[4,3-*b*]pyran-7-yl]-3-methylbut-2-enoyl]oxy]nonanoic acid,

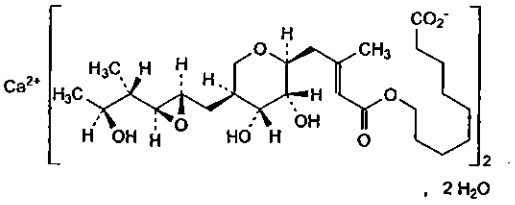


F. 7-[[(2*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5-[[(2*S*,3*S*)-3-[(1*S*,2*S*)-2-hydroxy-1-methylpropyl]oxiranyl]methyl]tetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyl]oxy]heptanoic acid.

Ph Eur

Mupirocin Calcium

(Ph. Eur. monograph 1451)



$C_{52}H_{86}CaO_{18},2H_2O$ 1075 *115074-43-6*

Action and use
Antibacterial.

Preparations
Mupirocin Cream
Mupirocin Nasal Ointment

Ph Eur

DEFINITION

Calcium bis[9-[[(2*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5[[(2*S*,3*S*)-3-[(1*S*,2*S*)-2-hydroxy-1methylpropyl]oxiranyl]methyl]tetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyl]oxy]nonanoate] dihydrate.

Substance produced by the growth of certain strains of *Pseudomonas fluorescens* or obtained by any other means.

Content
93.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Very slightly soluble in water, sparingly soluble in anhydrous ethanol and in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of mupirocin calcium.*

B. It gives reaction (a) of calcium (*2.3.1*).

TESTS

Specific optical rotation (*2.2.7*)
−20 to −16 (anhydrous substance).

Dissolve 0.50 g in *methanol R* and dilute to 10.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture Mix 50 volumes of *methanol R* and 50 volumes of a 13.6 g/L solution of *sodium acetate R* adjusted to pH 4.0 with *acetic acid R*.

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 50.0 mL with the solvent mixture.

Reference solution (b) Adjust 10 mL of reference solution (a) to pH 2.0 with *hydrochloric acid R* and allow to stand for 20 h.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 20 volumes of *water for chromatography R*, 30 volumes of *tetrahydrofuran R* and 50 volumes of a 10.5 g/L solution of *ammonium acetate R* adjusted to pH 5.7 with *acetic acid R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 240 nm.

Injection 20 µL.

Run time 3.5 times the retention time of mupirocin.

Relative retention With reference to mupirocin: impurity C = about 0.75.

System suitability Reference solution (b):
— *resolution*: minimum 7.0 between the 2nd of the 2 peaks due to hydrolysis products and the peak due to mupirocin.

Limits:
— *impurity C*: not more than 1.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (2.5 per cent);
— *any other impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent);
— *total*: not more than 2.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (4.5 per cent);
— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Chlorides (*2.4.4*)
Maximum 0.5 per cent.

Dissolve 10.0 mg in a mixture of 1 mL of *dilute nitric acid R* and 15 mL of *methanol R*.

Water (*2.5.12*)
3.0 per cent to 4.5 per cent, determined on 0.500 g.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in 5 mL of *methanol R* and dilute to 200.0 mL with a 7.5 g/L solution of *ammonium acetate R* adjusted to pH 5.7 with *acetic acid R*.

Reference solution (a) Dissolve 25.0 mg of *mupirocin lithium CRS* in 5 mL of *methanol R* and dilute to 200.0 mL with a 7.5 g/L solution of *ammonium acetate R* adjusted to pH 5.7 with *acetic acid R*.

Reference solution (b) Adjust 10 mL of the test solution to pH 2.0 with *hydrochloric acid R* and allow to stand for 20 h.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 19 volumes of *water for chromatography R*, 32 volumes of *tetrahydrofuran R* and 49 volumes of a 10.5 g/L solution of *ammonium acetate R* adjusted to pH 5.7 with *acetic acid R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µL.

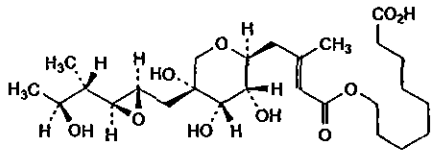
System suitability:
— *resolution*: minimum 7.0 between the 2nd of the 2 peaks due to hydrolysis products and the peak due to mupirocin in the chromatogram obtained with reference solution (b);
— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections of reference solution (a).

Calculate the percentage content of $C_{52}H_{86}CaO_{18}$ using the chromatogram obtained with reference solution (a), taking into account the assigned content of *mupirocin lithium CRS* and a conversion factor of 1.026.

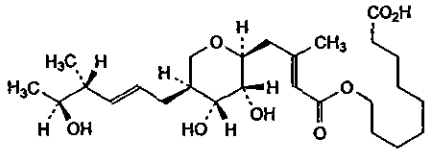
IMPURITIES

Specified impurities *C*.

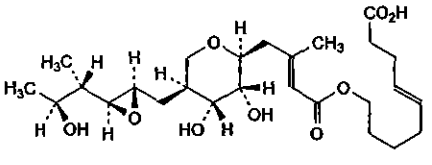
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, D, E, F, G, H, I.*



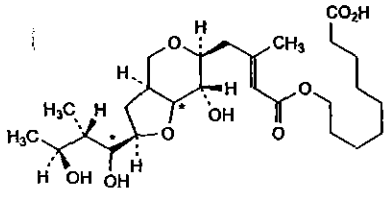
A. 9-[[(2*E*)-4-[(2*S*,3*R*,4*S*,5*R*)-3,4,5-trihydroxy-5-[[(2*S*,3*S*)3-[(1*S*,2*S*)-2-hydroxy-1-methylpropyl]oxiranyl] methyl]tetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyl] oxy]nonanoic acid (pseudomonic acid B),



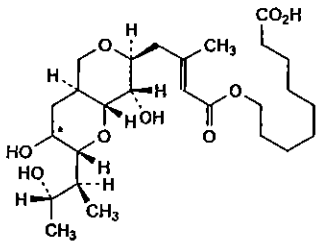
B. 9-[[(2*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5-[(2*E*,4*R*,5*S*)-5-hydroxy-4-methylhex-2-enyl]tetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyl]oxy]nonanoic acid (pseudomonic acid C),



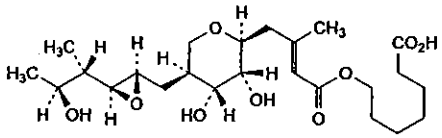
C. (4*E*)-9-[[(2*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5-[[(2*S*,3*S*)3-[(1*S*,2*S*)-2-hydroxy-1-methylpropyl]oxiranyl] methyl]tetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyl] oxy]non-4-enoic acid (pseudomonic acid D),



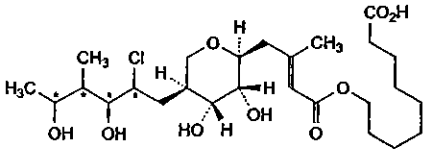
D. 9-[[(2*E*)-4-[(2*R*,3a*S*,6*S*,7*S*)-2-[(2*S*,3*S*)-1,3-dihydroxy-2-methylbutyl]-7-hydroxyhexahydro-4*H*-furo[3,2-*c*]pyran-6-yl]-3-methylbut-2-enoyl]oxy]nonanoic acid,



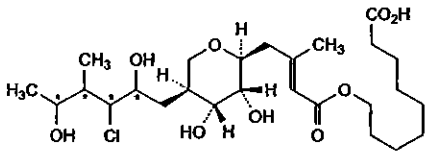
E. 9-[[(2*E*)-4-[(2*R*,3*RS*,4a*S*,7*S*,8*S*,8a*R*)-3,8-dihydroxy-2-[(1*S*,2*S*)-2-hydroxy-1-methylpropyl]hexahydro-2*H*,5*H*-pyrano[4,3-*b*]pyran-7-yl]-3-methylbut-2-enoyl]oxy]nonanoic acid,



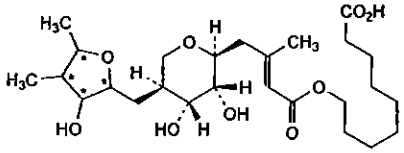
F. 7-[[(2*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5-[[(2*S*,3*S*)-3-[(1*S*,2*S*)-2-hydroxy-1-methylpropyl]oxiranyl]methyl]tetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyl]oxy]heptanoic acid,



G. 9-[[(2*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-5-(2-chloro-3,5-dihydroxy-4-methylhexyl)-3,4-dihydroxytetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyl]oxy]nonanoic acid,



H. 9-[[(2*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-5-(3-chloro-2,5-dihydroxy-4-methylhexyl)-3,4-dihydroxytetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyl]oxy]nonanoic acid,

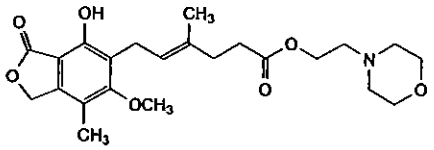


I. 9-[[(2*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5-[(3-hydroxy-4,5-dimethyltetrahydrofuran-2-yl)methyl]tetrahydro-2*H*-pyran-2-yl]-3-methylbut-2-enoyl]oxy]nonanoic acid.

Ph Eur

Mycophenolate Mofetil

(Ph. Eur. monograph 1700)



$C_{23}H_{31}NO_7$ 433.5 *128794-94-5*

Action and use
Inhibitor of nucleic acid synthesis; immunomodulator.

Preparations
Mycophenolate Mofetil Capsules
Mycophenolate Mofetil for Infusion
Mycophenolate Mofetil Oral Suspension
Mycophenolate Mofetil Tablets

Ph Eur

DEFINITION

2-(Morpholin-4-yl)ethyl (4*E*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, freely soluble in acetone, sparingly soluble in anhydrous ethanol.

mp
About 96 °C.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).
Comparison *mycophenolate mofetil CRS.*

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 0.10 g in *ethanol (96 per cent) R* and dilute to 10 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*). *Protect the solutions from light. Prepare the solutions immediately before use, or store them at 4-8 °C. Keep the temperature of the autosampler at 10 °C, allow the temperature of the solutions to equilibrate in the vials for 15 min before injection.*

Test solution Dissolve 20 mg of the substance to be examined in *acetonitrile R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *acetonitrile R.* Dilute 1.0 mL of this solution to 10.0 mL with *acetonitrile R.*

Reference solution (b) Dissolve 5 mg of *mycophenolate mofetil for peak identification CRS* (mycophenolate mofetil with impurities A, B, D, E, F, G and H) in *acetonitrile R* and dilute to 2.5 mL with the same solvent.

Column:
— *size:* l = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm),
— *temperature*: 45 °C.

Mobile phase Mix 350 mL of *acetonitrile R* with a mixture of 650 mL of *water R* and 2.0 mL of *triethylamine R* previously adjusted to pH 5.3 with *dilute phosphoric acid R*.

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 250 nm.

Injection 10 µL.

Run time 3 times the retention time of mycophenolate mofetil.

Relative retention With reference to mycophenolate mofetil (retention time = about 22 min): impurity F = about 0.3; impurity A = about 0.4; impurity H = about 0.5; impurity G = about 0.6; impurity B = about 0.8; impurity D = about 1.2; impurity E = about 1.6.

System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the peaks due to impurity A and impurity H,
— the chromatogram obtained is similar to the chromatogram supplied with *mycophenolate mofetil for peak identification CRS*.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity B by 2.1,
— *impurity F*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),
— *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent),
— *impurities A, D, E, G, H*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
— *total*: not more than 7 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.7 per cent),
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 3 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.400 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 43.35 mg of $C_{23}H_{31}NO_7$.

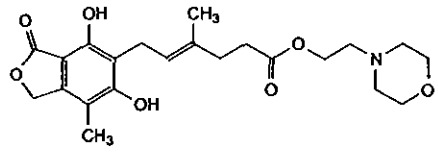
STORAGE

Protected from light.

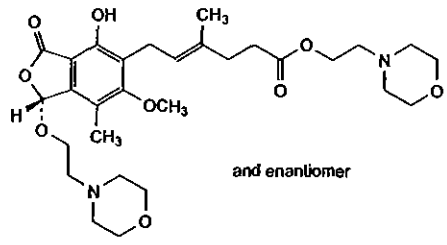
IMPURITIES

Specified impurities *A, B, D, E, F, G, H.*

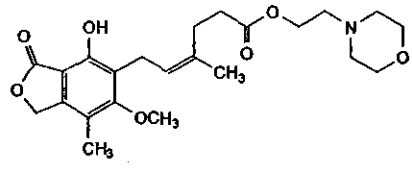
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) C.



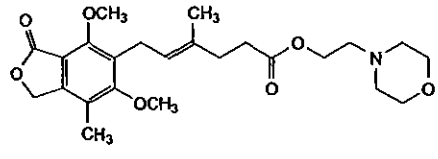
A. 2-(morpholin-4-yl)ethyl (4*E*)-6-(4,6-dihydroxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate,



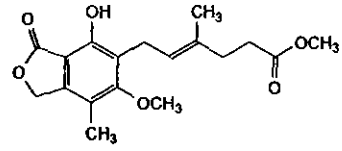
B. 2-(morpholin-4-yl)ethyl (4*E*)-6-{(1*RS*)-4-hydroxy-6-methoxy-7-methyl-1-[2-(morpholin-4-yl)ethoxy]-3-oxo-1,3-dihydroisobenzofuran-5-yl]-4-methylhex-4-enoate,



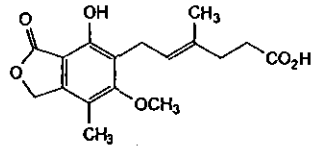
C. 2-(morpholin-4-yl)ethyl (4*Z*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate,



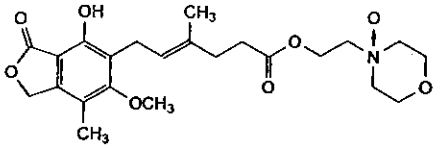
D. 2-(morpholin-4-yl)ethyl (4*E*)-6-(4,6-dimethoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate,



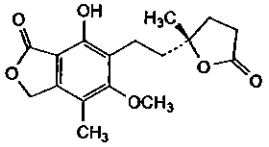
E. methyl (4*E*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate,



F. (4*E*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoic acid (mycophenolic acid),



G. 2-(morpholin-4-yl)ethyl (4*E*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate *N*-oxide,



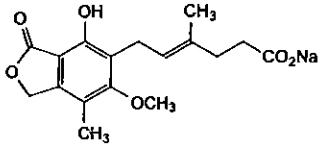
and enantiomer

H. 7-hydroxy-5-methoxy-4-methyl-6-[2-[(2*RS*)-2-methyl-5-oxotetrahydrofuran-2-yl]ethyl]isobenzofuran-1(3*H*)-one.

Ph Eur

Mycophenolate Sodium

(Ph. Eur. monograph 2813)



$C_{17}H_{19}NaO_6$ 342.3 *37415-62-6*

Action and use
Inhibitor of nucleic acid synthesis; immunomodulator.

Ph Eur

DEFINITION

Sodium (4*E*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydro-2-benzofuran-5-yl)-4-methylhex-4-enoate, the sodium salt of a fermentation product.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Slightly soluble in water and in anhydrous ethanol, very slightly soluble in heptane.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *mycophenolate sodium CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

B. It gives reaction (a) of sodium (*2.3.1*).

TESTS

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Buffer solution Dissolve 0.77 g of *ammonium acetate R* in 600 mL of *water for chromatography R*, adjust to pH 4.86 with a 60 g/L solution of *glacial acetic acid R* and dilute to 1000 mL with *water for chromatography R.*

Solvent mixture *water R, acetonitrile R* (40:60 *V/V*).

Test solution Dissolve 25.0 mg of the substance to be examined in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 10.0 mg of *mycophenolate sodium CRS* in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 5 mg of *mycophenolate for system suitability CRS* (containing impurities A and B) in the solvent mixture and dilute to 5 mL with the solvent mixture.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography compatible with 100 per cent aqueous mobile phases R* (3 µm);
— *temperature*: 45 °C.

Mobile phase:
— *mobile phase A*: buffer solution;
— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	71	29
15 - 34	71 → 21.3	29 → 78.7

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 250 nm.

Autosampler Set at 10 °C.

Injection 10 µL of the test solution and reference solutions (a) and (c).

Identification of impurities Use the chromatogram supplied with *mycophenolate for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A and B.

Relative retention With reference to mycophenolic acid (retention time = about 12 min): impurity B = about 0.93; impurity A = about 1.1.

System suitability Reference solution (c):
— *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to mycophenolic acid; minimum 10.0, where H_p = height above the baseline of the peak due to impurity B and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to mycophenolic acid.

Calculation of percentage contents:
— for each impurity, use the concentration of mycophenolate sodium in reference solution (a).

Limits:
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.1 per cent;
— *reporting threshold*: 0.05 per cent.

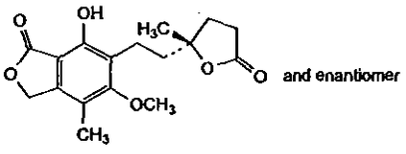
Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

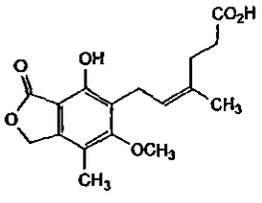
Injection Test solution and reference solution (b).

Calculate the percentage content of $C_{17}H_{19}NaO_6$ taking into account the assigned content of *mycophenolate sodium CRS*.

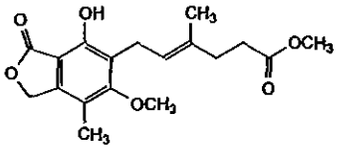
IMPURITIES
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C.*



A. 7-hydroxy-5-methoxy-4-methyl-6-[2-[(2*RS*)-2-methyl-5-oxooxolan-2-yl]ethyl]-2-benzofuran-1(3*H*)-one,



B. (4*Z*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydro-2-benzofuran-5-yl)-4-methylhex-4-enoic acid,

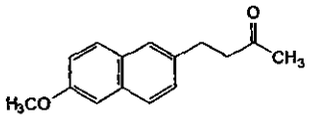


C. methyl (4*E*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydro-2-benzofuran-5-yl)-4-methylhex-4-enoate.

Ph Eur

Nabumetone

(Ph. Eur. monograph 1350)



$C_{15}H_{16}O_2$ 228.3 *42924-53-8*

Action and use
Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Preparations
Nabumetone Oral Suspension
Nabumetone Tablets

Ph Eur

DEFINITION
4-(6-Methoxynaphthalen-2-yl)butan-2-one.

Content
97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, freely soluble in acetone, slightly soluble in methanol.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *nabumetone CRS*.

TESTS
Related substances
Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 50.0 mg of the substance to be examined in *acetonitrile R* and dilute to 10.0 mL with the same solvent.

Test solution (b) Dilute 1.0 mL of test solution (a) to 25.0 mL with *acetonitrile R*. Dilute 1.0 mL of this solution to 5.0 mL with *acetonitrile R*.

Reference solution (a) Dissolve 20.0 mg of *nabumetone CRS* in *acetonitrile R* and dilute to 10.0 mL with the same solvent. Dilute 1.0 mL of this solution to 50.0 mL with *acetonitrile R*.

Reference solution (b) Dilute 0.5 mL of test solution (a) to 100.0 mL with *acetonitrile R*.

Reference solution (c) Dissolve 1.5 mg of *nabumetone impurity F CRS* in *acetonitrile R* and dilute to 100.0 mL with the same solvent.

Reference solution (d) Dissolve 4 mg of *nabumetone impurity D CRS* in *acetonitrile R* and dilute to 100 mL with the same solvent. To 5 mL of this solution, add 5 mL of test solution (b).

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (4 µm);
— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: mix 12 volumes of *tetrahydrofuran R*, 28 volumes of *acetonitrile for chromatography R* and 60 volumes of a 0.1 per cent *V/V* solution of *glacial acetic acid R* in *carbon dioxide-free water R* prepared from *distilled water R*;
— *mobile phase B*: mix 24 volumes of *tetrahydrofuran R*, 56 volumes of *acetonitrile for chromatography R* and 20 volumes of a 0.1 per cent *V/V* solution of *glacial acetic acid R* in *carbon dioxide-free water R* prepared from *distilled water R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 12	100	0
12 - 28	100 → 0	0 → 100
28 - 33	0	100

Flow rate 1 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL of test solution (a) and reference solutions (b), (c) and (d).

Retention time Nabumetone = about 11 min.

System suitability Reference solution (d):
— *resolution*: minimum 1.5 between the peaks due to nabumetone and impurity D.

Limits:
— *impurity F*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent);
— *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *sum of impurities other than F*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.12*)
Maximum 0.2 per cent, determined on 1.000 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution (b) and reference solution (a).

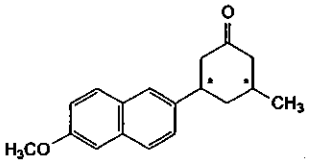
System suitability Reference solution (a):
— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections.

Calculate the percentage content of $C_{15}H_{16}O_2$ from the declared content of *nabumetone CRS*.

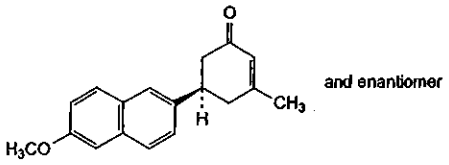
STORAGE
Protected from light.

IMPURITIES
Specified impurities *F*.

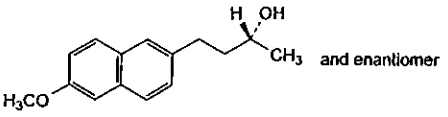
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, E.*



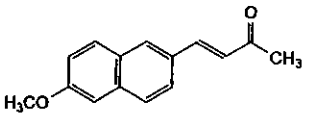
A. 3-(6-methoxynaphthalen-2-yl)-5-methylcyclohexanone,



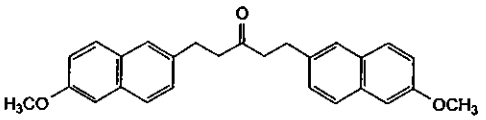
B. (5*RS*)-5-(6-methoxynaphthalen-2-yl)-3-methylcyclohex-2-enone,



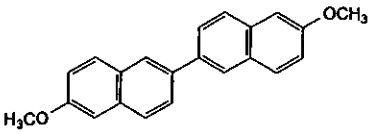
C. (2*RS*)-4-(6-methoxynaphthalen-2-yl)butan-2-ol,



D. (*E*)-4-(6-methoxynaphthalen-2-yl)but-3-en-2-one,



E. 1,5-bis(6-methoxynaphthalen-2-yl)pentan-3-one,

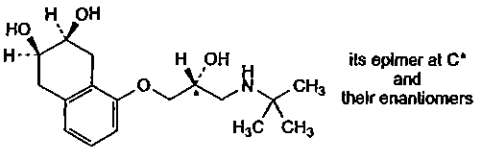


F. 6,6′-dimethoxy-2,2′-binaphthalenyl.

Ph Eur

Nadolol

(Ph. Eur. monograph 1789)



$C_{17}H_{27}NO_4$ 309.4 *42200-33-9*

Action and use
Beta-adrenoceptor antagonist.

Preparation
Nadolol Oral Suspension

Ph Eur

DEFINITION
cis-5-[(2*RS*)-3-[(1,1-Dimethylethyl)amino]-2-hydroxypropoxy]-1,2,3,4-tetrahydronaphthalene-2,3-diol.

It consists of 2 pairs of enantiomers that are present as 2 racemic compounds: racemate A and racemate B.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Slightly soluble in water, freely soluble in ethanol (96 per cent), practically insoluble in acetone.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *nadolol CRS*.

TESTS

Racemate content

Infrared absorption spectrophotometry (*2.2.24*).

Prepare a mull in *liquid paraffin R* of the substance to be examined (dried substance), adjusting the thickness of the mull to give an absorbance reading of 0.6 ± 0.1 at 1587 cm^{-1}. Record the spectrum from 1667 to 1111 cm^{-1}, using *liquid paraffin R* as reference. Measure the absorbance A_a, corresponding to racemate A, at the maximum at 1266 cm^{-1} and the absorbance A_b, corresponding to racemate B, at the maximum at 1250 cm^{-1}. The ratio A_a/A_b is 0.72 to 1.08 (corresponding to racemate A content of between 40 per cent and 60 per cent).

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Solvent mixture *acetonitrile R1, water R* (20:80 *V/V*).

Test solution Dissolve 0.100 g of the substance to be examined in 4.0 mL of the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 50.0 mL with the solvent mixture. Dilute 5.0 mL of this solution to 100.0 mL with the solvent mixture.

Reference solution (b) Dissolve the contents of a vial of *nadolol impurity mixture CRS* (impurities A and D) in 1.0 mL of reference solution (a).

Column:
- *size: l* = 0.25 m, Ø = 4.0 mm;
- *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature:* 40 °C.

Mobile phase:
- *mobile phase A:* 5.6 g/L solution of *sodium octanesulfonate R* adjusted to pH 3.5 with a 300 g/L solution of *phosphoric acid R*;
- *mobile phase B: acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 7	77	23
7 - 30	77 → 65	23 → 35
30 - 35	65 → 55	35 → 45
35 - 55	55	45

Flow rate 1 mL/min.

Detection Spectrophotometer at 206 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram supplied with *nadolol impurity mixture CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and D.

Relative retention With reference to nadolol (retention time = about 15 min): impurity A = about 0.2; impurity C (doublet) = about 0.47 and 0.53; impurity D = about 1.5.

System suitability Reference solution (b):
- *resolution:* minimum 8.0 between the peaks due to nadolol and impurity D.

Limits:
- *correction factor:* for the calculation of content, multiply the sum of the 2 peak areas of impurity C by 0.7;
- *impurities A, C, D:* for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *unspecified impurities:* for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total:* not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit:* 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 2.0 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 3 h.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

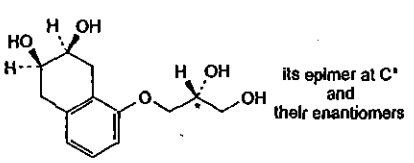
Dissolve 0.250 g in 100 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 30.94 mg of $C_{17}H_{27}NO_4$.

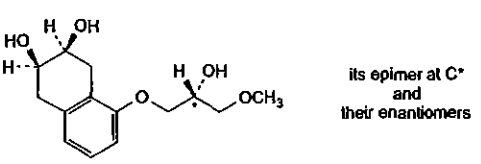
IMPURITIES

Specified impurities *A, C, D.*

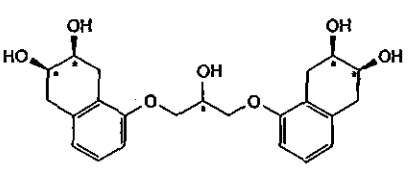
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, E, F, G.*



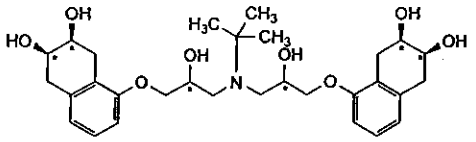
A. *cis*-5-[(2*RS*)-2,3-dihydroxypropoxy]-1,2,3,4-tetrahydronaphthalene-2,3-diol (tetraol),



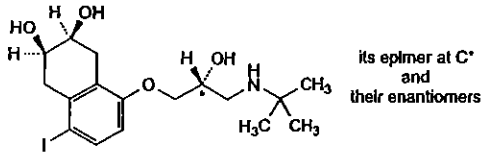
B. *cis*-5-[(2*RS*)-2-hydroxy-3-methoxypropoxy]-1,2,3,4-tetrahydronaphthalene-2,3-diol,



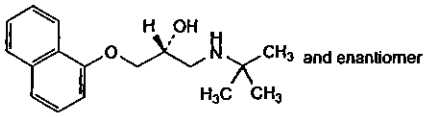
C. 5,5′-[(2*rs*)-2-hydroxypropane-1,3-diylbis(oxy)]bis(*cis*-1,2,3,4-tetrahydronaphthalene-2,3-diol) (3 diastereoisomers),



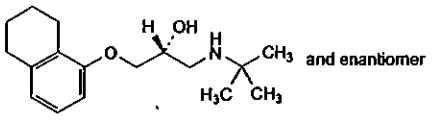
D. 5,5′-[[(1,1-dimethylethyl)imino]bis[(2-hydroxypropane-1,3-diyl)oxy]]bis(*cis*-1,2,3,4-tetrahydronaphthalene-2,3-diol) (10 stereoisomers),



E. *cis*-5-[(2*RS*)-3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-8-iodo-1,2,3,4-tetrahydronaphthalene-2,3-diol,



F. (2*RS*)-1-[(1,1-dimethylethyl)amino]-3-(naphthalen-1-yloxy)propan-2-ol,

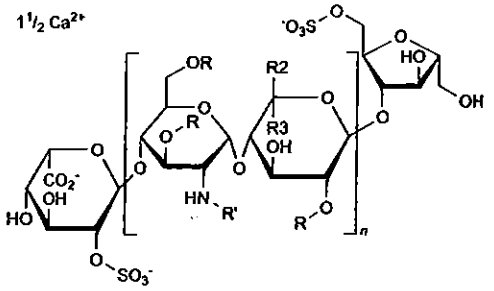


G. (2*RS*)-1-[(1,1-dimethylethyl)amino]-3-[(5,6,7,8-tetrahydronaphthalen-1-yl)oxy]propan-2-ol.

Ph Eur

Nadroparin Calcium

(Ph. Eur. monograph 1134)



Action and use
Low molecular weight heparin.

Ph Eur

DEFINITION
Calcium salt of low-molecular-mass heparin obtained by nitrous acid depolymerisation of heparin from pork intestinal mucosa, followed by fractionation to eliminate selectively most of the chains with a molecular mass lower than 2000. The majority of the components have a 2-*O*-sulfo-α-L-idopyranosuronic acid structure at the non-reducing end and a 6-*O*-sulfo-2,5-anhydro-D-mannitol structure at the reducing end of their chain.

Nadroparin calcium complies with the monograph Low-molecular-mass heparins (0828) with the modifications and additional requirements below.

The mass-average relative molecular mass ranges between 3600 and 5000 with a characteristic value of about 4300.

The degree of sulfatation is about 2 per disaccharide unit.

The potency is not less than 95 IU and not more than 130 IU of anti-factor Xa activity per milligram, calculated with reference to the dried substance. The ratio of anti-factor Xa activity to anti-factor IIa activity is between 2.5 and 4.0.

IDENTIFICATION
Carry out identification test A as described in the monograph *Low-molecular-mass heparins (0828)* using *nadroparin calcium CRS*.

Carry out identification test C as described in the monograph *Low-molecular-mass heparins (0828)*. The following requirements apply.

The mass-average relative molecular mass ranges between 3600 and 5000. The mass percentage of chains lower than 2000 is not more than 15 per cent. The mass percentage of chains between 2000 and 8000 ranges between 75 per cent and 95 per cent. The mass percentage of chains between 2000 and 4000 ranges between 35 per cent and 55 per cent.

TESTS

Appearance of solution
The solution is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution Y_5 (*2.2.2, Method II*).

Dissolve 0.5 g in *water R* and dilute to 10 mL with the same solvent.

Ethanol
Head-space gas chromatography (*2.2.28*).

Internal standard solution Dilute 1.0 mL of *2-propanol R* to 100.0 mL with *water R*. Dilute 1.0 mL of the solution to 50.0 mL with *water R*.

Blank solution 1.0 mL of *water R*.

Test solution (a) To 10.0 mg of the substance to be examined, add 1.0 mL of *water R*.

Test solution (b) To 10.0 mg of the substance to be examined, add 0.50 mL of *water R* and 0.50 mL of the internal standard solution.

Reference solution (a) Dilute 1.0 mL of *anhydrous ethanol R* to 100.0 mL with *water R*. Dilute 0.5 mL of the solution to 20.0 mL with *water R*.

Reference solution (b) To 0.50 mL of reference solution (a), add 0.50 mL of the internal standard solution.

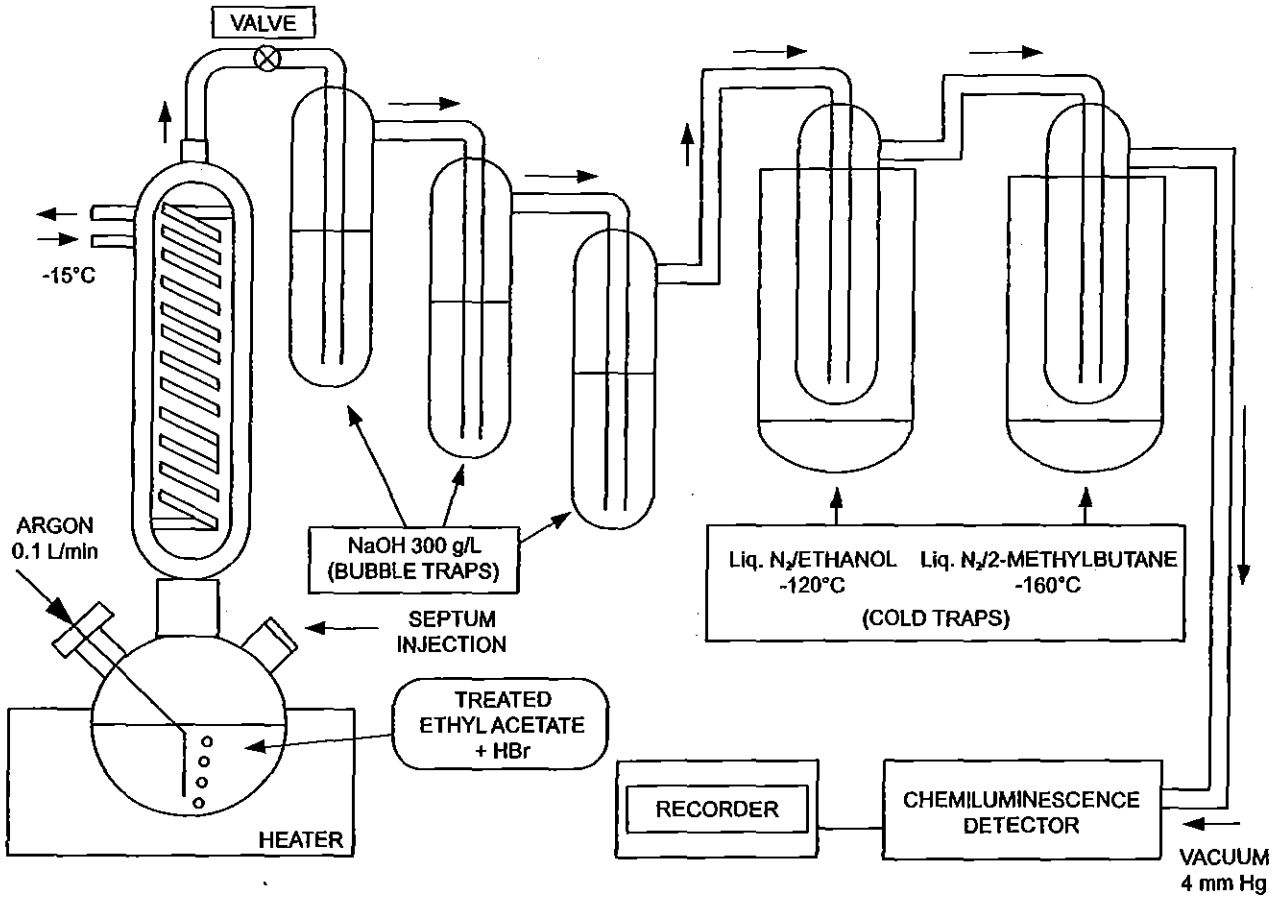
Column:
— *material*: nickel;
— *size*: *l* = 1.5 m, Ø = 2 mm;
— *stationary phase*: *ethylvinylbenzene-divinylbenzene copolymer R* (150-180 µm).

Carrier gas *helium for chromatography R* or *nitrogen for chromatography R*.

Flow rate 30 mL/min.

Static head-space conditions that may be used:
— *equilibration temperature*: 90 °C;
— *equilibration time*: 15 min;
— *pressurisation time*: 1 min.

Temperature:
— *column*: 150 °C;



Flask: round-bottom borosilicate glass flask equipped with a central rodavis joint, a torion joint on the left neck and a 15 mm screw joint on the right neck; septum: silicone material, diameter 14 mm and thickness 3.5 mm.
Condenser: height 21 cm and internal diameter 3 cm, with a lower rodavis joint and an upper torion joint.
Bubble traps: height 24 cm and internal diameter 2.5 cm; internal tubing: length 23 cm and internal diameter 0.5 cm. Equipped with a centrally positioned rotulex mounting with torion joints on the inlet and outlet.
Cold traps: height 16.5 cm and internal diameter 4 cm; internal tubing: length 14 cm and internal diameter 1.3 cm. Equipped with torion joints on the inlet and outlet and placed in an isothermic flask: internal depth 22 cm and internal diameter 8 cm.
Tubing: fluorinated ethylene propylene material, internal diameter 3.2 mm and thickness 0.8 mm.

Figure 1134.-1. – *Apparatus used for the assay of N-NO groups*

— *injection port and detector*: 250 °C.

Detection Flame ionisation.

Identification of peaks Use the chromatogram obtained with reference solution (b) to identify the peaks due to ethanol and 2-propanol.

Retention time Ethanol = about 2.5 min; 2-propanol = about 4 min.

Calculate the percentage content *m/m* of ethanol taking its density at 20 °C to be 0.792 g/mL.

Limit:
— *ethanol*: maximum 1.0 per cent *m/m*.

N-NO groups

Maximum 0.25 ppm.

The content of N-NO-groups is determined by cleavage of the N-NO bond with hydrobromic acid in ethyl acetate under a reflux condenser and detection of the released NO by chemiluminescence.

Description of the apparatus (Figure 1134.-1). Use a 500 mL borosilicate glass round-bottomed flask, above which is attached a condenser which is equipped with:
— on one side, a torion joint through which a stream of *argon R* can be introduced via a cannula;
— on the other side, a screw joint with a piston equipped with a septum through which the reference solution and test solution will be injected.

The round-bottomed flask is connected in series to 3 bubble traps which are themselves connected to 2 cold traps, which are in turn connected to a chemiluminescence detector. Suitable tubing ensures the junctions are leak-free.

Preparation of the chemiluminescence detector Switch on the chemiluminescence detector 48 h before use and start the vacuum pump. The vacuum must be less than 0.5 mm Hg. 1 h before use, open the oxygen valve at a pressure of 0.2 MPa and a flow rate of 9.4 mL/min.

Preparation of the bubble trap In each bubble trap, place 30 mL of a 300 g/L solution of *sodium hydroxide R* in *water R*.

Preparation of the cold traps.
— Trap at −120 °C: Slowly add liquid nitrogen to an isothermic flask containing 250 mL of *anhydrous ethanol R* whilst stirring with a wooden spatula until a paste is obtained. Place the cold trap in the isothermic flask prepared as described.
— Trap at −160 °C: Slowly add liquid nitrogen to an isothermic flask containing 250 mL of *2-methylbutane R* whilst stirring with a wooden spatula until a paste is obtained. Place the cold trap in the isothermic flask prepared as described.

Drying of the 500 mL borosilicate-glass round-bottomed flask and condenser Boil 50 mL of *ethyl acetate R* under reflux for 1 h under *argon R* without connecting the system to the chemiluminescence detector.

Test solution Dry the substance to be examined for 12 h over *diphosphorus pentoxide R* at 60 °C under vacuum. Dissolve 0.10 g of the treated substance to be examined in 1.0 mL of *treated formamide R*. Shake the solution obtained for 30 min.

Reference solution Dilute 0.1 mL of *nitrosodipropylamine solution R* in 6.0 mL of *anhydrous ethanol R*. Dilute 0.1 mL of the solution obtained in 1.0 mL of *treated formamide R*. (This solution is equivalent to 0.05 ppm of N-NO groups).

Place 50 mL of *treated ethyl acetate R* in the dry 500 mL borosilicate glass round-bottomed flask equipped with a septum. Connect the round-bottomed flask to the condenser which has been previously cooled to −15 °C for 2 h.

Connect the *argon R* cannula and adjust the flow rate to 0.1 L/min. Check that the system is leak-free. Only the connector to the chemiluminescence detector remains open in order to avoid excess pressure.

Heat the *treated ethyl acetate R* to boiling.

Evacuate the system by slowly turning the valve of the chemiluminescence detector. At the same time tighten the inlet on the chemiluminescence detector.

When the system is equilibrated, the vacuum reaches 4 mm Hg.

The signal of the zero adjuster on the chemiluminescence detector is set to 10 per cent of the full scale of the recorder.

Through the septum of the 500 mL borosilicate glass round-bottomed flask, sequentially inject 0.5 mL of *water R*, 2.0 mL of *dilute hydrobromic acid R* and then another 2.0 mL of *dilute hydrobromic acid R*, making sure that the recorder pen has returned to the baseline between each injection.

Inject 50.0 µL of the reference solution, then 50.0 µL of the test solution after the recorder pen has returned to the baseline.

Calculate the content of N-NO groups of the substance to be examined.

Free sulfates
Liquid chromatography (*2.2.29*).

Test solution Dissolve 30.0 mg of the substance to be examined in *water R* and dilute to 10.0 mL with the same solvent.

Reference solution Dissolve 1.4787 g of *anhydrous sodium sulfate R* in *water R* and dilute to 1000.0 mL with the same solvent. Dilute 1.0 mL of the solution to 200.0 mL with *distilled water R* (5 ppm of sulfate ions).

Column:
— *size*: *l* = 50 mm, Ø = 4.6 mm;
— *stationary phase*: anion-exchange resin.

Chemical neutralisation system Neutralisation micromembrane in line with the mobile phase for anion detection; continuously pump in counter-flow with a 2.45 g/L solution of *sulfuric acid R*, at a flow rate of 4 mL/min.

Mobile phase:
— *mobile phase A*: 1.91 g/L solution of *disodium tetraborate R*;
— *mobile phase B*: *0.1 M sodium hydroxide*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	100	0
15 - 15.5	100 → 0	0 → 100
15.5 - 25.5	0	100

Flow rate 1.0 mL/min.

Detection Conductivity detector with a sensitivity of 30 µS.

Injection 50 µL.

Identification of peaks Use the chromatogram obtained with the reference solution to identify the principal peak due to the sulfate ion.

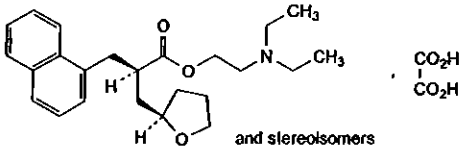
Retention time Sulfate ion = about 7.5 min. Change the composition of the mobile phase, if necessary, to obtain the prescribed retention time.

Limit:
— *free sulfates*: maximum 0.5 per cent.

Ph Eur

Naftidrofuryl Oxalate

(Naftidrofuryl Hydrogen Oxalate, Ph. Eur. monograph 1594)



and stereoisomers

$C_{26}H_{35}NO_7$ 473.6 *3200-06-4*

Action and use
Vasodilator.

Preparation
Naftidrofuryl Capsules

Ph Eur

DEFINITION
Mixture of 4 stereoisomers of 2-(diethylamino)ethyl 2-[(naphthalen-1-yl)methyl]-3-(tetrahydrofuran-2-yl) propanoate hydrogen oxalate.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Freely soluble in water, freely soluble or soluble in ethanol (96 per cent), slightly or sparingly soluble in acetone.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Dissolve 1.0 g in *water R* and dilute to 50 mL with the same solvent. Add 2 mL of *concentrated ammonia R* and shake with 3 quantities, each of 10 mL, of *methylene chloride R*. To the combined lower layers, add *anhydrous sodium sulfate R*, shake, filter and evaporate the filtrate by suitable means at a temperature not exceeding 30 °C. Use the residue obtained.

Comparison *Ph. Eur. reference spectrum of naftidrofuryl.*

B. Dissolve 0.5 g in *water R* and dilute to 10 mL with the same solvent. Add 2.0 mL of *calcium chloride solution R*. A white precipitate is formed. The precipitate dissolves after the addition of 3.0 mL of *hydrochloric acid R*.

TESTS

Absorbance (*2.2.25*)

Maximum 0.1 at 430 nm.

Dissolve 1.5 g in *water R* and dilute to 10 mL with the same solvent. If necessary use an ultrasonic bath.

Related substances

A. Liquid chromatography (*2.2.29*).

Test solution Dissolve 80.0 mg of the substance to be examined in the mobile phase and dilute to 20.0 mL with the mobile phase. Sonicate for 10 s. A precipitate is formed. Filter through a membrane filter (nominal pore size 0.45 µm), discarding the first 5 mL. *Use a freshly prepared solution.*

Reference solution (a) Dissolve 5.0 mg of *naftidrofuryl impurity A CRS* in *acetonitrile R* and dilute to 25.0 mL with the same solvent. Dilute 1.0 mL of the solution to 50.0 mL with the mobile phase.

Reference solution (b) Dissolve 5 mg of *naftidrofuryl impurity B CRS* and 5 mg of the substance to be examined in *acetonitrile R* and dilute to 50 mL with the same solvent. Dilute 1 mL of the solution to 50 mL with the mobile phase.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 60 mL of *methanol R* with 150 mL of *tetrabutylammonium buffer solution pH 7.0 R* and dilute to 1000 mL with *acetonitrile R*.

Flow rate 1 mL/min.

Detection Spectrophotometer at 283 nm.

Injection 20 µL.

Run time 2.3 times the retention time of naftidrofuryl.

Relative retention With reference to naftidrofuryl (retention time = about 7 min): impurity A = about 0.5; impurity B = about 0.8; impurity C = about 1.8.

System suitability Reference solution (b):
- *resolution*: minimum 3.0 between the peaks due to impurity B and naftidrofuryl.

Limits:
- *impurities A, B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
- *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

B. Gas chromatography (*2.2.28*).

Test solution (a) Dissolve 1.0 g of the substance to be examined in *water R* and dilute to 50 mL with the same solvent. Add 2 mL of *concentrated ammonia R* and shake with 3 quantities, each of 10 mL, of *methylene chloride R*. To the combined lower layers, add *anhydrous sodium sulfate R*, shake, filter and evaporate the filtrate by suitable means at a temperature not exceeding 30 °C. Take up the residue with *methylene chloride R* and dilute to 20.0 mL with the same solvent.

Test solution (b) Dilute 1.0 mL of test solution (a) to 10.0 mL with *methylene chloride R*.

Reference solution Dissolve 5 mg of *naftidrofuryl impurity F CRS* in *methylene chloride R* and dilute to 50 mL with the same solvent.

Column:
- *material*: fused silica;
- *size*: *l* = 25 m, Ø = 0.32 mm;
- *stationary phase*: *phenyl(5)methyl(95)polysiloxane R* (film thickness 0.45 µm).

Carrier gas *helium for chromatography R.*

Splitter flow rate 25 mL/min.

Flow rate 2.9 mL/min.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 4	210
	4 - 8	210 → 230
	8 - 18	230 → 260
	18 - 30	260
Injection port		290
Detector		290

Detection Flame ionisation.

Injection 1 µL.

Relative retention With reference to the second eluting peak of naftidrofuryl: impurity D = about 0.14; impurity B = about 0.55 (for the second eluting peak); impurity E = about 0.86; impurity F = about 1.04 (for the second eluting peak).

System suitability Test solution (b):
- *resolution*: minimum 1.0 between the 2 peaks due to the diastereoisomers of naftidrofuryl.

Limits Test solution (a):
- *impurity F*: for the sum of the areas of the 2 peaks, maximum 0.20 per cent of the sum of the areas of the 2 peaks due to naftidrofuryl (0.20 per cent);
- *impurity E*: maximum 0.20 per cent of the sum of the areas of the 2 peaks due to naftidrofuryl (0.20 per cent);
- *impurity D*: maximum 0.10 per cent of the sum of the areas of the 2 peaks due to naftidrofuryl (0.10 per cent);
- *any other impurity*: for each impurity, maximum 0.10 per cent of the sum of the areas of the 2 peaks due to naftidrofuryl (0.10 per cent);
- *total*: maximum 0.50 per cent of the sum of the areas of the 2 peaks due to naftidroduryl (0.50 per cent);
- *disregard limit*: 0.02 per cent of the sum of the areas of the 2 peaks due to naftidrofuryl (0.02 per cent); disregard any peaks due to impurity B.

Diastereoisomer ratio
Gas chromatography (*2.2.28*) as described in test B for related substances.

Limits Test solution (b):
— *first eluting naftidrofuryl diastereoisomer*: minimum 30 per cent of the sum of the areas of the 2 peaks due to naftidrofuryl.

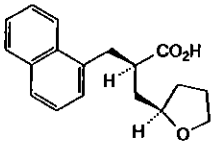
Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

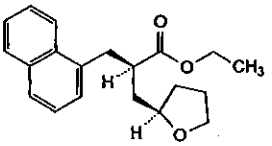
ASSAY
Dissolve 0.350 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 47.36 mg of $C_{26}H_{35}NO_7$.

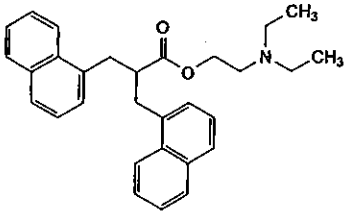
IMPURITIES
Specified impurities *A, B, C, D, E, F.*



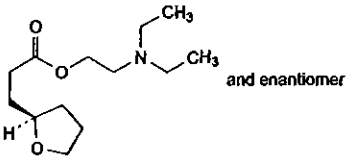
A. 2-[(naphthalen-1-yl)methyl]-3-(tetrahydrofuran-2-yl) propanoic acid,



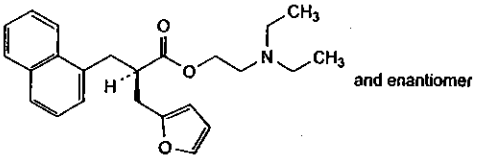
B. ethyl 2-[(naphthalen-1-yl)methyl]-3-(tetrahydrofuran-2-yl) propanoate,



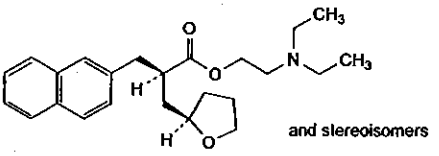
C. 2-(diethylamino)ethyl 3-(naphthalen-1-yl)-2-[(naphthalen-1-yl)methyl]propanoate,



D. 2-(diethylamino)ethyl 3-[(2*RS*)-tetrahydrofuran-2-yl] propanoate,



E. 2-(diethylamino)ethyl (2*RS*)-2-[(furan-2-yl)methyl]-3-(naphthalen-1-yl)propanoate,

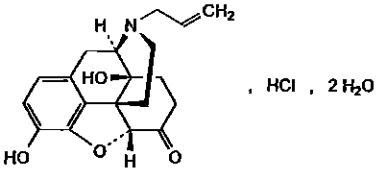


F. 2-(diethylamino)ethyl 2-[(naphthalen-2-yl)methyl]-3-(tetrahydrofuran-2-yl)propanoate.

Ph Eur

Naloxone Hydrochloride

(Naloxone Hydrochloride Dihydrate, Ph. Eur. monograph 0729)



$C_{19}H_{22}ClNO_4,2H_2O$ 399.9 *51481-60-8*

Action and use
Opioid receptor antagonist.

Preparation
Naloxone Injection

Ph Eur

DEFINITION
4,5α-Epoxy-3,14-dihydroxy-17-(prop-2-enyl)morphinan-6-one hydrochloride dihydrate.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white, hygroscopic, crystalline powder.

Solubility
Freely soluble in water, soluble in ethanol (96 per cent), practically insoluble in toluene.

IDENTIFICATION
First identification: A, C.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *naloxone hydrochloride dihydrate CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 8 mg of the substance to be examined in 0.5 mL of *water R* and dilute to 1 mL with *methanol R*.

Reference solution Dissolve 8 mg of *naloxone hydrochloride dihydrate CRS* in 0.5 mL of *water R* and dilute to 1 mL with *methanol R*.

Plate *TLC silica gel G plate R.*

Mobile phase Mix 5 volumes of *methanol R* and 95 volumes of the upper layer from a mixture of 60 mL of *dilute ammonia R2* and 100 mL of *butanol R.*

Application 5 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with a freshly prepared 5 g/L solution of *potassium ferricyanide R* in *ferric chloride solution R1*; examine in daylight.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 0.50 g in *carbon dioxide-free water R* and dilute to 25.0 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 10.0 mL of solution S add 0.05 mL of *methyl red solution R.* Not more than 0.2 mL of *0.02 M sodium hydroxide* or *0.02 M hydrochloric acid* is required to change the colour of the indicator.

Specific optical rotation (*2.2.7*)

−181 to −170 (anhydrous substance), determined on solution S.

Impurity D

Liquid chromatography (*2.2.29*).

Solution A Dissolve 1.58 g of *ammonium hydrogen carbonate R* in 950 mL of *water for chromatography R*, adjust to pH 9.0 with *concentrated ammonia R* and dilute to 1000 mL with *water for chromatography R.*

Test solution Dissolve 0.500 g of the substance to be examined in a 10.3 g/L solution of *hydrochloric acid R* and dilute to 20.0 mL with the same solution.

Reference solution (a) Dissolve 10.0 mg of *naloxone impurity D CRS* in a 10.3 g/L solution of *hydrochloric acid R* and dilute to 20.0 mL with the same solution. Dilute 5.0 mL of this solution to 100.0 mL with a 10.3 g/L solution of *hydrochloric acid R.*

Reference solution (b) Dilute 5.0 mL of reference solution (a) to 100.0 mL with a 10.3 g/L solution of *hydrochloric acid R.*

Reference solution (c) To 4.0 mL of the test solution add 2.0 mL of reference solution (a) and dilute to 20.0 mL with a 10.3 g/L solution of *hydrochloric acid R.*

Column:
- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature:* 40 °C.

Mobile phase:
- *mobile phase A: acetonitrile R1*, solution A (20:80 *V/V*);
- *mobile phase B: acetonitrile R1*, solution A (40:60 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 50	100	0
50 - 51	100 → 0	0 → 100
51 - 60	0	100

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µL of the test solution and reference solutions (b) and (c).

Relative retention With reference to naloxone (retention time = about 50 min): impurity D = about 0.8.

System suitability Reference solution (c):
- *symmetry factor:* maximum 1.8 for the peak due to impurity D.

Limit:
- *impurity D*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (75 ppm).

Related substances

Liquid chromatography (*2.2.29*).

Solution A Dissolve 1.10 g of *sodium octanesulfonate R* in 950 mL of *water for chromatography R*, adjust to pH 2.0 with a 50 per cent *V/V* solution of *phosphoric acid R*, filter and dilute to 1000 mL with *water for chromatography R.*

Test solution Dissolve 0.125 g of the substance to be examined in a 10.3 g/L solution of *hydrochloric acid R* and dilute to 25.0 mL with the same solution.

Reference solution (a) Dissolve the contents of a vial of *naloxone for peak identification CRS* (containing impurities A, B, C, D, E and F) in 1 mL of a 10.3 g/L solution of *hydrochloric acid R.*

Reference solution (b) Dilute 1.0 mL of the test solution to 20.0 mL with a 10.3 g/L solution of *hydrochloric acid R.* Dilute 1.0 mL of this solution to 25.0 mL with a 10.3 g/L solution of *hydrochloric acid R.*

Column:
- *size: l* = 0.125 m, Ø = 4.0 mm;
- *stationary phase: end-capped octylsilyl silica gel for chromatography R* (5 µm);
- *temperature:* 40 °C.

Mobile phase:
- *mobile phase A: acetonitrile R, tetrahydrofuran R*, solution A (2:4:94 *V/V/V*);
- *mobile phase B: tetrahydrofuran R, acetonitrile R*, solution A (4:17:79 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 40	100 → 0	0 → 100
40 - 50	0	100

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µL.

Relative retention With reference to naloxone (retention time = about 11 min): impurity C = about 0.6; impurity A = about 0.8; impurity F = about 0.9; impurity D = about 1.1; impurity E = about 3.0; impurity B = about 3.2.

Identification of impurities Use the chomatogram supplied with *naloxone for peak identification CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D, E and F.

System suitability Reference solution (a):
- *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to naloxone.

Limits:
- — *correction factor*: for the calculation of content, multiply the peak area of impurity E by 0.5;
- — *impurities A, B, C, E, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- — *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- — *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.8 per cent);
- — *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.12*)
7.5 per cent to 11.0 per cent, determined on 0.200 g.

Sulfated ash (*2.4.14*)
Maximum 0.2 per cent, determined on 0.50 g.

ASSAY

Dissolve 0.300 g in 50 mL of *ethanol (96 per cent) R* and add 5.0 mL of *0.01 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M ethanolic sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M ethanolic sodium hydroxide* is equivalent to 36.38 mg of $C_{19}H_{22}ClNO_4$.

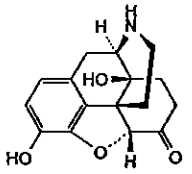
STORAGE

In an airtight container, protected from light.

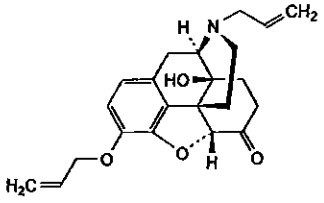
IMPURITIES

Specified impurities *A, B, C, D, E, F.*

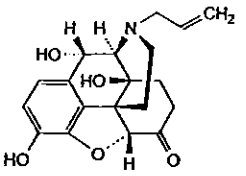
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *G.*



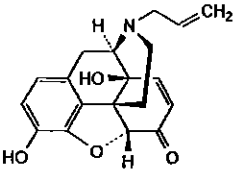
A. 4,5α-epoxy-3,14-dihydroxymorphinan-6-one (noroxymorphone),



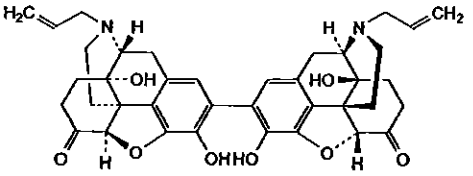
B. 4,5α-epoxy-14-hydroxy-17-(prop-2-enyl)-3-(prop-2-enyloxy)morphinan-6-one (3-*O*-allylnaloxone),



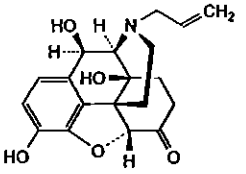
C. 4,5α-epoxy-3,10α,14-trihydroxy-17-(prop-2-enyl) morphinan-6-one (10α-hydroxynaloxone),



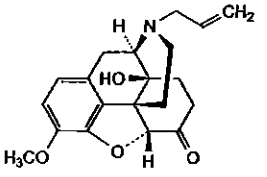
D. 7,8-didehydro-4,5α-epoxy-3,14-dihydroxy-17-(prop-2-enyl)morphinan-6-one (7,8-didehydronaloxone),



E. 4,5α:4′,5′α-diepoxy-3,3′,14,14′-tetrahydroxy-17,17′-bis (prop-2-enyl)-2,2′-bimorphinanyl-6,6′-dione (2,2′-binaloxone),



F. 4,5α-epoxy-3,10β,14-trihydroxy-17-(prop-2-enyl) morphinan-6-one (10β-hydroxynaloxone),

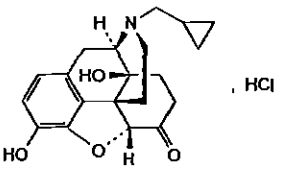


G. 4,5α-epoxy-14-hydroxy-3-methoxy-17-(prop-2-enyl) morphinan-6-one (3-*O*-methylnaloxone).

Ph Eur

Naltrexone Hydrochloride

(Ph. Eur. monograph 1790)



$C_{20}H_{24}ClNO_4$ 377.9

Action and use
Opioid receptor antagonist.

Ph Eur

DEFINITION
17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan-6-one hydrochloride. It may be anhydrous, a monohydrate or a dihydrate, a mixture or a solvate.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white powder, very hygroscopic.

Solubility
Freely soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Dissolve 20 mg in *water R* and dilute to 5 mL with the same solvent. Make alkaline with *dilute ammonia R1*. Shake with 10 mL of *methylene chloride R*, separate the organic layer and evaporate the solvent. Dry the residue obtained *in vacuo*.

Comparison *naltrexone hydrochloride CRS*.

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S
Dissolve 0.40 g in *carbon dioxide-free water R* and dilute to 20.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 or B_6 (*2.2.2, Method II*).

Acidity and alkalinity
To 10 mL of solution S, add 0.05 mL of *methyl red solution R*. Not more than 0.2 mL of *0.02 M sodium hydroxide* or *0.02 M hydrochloric acid* is required to change the colour of the indicator.

Specific optical rotation (*2.2.7*)
−187 to −195 (anhydrous substance).

Dissolve 0.40 g in *water R* and dilute to 20.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in *0.1 M hydrochloric acid* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dissolve 5.0 mg of *naltrexone impurity C CRS* in *0.1 M hydrochloric acid* and dilute to 2.5 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of the test solution and 1.0 mL of reference solution (a) to 100.0 mL with *0.1 M hydrochloric acid*. Dilute 1.0 mL of this solution to 10.0 mL with *0.1 M hydrochloric acid*.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R1* (5 µm);
— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: 1.1 g/L solution of *sodium octanesulfonate R* adjusted to pH 2.3 with *phosphoric acid R*;
— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 45	90 → 55	10 → 45
45 - 47	55 → 90	45 → 10
47 - 55	90	10

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 230 nm.

Equilibration 8 min.

Injection 10 µL.

Relative retention With reference to naltrexone (retention time = about 16 min): impurity A = about 0.4; impurity B = about 0.7; impurity F = about 0.8; impurity G = about 0.9; impurity C = about 1.05; impurity H = about 1.1; impurity I = about 1.2; impurity J = about 1.3; impurity D = about 1.4; impurity E = 1.7.

System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the peaks due to naltrexone and impurity C.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity D by 0.4;
— *impurities C, D, E, F, G*: for each impurity, not more than twice the area of the peak due to naltrexone in the chromatogram obtained with reference solution (b) (0.2 per cent);
— *impurities A, B, H, I, J*: for each impurity, not more than the area of the peak due to naltrexone in the chromatogram obtained with reference solution (b) (0.1 per cent);
— *any other impurity*: for each impurity, not more than the area of the peak due to naltrexone in the chromatogram obtained with reference solution (b) (0.1 per cent);
— *total*: not more than 10 times the area of the peak due to naltrexone in the chromatogram obtained with reference solution (b) (1.0 per cent);
— *disregard limit*: 0.5 times the area of the peak due to naltrexone in the chromatogram obtained with reference solution (b) (0.05 per cent).

Ethanol (*2.4.24, System A*)
Maximum 3.0 per cent.

Test solution Dissolve 0.25 g of the substance to be examined in *water R* and dilute to 10.0 mL with the same solvent.

Reference solution Dilute 0.750 g of *anhydrous ethanol R* to 1000.0 mL with *water R*.

Water (*2.5.12*)
Maximum 10.0 per cent, determined on 0.200 g.

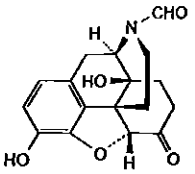
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.200 g in 60 mL of *ethanol (96 per cent) R*, add 1.0 mL of *0.1 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. The curve shows 3 points of inflexion. Read the volume added between the first 2 points of inflexion.

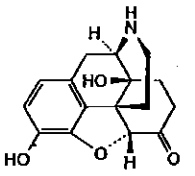
1 mL of *0.1 M sodium hydroxide* is equivalent to 37.79 mg of $C_{20}H_{24}ClNO_4$.

STORAGE
In an airtight container. Protected from light.

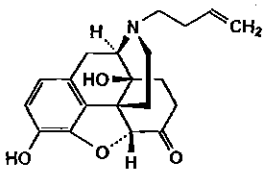
IMPURITIES
Specified impurities *A, B, C, D, E, F, G, H, I, J.*



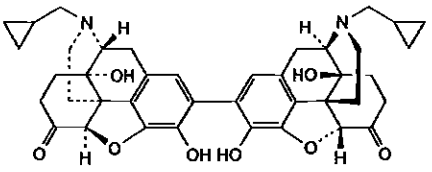
A. 17-formyl-4,5α-epoxy-3,14-dihydroxymorphinan-6-one,



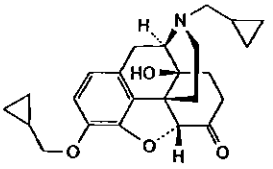
B. 4,5α-epoxy-3,14-dihydroxymorphinan-6-one (noroxymorphone),



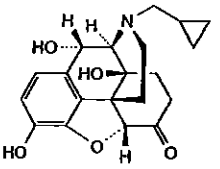
C. 17-but-3-enyl-4,5α-epoxy-3,14-dihydroxymorphinan-6-one,



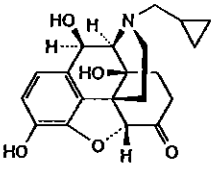
D. 17,17′-bis(cyclopropylmethyl)-4,5α:4′,5′α-diepoxy3,3′,14,14′-tetrahydroxy-2,2′-bimorphinanyl-6,6′-dione (pseudonaltrexone),



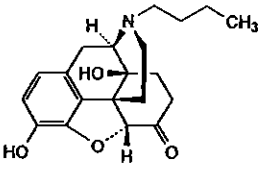
E. 3-(cyclopropylmethoxy)-17-(cyclopropylmethyl)-4,5α-epoxy-14-hydroxymorphinan-6-one,



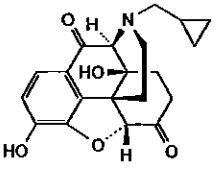
F. 17-(cyclopropylmethyl)-4,5α-epoxy-3,10α,14-trihydroxymorphinan-6-one,



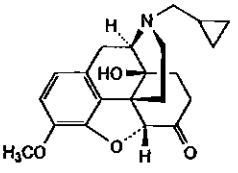
G. 17-(cyclopropylmethyl)-4,5α-epoxy-3,10β,14-trihydroxymorphinan-6-one,



H. 17-butyl-4,5α-epoxy-3,14-dihydroxymorphinan-6-one,



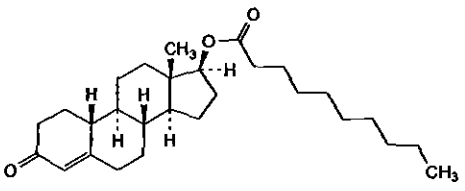
I. 17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan-6,10-dione,



J. 17-(cyclopropylmethyl)-4,5α-epoxy-14-hydroxy-3-methoxymorphinan-6-one.

Nandrolone Decanoate

(Ph. Eur. monograph 1992)



$C_{28}H_{44}O_3$ 428.7 *360-70-3*

Action and use
Anabolic steroid; androgen.

Ph Eur

DEFINITION
3-Oxoestr-4-en-17β-yl decanoate.

Content
97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, very soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION
A. Melting point (*2.2.14*): 34 °C to 38 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *nandrolone decanoate CRS.*

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Dissolve 0.20 g in 10 mL of *methanol R.*

Specific optical rotation (*2.2.7*)
+ 35.0 to + 40.0 (dried substance).

Dissolve 0.200 g in *anhydrous ethanol R* and dilute to 20.0 mL with the same solvent.

Impurities A, B, C
Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 50 mg of the substance to be examined in *methylene chloride R* and dilute to 5.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 10.0 mL with *methylene chloride R.*

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 10.0 mL with *methylene chloride R.*

Reference solution (c) Dilute 1.0 mL of reference solution (a) to 20.0 mL with *methylene chloride R.*

Reference solution (d) Dissolve 5 mg of *nandrolone decanoate for system suitability CRS* (containing impurities A, B, C) in 0.5 mL of *methylene chloride R.*

Plate *TLC silica gel plate R.*

Mobile phase *acetone R, heptane R* (30:70 *V/V*).

Application 10 µL of the test solution and reference solutions (b), (c) and (d).

Development Over 2/3 of the plate.

Drying In air.

Detection Treat with *alcoholic solution of sulfuric acid R* and heat at 130 °C until the spots appear. Examine in ultraviolet light at 366 nm.

Retardation factors Nandrolone decanoate = about 0.37; impurity A = about 0.45; impurity B = about 0.55; impurity C = about 0.58.

System suitability Reference solution (d):
— the chromatogram shows 4 clearly separated spots.

Limits:
— *impurity A*: any spot due to impurity A is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (1.0 per cent);
— *impurities B, C*: any spot due to impurity B or C is not more intense than the principal spot in the chromatogram obtained with reference solution (c) (0.5 per cent).

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *methanol R.* Dilute 1.0 mL of this solution to 10.0 mL with *methanol R.*

Reference solution (b) Dissolve 5 mg of *nandrolone decanoate for peak identification CRS* (containing impurities D, F, G, H, I, K, L) in *methanol R* and dilute to 2.0 mL with the same solvent.

Column:
— *size*: *l* = 0.15 m, Ø = 3.9 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: *water for chromatography R;*
— *mobile phase B*: *acetonitrile R;*

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	35	65
5 - 40	35 → 0	65 → 100
40 - 75	0	100
75 - 80	0 → 35	100 → 65

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL.

Relative retention With reference to nandrolone decanoate (retention time = about 30 min): impurity D = about 0.05; impurity F = about 0.6; impurity K = about 0.7; impurity L = about 0.9; impurity G = about 0.97; impurity H = about 1.1; impurity I = about 1.2.

System suitability Reference solution (b):
— *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to nandrolone decanoate.

Limits:
— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity D = 0.5; impurity F = 0.6; impurity H = 1.1; impurity I = 1.3; impurity K = 0.8;

— *impurities D, F, G, H, I, K, L*: for each impurity, not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 15 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at a pressure not exceeding 0.7 kPa for 4 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 10.0 mg in *anhydrous ethanol R* and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of this solution to 50.0 mL with *anhydrous ethanol R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 240 nm. Calculate the content of $C_{28}H_{44}O_3$ taking the specific absorbance to be 407.

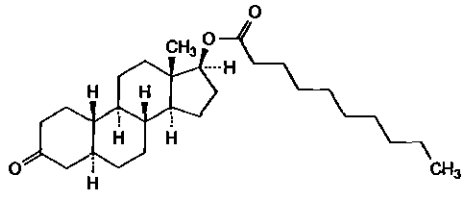
STORAGE

Under nitrogen, protected from light and at a temperature of 2 °C to 8 °C.

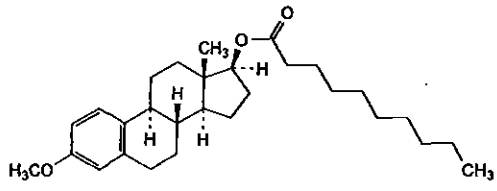
IMPURITIES

Specified impurities *A, B, C, D, F, G, H, I, K, L.*

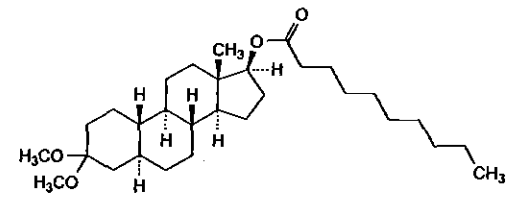
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *E, J.*



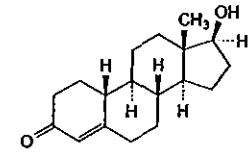
A. 3-oxo-5α-estran-17β-yl decanoate,



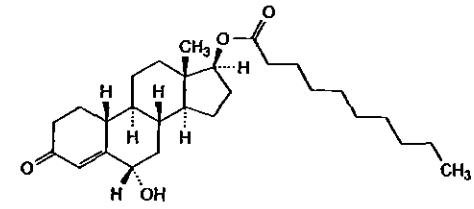
B. 3-methoxyestra-1,3,5(10)-trien-17β-yl decanoate,



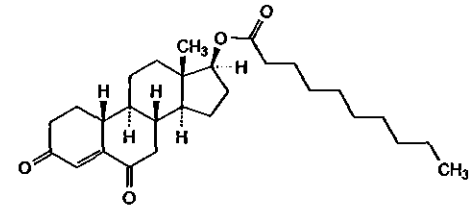
C. 3,3-dimethoxy-5α-estran-17β-yl decanoate,



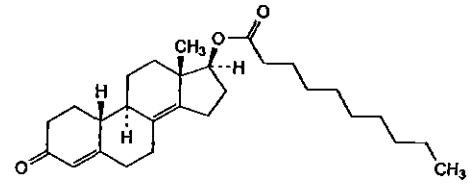
D. 17β-hydroxyestr-4-en-3-one,



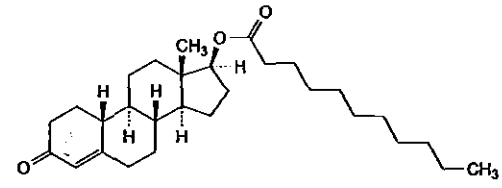
E. 6α-hydroxy-3-oxoestr-4-en-17β-yl decanoate,



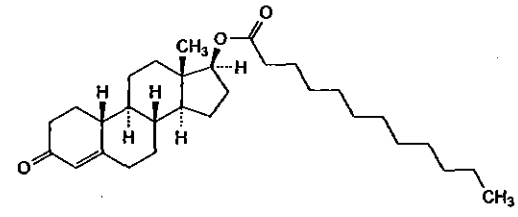
F. 3,6-dioxoestr-4-en-17β-yl decanoate,



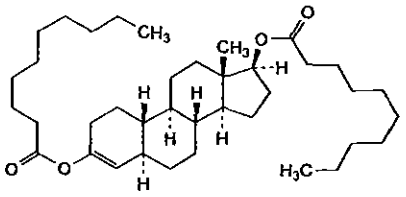
G. 3-oxoestra-4,8(14)-dien-17β-yl decanoate,



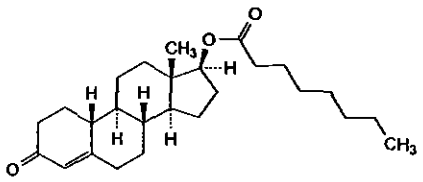
H. 3-oxoestr-4-en-17β-yl undecanoate,



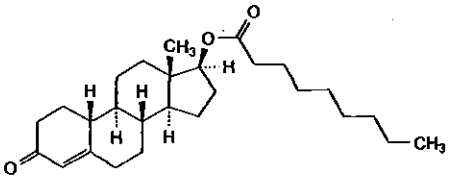
I. 3-oxoestr-4-en-17β-yl dodecanoate,



J. 5α-estr-3-ene-3,17β-diyl didecanoate,



K. 3-oxoestr-4-en-17β-yl octanoate,

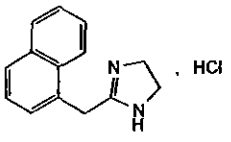


L. 3-oxoestr-4-en-17β-yl nonanoate.

Ph Eur

Naphazoline Hydrochloride

(Ph. Eur. monograph 0730)



$C_{14}H_{15}ClN_2$ 246.7 *550-99-2*

Action and use
Alpha-adrenoceptor agonist.

Ph Eur

DEFINITION
2-(Naphthalen-1-ylmethyl)-4,5-dihydro-1*H*-imidazole hydrochloride.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water, soluble in ethanol (96 per cent).

mp
About 259 °C, with decomposition.

IDENTIFICATION
First identification: B.

Second identification: A, C.

A. Dissolve 50.0 mg in *0.01 M hydrochloric acid* and dilute to 250.0 mL with the same acid. Dilute 25.0 mL of the solution to 100.0 mL with *0.01 M hydrochloric acid*. Examined between 230 nm and 350 nm (*2.2.25*), the solution shows 4 absorption maxima, at 270 nm, 280 nm, 287 nm and 291 nm. The ratios of the absorbances measured at the maxima at 270 nm, 287 nm and 291 nm to that measured at the maximum at 280 nm are 0.82 to 0.86, 0.67 to 0.70 and 0.65 to 0.69, respectively.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *naphazoline hydrochloride CRS.*

C. It gives reaction (a) of chlorides (*2.3.1*).

TESTS
Solution S
Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity
To 20 mL of solution S add 0.2 mL of *0.01 M sodium hydroxide* and 0.1 mL of *methyl red solution R*. The solution is yellow. Not more than 0.6 mL of *0.01 M hydrochloric acid* is required to change the colour of the solution to red.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dissolve 5 mg of *1-naphthylacetic acid R* in the mobile phase, add 5 mL of the test solution and dilute to 100 mL with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *naphazoline impurity A CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 100.0 mL with the mobile phase.

Reference solution (c) Dilute 1.0 mL of the test solution to 10.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 100.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.0 mm;
— *stationary phase*: *base-deactivated end-capped octylsilyl silica gel for chromatography R* (4 µm) with a pore size of 6 nm.

Mobile phase Dissolve 1.1 g of *sodium octanesulfonate R* in a mixture of 5 mL of *glacial acetic acid R*, 300 mL of *acetonitrile R* and 700 mL of *water R*.

Flow rate 1 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µL.

Run time 3 times the retention time of naphazoline.

Retention time Naphazoline = about 14 min.

System suitability Reference solution (a):
— *resolution*: minimum 5.0 between the peaks due to naphazoline and impurity B.

Limits:
— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
— *unspecified impurities*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

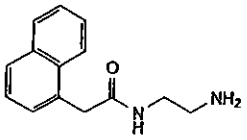
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.200 g in a mixture of 5.0 mL of *0.01 M hydrochloric acid* and 50 mL of *ethanol (96 per cent) R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

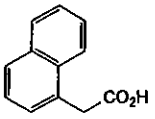
1 mL of *0.1 M sodium hydroxide* is equivalent to 24.67 mg of $C_{14}H_{15}ClN_2$.

STORAGE
Protected from light.

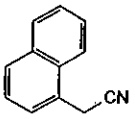
IMPURITIES
Specified impurities A.
Other detectable impurities: B, C, D.



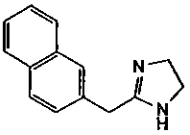
A. *N*-(2-aminoethyl)-2-(naphthalen-1-yl)acetamide (naphthylacetylethylenediamine),



B. (naphthalen-1-yl)acetic acid (1-naphthylacetic acid),



C. (naphthalen-1-yl)acetonitrile (1-naphthylacetonitrile),

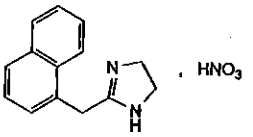


D. 2-(naphthalen-2-ylmethyl)-4,5-dihydro-1*H*-imidazole (β-naphazoline).

Ph Eur

Naphazoline Nitrate

(Ph. Eur. monograph 0147)



$C_{14}H_{15}N_3O_3$ 273.3 *5144-52-5*

Action and use
Alpha-adrenoceptor agonist.

Ph Eur

DEFINITION
2-(Naphthalen-1-ylmethyl)-4,5-dihydro-1*H*-imidazole nitrate.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Sparingly soluble in water, soluble in ethanol (96 per cent).

IDENTIFICATION
First identification: C.
Second identification: A, B, D.

A. Melting point (*2.2.14*): 167 °C to 170 °C.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 50.0 mg in *0.01 M hydrochloric acid* and dilute to 250.0 mL with the same acid. Dilute 25.0 mL of the solution to 100.0 mL with *0.01 M hydrochloric acid.*

Spectral range 230-350 nm.

Absorption maximum At 270 nm, 280 nm, 287 nm and 291 nm.

Absorbance ratio:
— A_{270}/A_{280} = 0.82 to 0.86,
— A_{291}/A_{280} = 0.65 to 0.69.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *naphazoline nitrate CRS.*

D. Dissolve 45 mg of the substance to be examined in 2 mL of *water R*. Add 1 mL of *sulfuric acid R*. Shake carefully and allow to cool. Add 1 mL of *ferrous sulfate solution R2* dropwise along the walls of the container. At the junction of the 2 liquids, a brown colour develops.

TESTS
Solution S
Dissolve 0.5 g in *carbon dioxide-free water R*, warming gently, and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)
5.0 to 6.5 for solution S.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dissolve 5 mg of *1-naphthylacetic acid R* in the mobile phase, add 5 mL of the test solution and dilute to 100 mL with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *naphazoline impurity A CRS* in the mobile phase and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of this solution to 100.0 mL with the mobile phase.

Reference solution (c) Dilute 2.0 mL of the test solution to 10.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 100.0 mL with the mobile phase.

Column:
- *size: l* = 0.25 m, Ø = 4.0 mm,
- *stationary phase: base-deactivated end-capped octylsilyl silica gel for chromatography R* (4 µm) with a pore size of 6 nm.

Mobile phase Dissolve 1.1 g of *sodium octanesulfonate R* in a mixture of 5 mL of *glacial acetic acid R*, 300 mL of *acetonitrile R* and 700 mL of *water R*.

Flow rate 1 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µL.

Run time 3 times the retention time of naphazoline.

Relative retention With reference to naphazoline (retention time = about 14 min): impurity A = about 0.76; impurity D = about 1.24; impurity B = about 1.27; impurity C = about 2.8.

System suitability Reference solution (a):
- *resolution*: minimum 5.0 between the peaks due to naphazoline and impurity B.

Limits:
- *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),
- *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent),
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent),
- *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent); disregard the peak due to the nitrate ion.

Chlorides *(2.4.4)*
Maximum 330 ppm, determined on solution S.

Loss on drying *(2.2.32)*
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash *(2.4.14)*
Maximum 0.1 per cent, determined on 1.0 g.

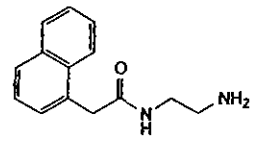
ASSAY
Dissolve 0.200 g in 30 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

1 mL of *0.1 M perchloric acid* is equivalent to 27.33 mg of $C_{14}H_{15}N_3O_3$.

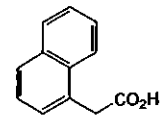
STORAGE
Protected from light.

IMPURITIES
Specified impurities *A.*

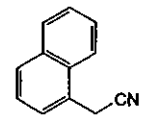
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D.*



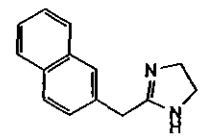
A. *N*-(2-aminoethyl)-2-(naphthalen-1-yl)acetamide (naphthylacetylethylenediamine),



B. (naphthalen-1-yl)acetic acid (1-naphthylacetic acid),



C. (naphthalen-1-yl)acetonitrile (1-naphthylacetonitrile),

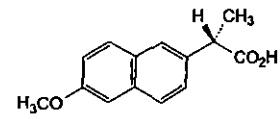


D. 2-(naphthalen-2-ylmethyl)-4,5-dihydro-1*H*-imidazole (β-naphazoline).

Ph Eur

Naproxen

(Ph. Eur. Monograph 0731)



$C_{14}H_{14}O_3$ 230.3 *22204-53-1*

Action and use
Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Preparations
Naproxen Oral Suspension
Naproxen Tablets
Naproxen Gastro-resistant Tablets

Ph Eur

DEFINITION
(2*S*)-2-(6-Methoxynaphthalen-2-yl)propanoic acid.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, soluble in ethanol (96 per cent) and in methanol.

IDENTIFICATION

First identification: A, D.

Second identification: A, B, C.

A. Specific optical rotation (*2.2.7*): + 59 to + 62 (dried substance).

Dissolve 0.50 g in *ethanol (96 per cent) R* and dilute to 25.0 mL with the same solvent.

B. Melting point (*2.2.14*): 154 °C to 158 °C.

C. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 40.0 mg in *methanol R* and dilute to 100.0 mL with the same solvent. Dilute 10.0 mL of the solution to 100.0 mL with *methanol R*.

Spectral range 230-350 nm.

Absorption maxima At 262 nm, 271 nm, 316 nm and 331 nm.

Specific absorbances at the absorption maxima:
- — at 262 nm: 216 to 238;
- — at 271 nm: 219 to 241;
- — at 316 nm: 61 to 69;
- — at 331 nm: 79 to 87.

D. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *naproxen CRS*.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_7 (*2.2.2, Method II*).

Dissolve 1.25 g in *methanol R* and dilute to 25 mL with the same solvent.

Enantiomeric purity

Liquid chromatography (*2.2.29*). *Protect the solutions from light.*

Test solution Dissolve 25.0 mg of the substance to be examined in *tetrahydrofuran R* and dilute to 50.0 mL with the same solvent. Dilute 2.0 mL of the solution to 20.0 mL with the mobile phase.

Reference solution (a) Dilute 2.5 mL of the test solution to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve 5 mg of *racemic naproxen CRS* in 10.0 mL of *tetrahydrofuran R* and dilute to 100.0 mL with the mobile phase.

Column:
- — *size*: *l* = 0.25 m, Ø = 4.6 mm;
- — *stationary phase*: *silica gel π-acceptor/π-donor for chiral separations R* (5 µm) (*S,S*);
- — *temperature*: 25 °C.

Mobile phase *glacial acetic acid R, acetonitrile R, 2-propanol R, hexane R* (0.5:5:10:84.5 *V/V/V/V*).

Flow rate 2 mL/min.

Detection Spectrophotometer at 263 nm.

Injection 20 µL.

Run time 1.5 times the retention time of naproxen (retention time = about 5 min).

System suitability Reference solution (b):
- — *resolution*: minimum 3 between the peaks due to impurity G and naproxen.

Limit:
- — *impurity G*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (2.5 per cent).

Related substances

Liquid chromatography (*2.2.29*). *Protect the solutions from light.*

Test solution Dissolve 12 mg of the substance to be examined in the mobile phase and dilute to 20 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 50.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 20.0 mL with the mobile phase.

Reference solution (b) Dissolve 6 mg of *bromomethoxynaphthalene R* (impurity N), 6.0 mg of *naproxen impurity L CRS*, 6 mg of *6-methoxy-2-naphthoic acid R* (impurity O) and 6 mg of *(1RS)-1-(6-methoxynaphthalen-2-yl)ethanol R* (impurity K) in *acetonitrile R* and dilute to 10.0 mL with the same solvent. To 1.0 mL of the solution add 1.0 mL of the test solution and dilute to 50.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 20.0 mL with the mobile phase.

Column:
- — *size*: *l* = 0.10 m, Ø = 4.0 mm;
- — *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm);
- — *temperature*: 50 °C.

Mobile phase Mix 42 volumes of *acetonitrile R* and 58 volumes of a 1.36 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 2.0 with *phosphoric acid R*.

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µL.

Run time 1.5 times the retention time of impurity N.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities K, L, N and O.

Relative retention With reference to naproxen (retention time = about 2.5 min): impurity O = about 0.8; impurity K = about 0.9; impurity L = about 1.4; impurity N = about 5.3.

System suitability Reference solution (b):
- — *resolution*: minimum 2.2 between the peaks due to impurity K and naproxen.

Limits:
- — *correction factor*: for the calculation of content, multiply the peak area of impurity O by 2.0;
- — *impurity O*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);
- — *impurity L*: not more than 1.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
- — *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- — *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in a mixture of 25 mL of *water R* and 75 mL of *methanol R*. Titrate with *0.1 M sodium hydroxide*, using 1 mL of *phenolphthalein solution R* as indicator.

1 mL of *0.1 M sodium hydroxide* is equivalent to 23.03 mg of $C_{14}H_{14}O_3$.

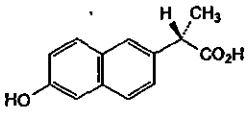
STORAGE

Protected from light.

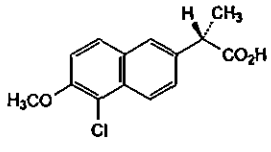
IMPURITIES

Specified impurities G, L, O.

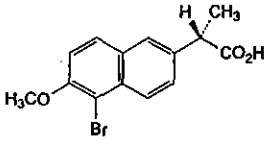
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) A, B, C, D, E, F, H, I, J, K, M, N.



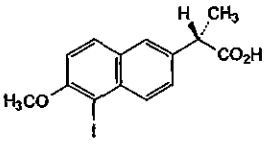
A. (2*S*)-2-(6-hydroxynaphthalen-2-yl)propanoic acid,



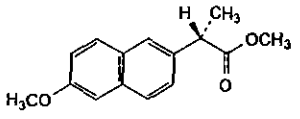
B. (2*S*)-2-(5-chloro-6-methoxynaphthalen-2-yl)propanoic acid,



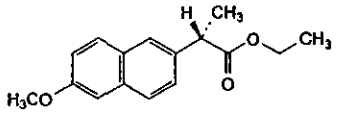
C. (2*S*)-2-(5-bromo-6-methoxynaphthalen-2-yl)propanoic acid,



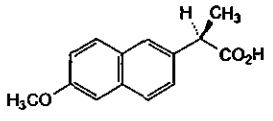
D. (2*S*)-2-(5-iodo-6-methoxynaphthalen-2-yl)propanoic acid,



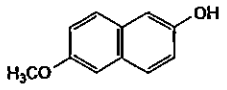
E. methyl (2*S*)-2-(6-methoxynaphthalen-2-yl)propanoate,



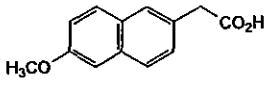
F. ethyl (2*S*)-2-(6-methoxynaphthalen-2-yl)propanoate,



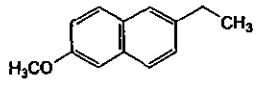
G. (2*R*)-2-(6-methoxynaphthalen-2-yl)propanoic acid ((*R*)-enantiomer),



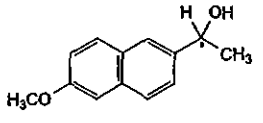
H. 6-methoxynaphthalen-2-ol,



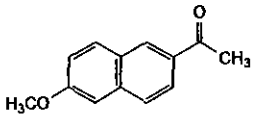
I. (6-methoxynaphthalen-2-yl)acetic acid,



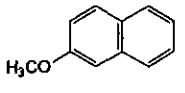
J. 2-ethyl-6-methoxynaphthalene,



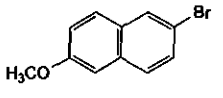
K. (1*RS*)-1-(6-methoxynaphthalen-2-yl)ethanol,



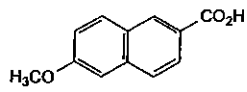
L. 1-(6-methoxynaphthalen-2-yl)ethanone,



M. 2-methoxynaphthalene (nerolin),



N. 2-bromo-6-methoxynaphthalene,

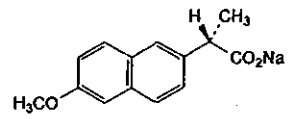


O. 6-methoxynaphthalene-2-carboxylic acid (6-methoxy-2-naphthoic acid).

Ph Eur

Naproxen Sodium

(Ph. Eur. monograph 1702)



$C_{14}H_{13}O_3Na$ 252.2 *26159-34-2*

Ph Eur

DEFINITION

Sodium (2*S*)-2-(6-methoxynaphthalen-2-yl)propanoate.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, hygroscopic, crystalline powder.

Solubility

Freely soluble in water, freely soluble or soluble in methanol, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: A, C, D.

Second identification: A, B, D.

A. Specific optical rotation (*2.2.7*): −17.0 to −14.7 (dried substance).

Dissolve 0.50 g in a 4.2 g/L solution of *sodium hydroxide R* and dilute to 25.0 mL with the same solution.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 40.0 mg in *methanol R* and dilute to 100.0 mL with the same solvent. Dilute 10.0 mL of this solution to 100.0 mL with *methanol R*.

Spectral range 230-350 nm.

Absorption maxima At 262 nm, 271 nm, 316 nm and 331 nm.

Specific absorbance at the absorption maxima:
- at 262 nm: 207 to 227;
- at 271 nm: 200 to 220;
- at 316 nm: 56 to 68;
- at 331 nm: 72 to 84.

C. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Dissolve 50 mg in 5 mL of *water R*. Add 1 mL of *dilute sulfuric acid R* and 5 mL of *ethyl acetate R*. Shake vigorously. Allow the 2 layers to separate. Evaporate the upper layer to dryness and subsequently dry at 60 °C for 15 min. Record the spectrum using the residue.

Comparison *naproxen CRS*.

D. It gives reaction (a) of sodium (*2.3.1*).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_7 (*2.2.2, Method II*).

Dissolve 1.25 g in *water R* and dilute to 25 mL with the same solvent.

pH (*2.2.3*)

7.0 to 9.8.

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent.

Enantiomeric purity

Liquid chromatography (*2.2.29*). *Protect the solutions from light.*

Test solution Dissolve 25.0 mg of the substance to be examined in 15 mL of *water R* and add 1 mL of *hydrochloric acid R*. Shake with 2 quantities, each of 10 mL, of *ethyl acetate R*, combine the upper layers and evaporate to dryness under reduced pressure. Dissolve the residue in 50.0 mL of *tetrahydrofuran R*. Dilute 2.0 mL of this solution to 20.0 mL with the mobile phase.

Reference solution (a) Dilute 2.5 mL of the test solution to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve 5 mg of *racemic naproxen CRS* in 10 mL of *tetrahydrofuran R* and dilute to 100 mL with the mobile phase.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *silica gel π-acceptor/π-donor for chiral separations R* (5 µm) (*S,S*);
- *temperature*: 25 °C.

Mobile phase *glacial acetic acid R, acetonitrile R, 2-propanol R, hexane R* (5:50:100:845 *V/V/V/V*).

Flow rate 2 mL/min.

Detection Spectrophotometer at 263 nm.

Injection 20 µL.

Run time 1.5 times the retention time of naproxen (retention time = about 5 min).

System suitability Reference solution (b):
- *resolution*: minimum 3 between the peaks due to impurity G and naproxen.

Limit:
- *impurity G*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (2.5 per cent).

Related substances

Liquid chromatography (*2.2.29*). *Protect the solutions from light.*

Test solution Dissolve 12 mg of the substance to be examined in the mobile phase and dilute to 20 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 50.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 20.0 mL with the mobile phase.

Reference solution (b) Dissolve 6 mg of *bromomethoxynaphthalene R* (impurity N), 6.0 mg of *naproxen impurity L CRS* and 6 mg of *(1RS)-1-(6-methoxynaphthalen-2-yl)ethanol R* (impurity K) in *acetonitrile R* and dilute to 10 mL with the same solvent. To 1 mL of the solution add 1 mL of the test solution and dilute to 50 mL with the mobile phase. Dilute 1 mL of this solution to 20 mL with the mobile phase.

Column:
— *size*: *l* = 0.10 m, Ø = 4.0 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm);
— *temperature*: 50 °C.

Mobile phase Mix 42 volumes of *acetonitrile R* and 58 volumes of a 1.36 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 2.0 with *phosphoric acid R*.

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µL.

Run time 1.5 times the retention time of impurity N.

Relative retention With reference to naproxen (retention time = about 2.5 min): impurity K = about 0.9; impurity L = about 1.4; impurity N = about 5.3.

System suitability Reference solution (b):
— *resolution*: minimum 2.2 between the peaks due to impurity K and naproxen.

Limits:
— *impurity L*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

ASSAY

Dissolve 0.200 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 25.22 mg of $C_{14}H_{13}O_3Na$.

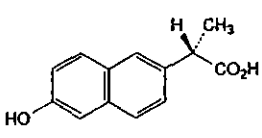
STORAGE

In an airtight container, protected from light.

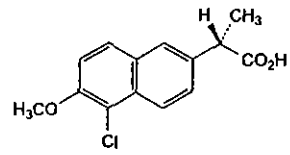
IMPURITIES

Specified impurities *G, L.*

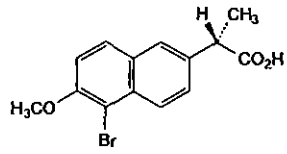
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, E, F, H, I, J, K, M, N.*



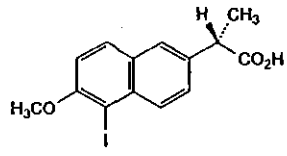
A. (2*S*)-2-(6-hydroxynaphthalen-2-yl)propanoic acid,



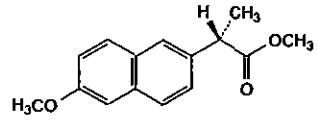
B. (2*S*)-2-(5-chloro-6-methoxynaphthalen-2-yl)propanoic acid,



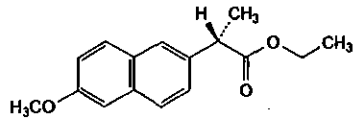
C. (2*S*)-2-(5-bromo-6-methoxynaphthalen-2-yl)propanoic acid,



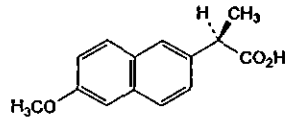
D. (2*S*)-2-(5-iodo-6-methoxynaphthalen-2-yl)propanoic acid,



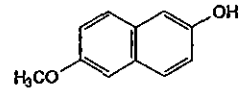
E. methyl (2*S*)-2-(6-methoxynaphthalen-2-yl)propanoate,



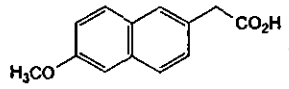
F. ethyl (2*S*)-2-(6-methoxynaphthalen-2-yl)propanoate,



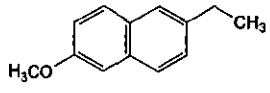
G. (2*R*)-2-(6-methoxynaphthalen-2-yl)propanoic acid,



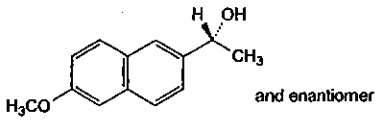
H. 6-methoxynaphthalen-2-ol,



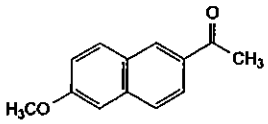
I. (6-methoxynaphthalen-2-yl)acetic acid,



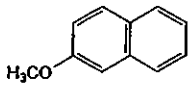
J. 2-ethyl-6-methoxynaphthalene,



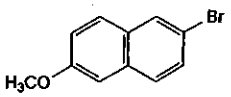
K. (1*RS*)-1-(6-methoxynaphthalen-2-yl)ethanol,



L. 1-(6-methoxynaphthalen-2-yl)ethanone,



M. 2-methoxynaphthalene (nerolin),

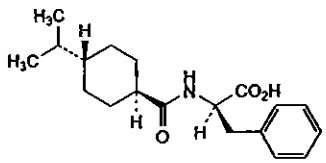


N. 2-bromo-6-methoxynaphthalene.

Ph Eur

Nateglinide

(Ph. Eur. monograph 2575)



$C_{19}H_{27}NO_3$ 317.4 *105816-04-4*

Action and use
Stimulates insulin release; treatment of diabetes mellitus.

Ph Eur

DEFINITION
N-[[*trans*-4-(1-Methylethyl)cyclohexyl]carbonyl]-D-phenylalanine.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white powder.

Solubility
Practically insoluble in water, freely soluble in methanol and in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION
Carry out either tests A, B or tests B, C.

A. Specific optical rotation (*2.2.7*): −40.0 to −36.5 (dried substance).

Dissolve 0.200 g in a 4 g/L solution of *sodium hydroxide R* and dilute to 20.0 mL with the same solution.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *nateglinide CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

C. Test B for related substances (see Tests).

TESTS
Related substances
A. Impurity A and unspecified impurities. Liquid chromatography (*2.2.29*).

Test solution Dissolve 60.0 mg of the substance to be examined in 1 mL of *acetonitrile R1* and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dissolve 3.0 mg of *nateglinide impurity A CRS* in 1 mL of *acetonitrile R1* and dilute to 25.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 10.0 mL with the mobile phase.

Reference solution (c) Dissolve 3 mg of the substance to be examined in 1 mL of *acetonitrile R1*, add 4.0 mL of reference solution (a) and dilute to 10 mL with the mobile phase.

Reference solution (d) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:
- *size*: *l* = 0.05 m, Ø = 3.9 mm;
- *stationary phase*: spherical *end-capped octylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase Mix 35 volumes of *acetonitrile R1* and 65 volumes of a 7.8 g/L solution of *sodium dihydrogen phosphate monohydrate R* previously adjusted to pH 2.5 with *phosphoric acid R*.

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 100 µL of the test solution and reference solutions (b), (c) and (d).

Run time 5 times the retention time of nateglinide.

Relative retention With reference to nateglinide (retention time = about 7 min): impurity A = about 0.5.

System suitability Reference solution (c):
- *resolution*: minimum 5.0 between the peaks due to impurity A and nateglinide.

Limits:
- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.10 per cent);
- *sum of unspecified impurities*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (d) (0.2 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.05 per cent).

B. Impurity B. Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.200 g of the substance to be examined in *methanol R2* and dilute to 20.0 mL with the same solvent.

Reference solution (a) Dissolve 5 mg of *nateglinide impurity B CRS* in *methanol R2* and dilute to 10.0 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of the test solution to 50.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (c) Dissolve 0.10 g of the substance to be examined in *methanol R2*. Add 1.0 mL of reference solution (a) and dilute to 10.0 mL with *methanol R2*.

Column:
- *size*: l = 0.25 m, Ø = 4.0 mm;
- *stationary phase*: urea type silica gel for chiral chromatography R (5 µm);
- *temperature*: 40 °C.

Mobile phase Dissolve 0.77 g of *ammonium acetate R* in *methanol R2* and dilute to 1000 mL with the same solvent.

Flow rate 0.8 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µL of the test solution and reference solutions (b) and (c).

Run time 1.5 times the retention time of nateglinide.

Relative retention With reference to nateglinide (retention time = about 21 min): impurity B = about 0.9.

System suitability Reference solution (c):
- *peak-to-valley ratio*: minimum 3, where H_p = height above the baseline of the peak due to impurity B and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to nateglinide.

Limit:
- *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent).

C. Impurities C and D. Liquid chromatography (*2.2.29*).

Sodium phosphate buffer Dissolve 8.5 g of *anhydrous disodium hydrogen phosphate R* in 950 mL of *water R*. Adjust to pH 7.5 with *phosphoric acid R* and dilute to 1000 mL with *water R*.

Test solution Dissolve 50.0 mg of the substance to be examined in 25 mL of *methanol R2* and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 5.0 mg of *phenylalanine CRS* (impurity D) and 5 mg of *nateglinide impurity C CRS* in *methanol R2* and dilute to 25.0 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of the test solution to 50.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (c) Dissolve 20 mg of the substance to be examined in 10 mL of *methanol R2*, add 1.0 mL of reference solution (a) and dilute to 20.0 mL with sodium phosphate buffer.

Reference solution (d) Dilute 1.0 mL of reference solution (a) to 100.0 mL with the mobile phase.

Reference solution (e) Dissolve 50.0 mg of *nateglinide CRS* in 25 mL of *methanol R2* and dilute to 50.0 mL with the mobile phase.

Column:
- *size*: l = 0.15 m, Ø = 6.0 mm;
- *stationary phase*: *polymethacrylate gel R* (6 µm);
- *temperature*: 30 °C.

Mobile phase *methanol R2*, sodium phosphate buffer (45:55 *V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µL of the test solution and reference solutions (b), (c) and (d).

Run time 1.4 times the retention time of nateglinide.

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peaks due to impurities C and D.

Relative retention With reference to nateglinide (retention time = about 18 min): impurity D = about 0.2; impurity C = about 0.9.

System suitability Reference solution (c):
- *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to nateglinide.

Limits:
- *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *impurity D*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.2 per cent).

Limits:
- *total for impurities A, B, C, D and sum of unspecified impurities*: maximum 0.5 per cent;
- *disregard limit for impurities A, B, C and D*: 0.05 per cent for each impurity.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in test C for related substances with the following modification.

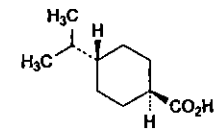
Injection Test solution and reference solution (e).

Calculate the percentage content of $C_{19}H_{27}NO_3$ taking into account the assigned content of *nateglinide CRS*.

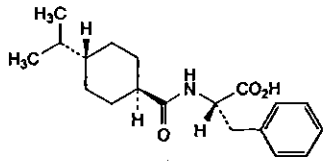
IMPURITIES

Specified impurities *A, B, C, D.*

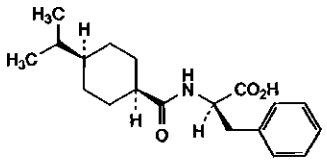
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *E, F, G.*



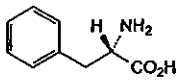
A. *trans*-4-(1-methylethyl)cyclohexanecarboxylic acid,



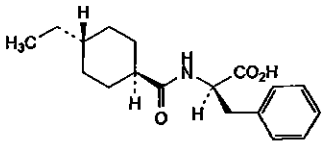
B. *N*-[[*trans*-4-(1-methylethyl)cyclohexyl]carbonyl]-L-phenylalanine (L-phenylalanine isomer),



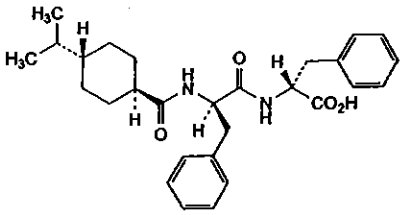
C. *N*-[[*cis*-4-(1-methylethyl)cyclohexyl]carbonyl]-D-phenylalanine (*cis*-isomer),



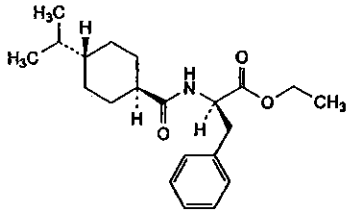
D. (2*S*)-2-amino-3-phenylpropanoic acid (phenylalanine),



E. *N*-[(*trans*-4-ethylcyclohexyl)carbonyl]-D-phenylalanine,



F. *N*-[[*trans*-4-(1-methylethyl)cyclohexyl]carbonyl]-D-phenylalanyl-D-phenylalanine,

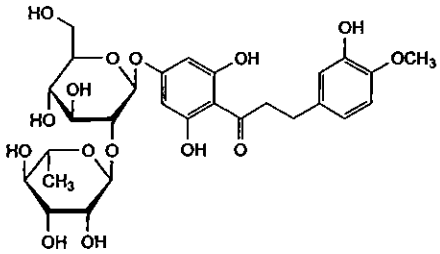


G. ethyl *N*-[[*trans*-4-(1-methylethyl)cyclohexyl]carbonyl]-D-phenylalaninate.

Ph Eur

Neohesperidin-Dihydrochalcone

(Ph. Eur. monograph 1547)



$C_{28}H_{36}O_{15}$ 613 *20702-77-6*

Ph Eur

DEFINITION

1-[4-[[2-*O*-(6-Deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-2,6-dihydroxyphenyl]-3-(3-hydroxy-4-methoxyphenyl)propan-1-one.

Content

96.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or yellowish-white powder.

Solubility

Practically insoluble in water, freely soluble in dimethyl sulfoxide, soluble in methanol, practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *neohesperidin-dihydrochalcone CRS.*

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_4 (*2.2.2, Method II*).

Dissolve 0.25 g in *methanol R* and dilute to 25 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 0.10 g of the substance to be examined in *dimethyl sulfoxide R* and dilute to 50.0 mL with the same solvent.

Test solution (b) Dilute 10.0 mL of test solution (a) to 20.0 mL with *dimethyl sulfoxide R.*

Reference solution (a) Dissolve 50.0 mg of *neohesperidin-dihydrochalcone CRS* in *dimethyl sulfoxide R* and dilute to 50.0 mL with the same solvent.

Reference solution (b) Dissolve 4.0 mg of *neohesperidin-dihydrochalcone impurity B CRS* in *dimethyl sulfoxide R* and dilute to 100.0 mL with the same solvent.

Reference solution (c) Dilute 1.0 mL of test solution (a) to 100.0 mL with *dimethyl sulfoxide R.*

Reference solution (d) In order to prepare *in situ* impurity F and impurity G, suspend 0.10 g of the substance to be examined in 10.0 mL of a 100 g/L solution of *sulfuric acid R.*

Heat the sample for 5 min on a water-bath. Dilute immediately 1.0 mL of the resulting solution to 50.0 mL with *dimethyl sulfoxide R*.

Column:
— *size*: *l* = 0.15 m, Ø = 3.9 mm,
— *stationary phase*: spherical *octadecylsilyl silica gel for chromatography R* (4 µm) with a carbon loading of 7 per cent,
— *temperature*: 30 °C.

Mobile phase Mix 20 volumes of *acetonitrile R* and 80 volumes of a solution prepared by adding 5.0 mL of *glacial acetic acid R* to 1000.0 mL of *water R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 282 nm.

Injection 10 µL; inject test solution (a) and reference solutions (a), (b), (c) and (d).

Run time 5 times the retention time of neohesperidin-dihydrochalcone which is about 10 min.

Relative retention With reference to neohesperidin-dihydrochalcone: impurity B = about 0.4; impurity D = about 0.7; impurity F = about 1.2; impurity G = about 3.7.

System suitability:
— *resolution*: minimum of 2.5 between the first peak (neohesperidin-dihydrochalcone) and the second peak (impurity F) in the chromatogram obtained with reference solution (d),
— chromatogram obtained with reference solution (a) is similar to the chromatogram provided with *neohesperidin-dihydrochalcone CRS*.

Limits:
— *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent),
— *impurity D*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (2 per cent),
— *any other impurity*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent),
— *total of all impurities apart from impurity B*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (2.5 per cent),
— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Water (*2.5.12*)
Maximum 12.0 per cent, determined on 0.200 g.

Sulfated ash (*2.4.14*)
Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances.

Injection 10 µL; inject test solution (b) and reference solutions (a) and (d).

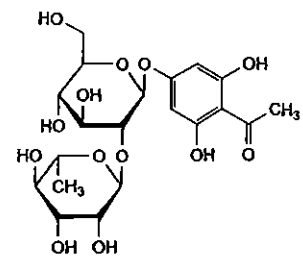
System suitability:
— *resolution*: minimum of 2.5 between the first peak (neohesperidin-dihydrochalcone) and the second peak (impurity F) in the chromatogram obtained with reference solution (d),
— *repeatability*: reference solution (a).

Calculate the percentage content of $C_{28}H_{36}O_{15}$ using the chromatogram obtained with reference solution (a) and the stated content of $C_{28}H_{36}O_{15}$ in *neohesperidin-dihydrochalcone CRS*, correcting for the water content of the substance to be examined.

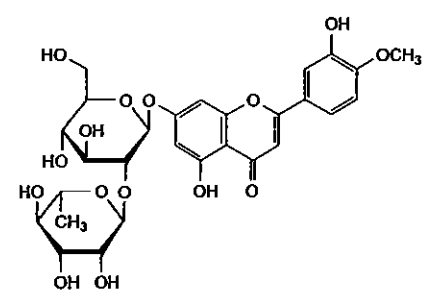
STORAGE

Protected from light.

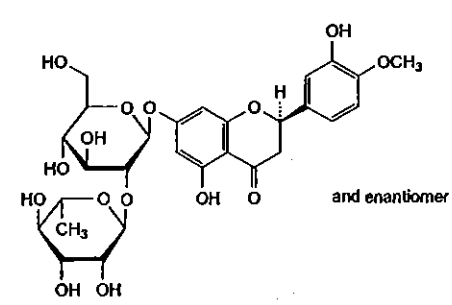
IMPURITIES



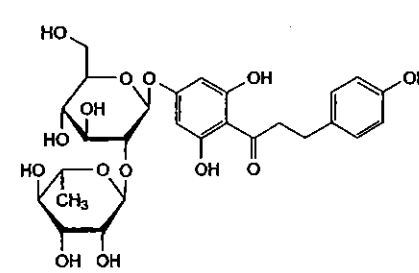
A. 1-[4-[[2-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-2,6-dihydroxyphenyl]ethanone (phloroacetophenone neohesperidoside),



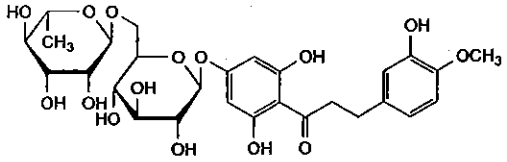
B. 7-[[2-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-1-benzopyran-4-one (neodiosmin),



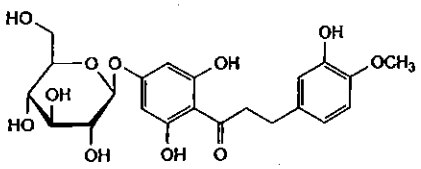
C. (2*RS*)-7-[[2-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-2,3-dihydro-4*H*-1-benzopyran-4-one (neohesperidin),



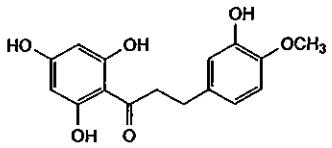
D. 1-[4-[[2-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-2,6-dihydroxyphenyl]-3-(4-hydroxyphenyl)propan-1-one (naringin-dihydrochalcone),



E. 1-[4-{[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy}-2,6-dihydroxyphenyl]-3-(3-hydroxy-4-methoxyphenyl)propan-1-one (hesperidin-dihydrochalcone),



F. 1-[4-(β-D-glucopyranosyloxy)-2,6-dihydroxyphenyl]-3-(3-hydroxy-4-methoxyphenyl)propan-1-one (hesperetin-dihydrochalcone 7′-glucoside),



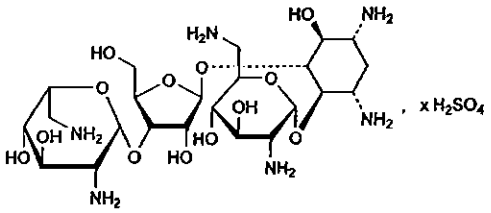
G. 3-(3-hydroxy-4-methoxyphenyl)-1-(2,4,6-trihydroxyphenyl)propan-1-one (hesperetin-dihydrochalcone).

Ph Eur

Neomycin Sulfate

Neomycin Sulphate

(Ph. Eur. monograph 0197)



$C_{23}H_{46}N_6O_{13}$,xH_2SO_4 615 (base)

Action and use
Aminoglycoside antibacterial.

Preparations
Dexamethasone and Neomycin Ear Spray
Hydrocortisone and Neomycin Cream
Hydrocortisone Acetate and Neomycin Ear Drops
Hydrocortisone Acetate and Neomycin Eye Drops
Hydrocortisone Acetate and Neomycin Eye Ointment
Neomycin Eye Drops
Neomycin Eye Ointment
Neomycin Tablets

Ph Eur

DEFINITION
Mixture of sulfates of substances produced by the growth of certain selected strains of *Streptomyces fradiae*, the main component being the sulfate of 2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-D-streptamine (neomycin B).

Content
Minimum of 680 IU/mg (dried substance).

CHARACTERS
Appearance
White or yellowish-white powder, hygroscopic.

Solubility
Very soluble in water, very slightly soluble in alcohol, practically insoluble in acetone.

IDENTIFICATION
A. Examine the chromatograms obtained in the test for related substances.

Results:
- — the retention time of the principal peak in the chromatogram obtained with the test solution is approximately the same as that of the principal peak in the chromatogram obtained with reference solution (e);
- — it complies with the limits given for impurity C.

B. It gives reaction (a) of sulfates (*2.3.1*).

TESTS
pH (*2.2.3*)
5.0 to 7.5.

Dissolve 0.1 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Specific optical rotation (*2.2.7*)
+ 53.5 to + 59.0 (dried substance).

Dissolve 1.00 g in *water R* and dilute to 10.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 25.0 mg of *framycetin sulfate CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (b) Dilute 5.0 mL of reference solution (a) to 100.0 mL with the mobile phase.

Reference solution (c) Dilute 1.0 mL of reference solution (a) to 100.0 mL with the mobile phase.

Reference solution (d) Dissolve the contents of a vial of *neamine CRS* (corresponding to 0.5 mg) in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (e) Dissolve 10 mg of *neomycin sulfate CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase.

Column:
- — *size*: *l* = 0.25 m, Ø = 4.6 mm;
- — *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (5 µm);
- — *temperature*: 25 °C.

Mobile phase Mix 20.0 mL of *trifluoroacetic acid R*, 6.0 mL of *carbonate-free sodium hydroxide solution R* and 500 mL of

water R, allow to equilibrate, dilute to 1000 mL with *water R* and degas.

Flow rate 0.7 mL/min.

Post-column solution *carbonate-free sodium hydroxide solution R* diluted 1 in 25 previously degassed, which is added pulse-less to the column effluent using a 375 µL polymeric mixing coil.

Flow rate 0.5 mL/min.

Detection Pulsed amperometric detector with a gold indicator electrode, a silver-silver chloride reference electrode and a stainless steel auxiliary electrode which is the cell body, held at respectively 0.00 V detection, + 0.80 V oxidation and −0.60 V reduction potentials, with pulse durations according to the instrument used.

Injection 10 µL; inject the test solution and the reference solutions (b), (c), (d) and (e).

Run time 1.5 times the retention time of neomycin B.

Relative retention With reference to neomycin B (retention time = about 10 min): impurity A = about 0.65; impurity C = about 0.9; impurity G = about 1.1.

System suitability:
- *resolution*: minimum of 2.0 between the peaks due to impurity C and to neomycin B in the chromatogram obtained with reference solution (e); if necessary, adjust the volume of the carbonate-free sodium hydroxide solution in the mobile phase;
- *signal-to-noise ratio*: minimum 10 for the principal peak in the chromatogram obtained with reference solution (c).

Limits:
- *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (2.0 per cent);
- *impurity C*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (15.0 per cent) and not less than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent);
- *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (5.0 per cent);
- *total of other impurities*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (15.0 per cent);
- *disregard limit*: area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent).

Sulfate

27.0 per cent to 31.0 per cent (dried substance).

Dissolve 0.250 g in 100 mL of *water R* and adjust the solution to pH 11 using *concentrated ammonia R*. Add 10.0 mL of *0.1 M barium chloride* and about 0.5 mg of *phthalein purple R*. Titrate with *0.1 M sodium edetate* adding 50 mL of *alcohol R* when the colour of the solution begins to change, continuing the titration until the violet-blue colour disappears.

1 mL of *0.1 M barium chloride* is equivalent to 9.606 mg of SO_4.

Loss on drying (*2.2.32*)

Maximum 8.0 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C at a pressure not exceeding 0.7 kPa for 3 h.

Sulfated ash (*2.4.14*)

Maximum 1.0 per cent, determined on 1.0 g.

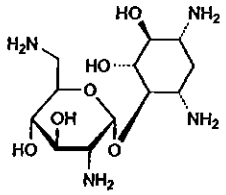
ASSAY

Carry out the microbiological assay of antibiotics (*2.7.2*). Use *neomycin sulfate for microbiological assay CRS* as the chemical reference substance.

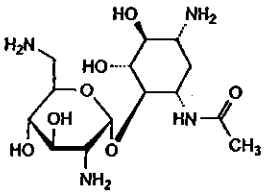
STORAGE

In an airtight container, protected from light.

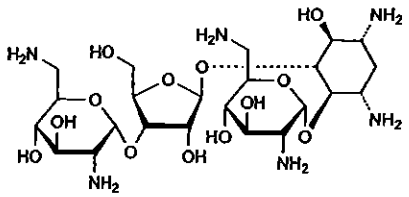
IMPURITIES



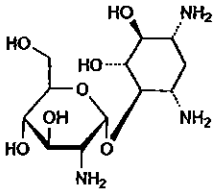
A. 2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-D-streptamine (neamine or neomycin A-LP),



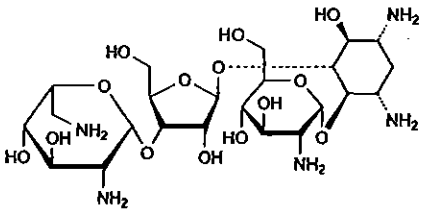
B. 3-*N*-acetyl-2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-D-streptamine (3-acetylneamine),



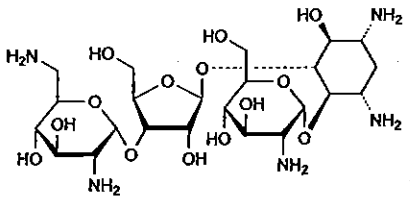
C. 2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-β-D-ribofuranosyl]-D-streptamine (neomycin C),



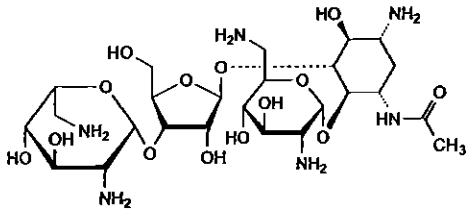
D. 4-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-2-deoxy-D-streptamine (paromamine or neomycin D),



E. 4-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-2-deoxy-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-D-streptamine (paromomycin I or neomycin E),



F. 4-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-2-deoxy-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-β-D-ribofuranosyl]-D-streptamine (paromomycin II or neomycin F),

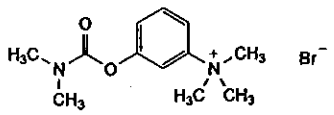


G. 3-*N*-acetyl-2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-D-streptamine (neomycin B-LP).

Ph Eur

Neostigmine Bromide

(Ph. Eur. monograph 0046)



$C_{12}H_{19}BrN_2O_2$ 303.2 *114-80-7*

Action and use
Cholinesterase inhibitor.

Preparation
Neostigmine Tablets

Ph Eur

DEFINITION

3-[(Dimethylcarbamoyl)oxy]-*N*,*N*,*N*-trimethylanilinium bromide.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder or colourless crystals, hygroscopic.

Solubility
Very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 20 mg in *0.5 M sulfuric acid* and dilute to 100 mL with the same acid.

Spectral range 230-350 nm.

Absorption maxima At 260 nm and 266 nm.

Specific absorbance at the absorption maxima:
— at 260 nm: about 16;
— at 266 nm: about 14.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *neostigmine bromide CRS*.

C. To 50 mg add 0.4 g of *potassium hydroxide R* and 2 mL of *ethanol (96 per cent) R* and heat on a water-bath for 3 min, replacing the evaporated ethanol (96 per cent). Cool and add 2 mL of *water R* and 2 mL of *diazobenzenesulfonic acid solution R1*. An orange-red colour develops.

D. It gives the reactions of bromides (*2.3.1*).

TESTS

Solution S
Dissolve 2.5 g in *distilled water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 50 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 4 mg of *3-dimethylaminophenol R* (impurity B) in 50 mL of the mobile phase. Dilute 1 mL of the solution to 200 mL with the mobile phase.

Reference solution (c) Dissolve the contents of a vial of *neostigmine impurity A CRS* in 1 mL of reference solution (b).

Reference solution (d) Mix 1 mL of the mobile phase and 1 mL of reference solution (a).

Column:
— *size*: *l* = 0.25 m, Ø = 4.0 mm;
— *stationary phase*: *base-deactivated octylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 30 °C.

Mobile phase To 710 mL of a 3.6 g/L solution of *sodium dihydrogen phosphate R* previously adjusted to pH 3.2 with *phosphoric acid R*, add 4.3 g of *sodium dodecyl sulfate R* and 290 mL of *acetonitrile R1*.

Flow rate 1.6 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 50 µL of the test solution and reference solutions (a), (c) and (d).

Run time Twice the retention time of neostigmine.

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A and B.

Relative retention With reference to neostigmine (retention time = about 20 min): impurity B = about 0.56; impurity A = about 0.61.

System suitability:
— *resolution*: minimum 1.5 between the peaks due to impurities B and A in the chromatogram obtained with reference solution (c);
— *signal-to-noise ratio*: minimum 25 for the principal peak in the chromatogram obtained with reference solution (d).

Calculation of percentage contents:
— for each impurity, use the concentration of neostigmine bromide in reference solution (a);
— *correction factor*: multiply the peak area of impurity B by 0.5.

Limits:
— *impurity B*: maximum 0.01 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.3 per cent;
— *reporting threshold*: 0.05 per cent; disregard the peak due to impurity B.

Sulfates (*2.4.13*)
Maximum 200 ppm, determined on solution S.

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

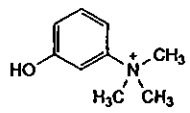
ASSAY
Dissolve 0.225 g in 2 mL of *anhydrous formic acid R*. Add 50 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 30.32 mg of $C_{12}H_{19}BrN_2O_2$.

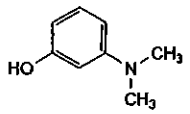
STORAGE
In an airtight container, protected from light.

IMPURITIES
Specified impurities B.

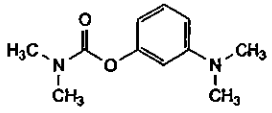
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) A, C.



A. 3-hydroxy-*N*,*N*,*N*-trimethylanilinium,



B. 3-(dimethylamino)phenol,

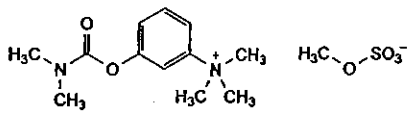


C. 3-(dimethylamino)phenyl dimethylcarbamate.

Ph Eur

Neostigmine Metilsulfate

(Ph. Eur. monograph 0626)



$C_{13}H_{22}N_2O_6S$ 334.4 *51-60-5*

Action and use
Cholinesterase inhibitor.

Preparation
Neostigmine Injection

Ph Eur

DEFINITION
3-[(Dimethylcarbamoyl)oxy]-*N*,*N*,*N*-trimethylanilinium methyl sulfate.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder or colourless crystals, hygroscopic.

Solubility
Very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION
First identification: A, C.

Second identification: A, B, D, E.

A. Melting point (*2.2.14*): 144 °C to 149 °C.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 50 mg in *0.5 M sulfuric acid* and dilute to 100 mL with the same acid.

Spectral range 230-350 nm.

Absorption maxima 261 nm and 267 nm.

Resolution (*2.2.25*): minimum 1.9 for the absorbance ratio.

Absorbance ratio A_{267}/A_{261} = 0.84 to 0.87.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *neostigmine metilsulfate CRS*.

D. To 50 mg add 0.4 g of *potassium hydroxide R* and 2 mL of *ethanol (96 per cent) R* and heat on a water-bath for 3 min, replacing the evaporated ethanol (96 per cent). Cool and add 2 mL of *water R* and 2 mL of *diazobenzenesulfonic acid solution R1*. An orange-red colour develops.

E. Dissolve 0.1 g in 5 mL of *distilled water R* and add 1 mL of *barium chloride solution R1*. No precipitate is formed. Add 2 mL of *hydrochloric acid R* and heat in a water-bath for 10 min. A fine, white precipitate is formed.

TESTS
Solution S
Dissolve 2.5 g in *distilled water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity
To 4.0 mL of solution S add 6.0 mL of *water R* and 0.1 mL of *phenolphthalein solution R1*. The solution is colourless. Add 0.3 mL of *0.01 M sodium hydroxide*; the solution

becomes red. Add 0.4 mL of *0.01 M hydrochloric acid*; the solution becomes colourless. Add 0.1 mL of *methyl red solution R*; the solution becomes red or yellowish-red.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 4 mg of *3-dimethylaminophenol R* (impurity B) in 50 mL of the mobile phase. Dilute 1 mL of the solution to 200 mL with the mobile phase.

Reference solution (c) Dissolve the contents of a vial of *neostigmine impurity A CRS* in 1 mL of reference solution (b).

Reference solution (d) Mix 1 mL of the mobile phase and 1 mL of reference solution (a).

Column:
- *size*: *l* = 0.25 m, Ø = 4.0 mm;
- *stationary phase*: *base-deactivated octylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 30 °C.

Mobile phase To 710 mL of a 3.6 g/L solution of *sodium dihydrogen phosphate R* previously adjusted to pH 3.2 with *phosphoric acid R*, add 4.3 g of *sodium dodecyl sulfate R* and 290 mL of *acetonitrile for chromatography R*.

Flow rate 1.6 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 50 µL of the test solution and reference solutions (a), (c) and (d).

Run time Twice the retention time of neostigmine.

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A and B.

Relative retention With reference to neostigmine (retention time = about 20 min): impurity B = about 0.56; impurity A = about 0.61.

System suitability:
- *resolution*: minimum 1.5 between the peaks due to impurities B and A in the chromatogram obtained with reference solution (c);
- *signal-to-noise ratio*: minimum 25 for the principal peak in the chromatogram obtained with reference solution (d).

Calculation of percentage contents:
- *correction factor*: multiply the peak area of impurity B by 0.5;
- for each impurity, use the concentration of neostigmine metilsulfate in reference solution (a).

Limits:
- *impurity B*: maximum 0.01 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.2 per cent;
- *reporting threshold*: 0.05 per cent, except for impurity B.

Sulfates (*2.4.13*)

Maximum 200 ppm, determined on solution S.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 150 mL of *water R* and add 100 mL of *dilute sodium hydroxide solution R*. Distil, collecting the distillate in 40 mL of a 40 g/L solution of *boric acid R* until the total volume in the collecting vessel is about 250 mL. Titrate the solution in the collecting vessel with *0.1 M hydrochloric acid*, using 0.25 mL of *methyl red mixed solution R* as indicator. Carry out a blank test.

1 mL of *0.1 M hydrochloric acid* is equivalent to 33.44 mg of $C_{13}H_{22}N_2O_6S$.

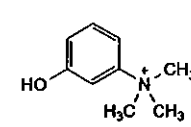
STORAGE

In an airtight container, protected from light.

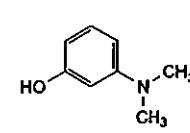
IMPURITIES

Specified impurities *B*.

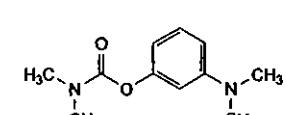
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, C*.



A. 3-hydroxy-*N*,*N*,*N*-trimethylanilinium,



B. 3-(dimethylamino)phenol,



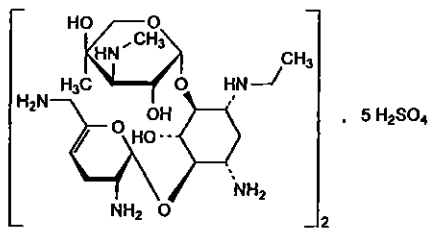
C. 3-(dimethylamino)phenyl dimethylcarbamate.

Ph Eur

Netilmicin Sulfate

Netilmicin Sulphate

(Ph. Eur. monograph 1351)



$C_{42}H_{92}N_{10}O_{34}S_5$ 1442 *56391-57-2*

Action and use
Aminoglycoside antibacterial.

Ph Eur

DEFINITION

Bis[2-deoxy-6-*O*-[3-deoxy-4-*C*-methyl-3-(methylamino)-β-L-arabinopyranosyl]-4-*O*-(2,6-diamino-2,3,4,6-tetradeoxy-α-D-*glycero*-hex-4-enopyranosyl)-1-*N*-ethyl-D-streptamine] pentasulfate.

Substance obtained by synthesis from sisomicin.

Semi-synthetic product derived from a fermentation product.

Content
95.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
White or yellowish-white, very hygroscopic powder.

Solubility
Very soluble in water, practically insoluble in acetone and in ethanol (96 per cent).

IDENTIFICATION

A. Examine the chromatograms obtained in the test for related substances.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

B. It gives reaction (a) of sulfates (*2.3.1*).

TESTS

Solution S
Dissolve 0.80 g in *carbon dioxide-free water R* and dilute to 20.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and its absorbance (*2.2.25*) at 400 nm is not greater than 0.08.

pH (*2.2.3*)
3.5 to 5.5 for solution S.

Related substances
Liquid chromatography (*2.2.29*). *Perform weighing steps as quickly as possible and immediately after opening the sample container.*

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 mL with the mobile phase.

Reference solution (a) Dissolve the contents of a vial of *netilmicin sulfate for LC assay CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (b) Dissolve 25.0 mg of *sisomicin sulfate CRS* (impurity A) in the mobile phase and dilute to 25.0 mL with the mobile phase.

Reference solution (c) Dissolve 25.0 mg of *1-N-ethylgaramine sulfate CRS* (impurity B) in the mobile phase and dilute to 25.0 mL with the mobile phase.

Reference solution (d) Dilute 0.6 mL of reference solution (a), 1.0 mL of reference solution (b) and 1.0 mL of reference solution (c) to 50.0 mL with the mobile phase.

Reference solution (e) Dissolve 5.0 mg of *netilmicin for peak identification CRS* (containing impurities E and F) in the mobile phase and dilute to 2.5 mL with the mobile phase.

Column:
— *size*: l = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 50 °C.

Mobile phase Solution in *carbon dioxide-free water R* containing 0.3 g/L of *sodium octanesulfonate R*, 20 g/L of *anhydrous sodium sulfate R1*, 20 mL/L of *tetrahydrofuran R* and 50 mL/L of *0.2 M potassium dihydrogen phosphate R*, adjusted to pH 3.0 with a 22.5 g/L solution of *phosphoric acid R*; degas.

Flow rate 1.0 mL/min.

Post-column solution Mixture of 1 volume of *carbonate-free sodium hydroxide solution R* and 24 volumes of previously degassed *carbon dioxide-free water R*, which is added pulse-free to the column effluent using a 375 µL polymeric mixing coil.

Flow rate of post-column solution 0.3 mL/min.

Detection Pulsed amperometric detector or equivalent with a gold indicator electrode, a silver-silver chloride reference electrode and a stainless steel auxiliary electrode which is the cell body, held at respectively + 0.05 V detection, + 0.75 V oxidation and − 0.15 V reduction potentials, with pulse durations according to the instrument used.

Injection 20 µL of the test solution and reference solutions (d) and (e).

Run time 3 times the retention time of netilmicin.

Identification of impurities Use the chromatogram obtained with reference solution (d) to identify the peaks due to impurities A and B; use the chromatogram supplied with *netilmicin for peak identification CRS* and the chromatogram obtained with reference solution (e) to identify the peaks due to impurities E and F.

Relative retention With reference to netilmicin (retention time = about 16 min): impurity B = about 0.4; impurity A = about 0.7; impurity E = about 1.9; impurity F = about 2.1.

System suitability Reference solution (d):
— *resolution*: minimum 10.0 between the peaks due to impurities B and A; minimum 6.0 between the peaks due to impurity A and netilmicin; if necessary, adjust the concentration of sodium octanesulfonate in the mobile phase.

Calculation of percentage contents:
— for impurities A and B, use the concentration of each impurity in reference solution (d);
— for impurities other than A and B, use the concentration of netilmicin sulfate in reference solution (d).

Limits:
— *impurities A, B, E, F*: for each impurity, maximum 1.0 per cent;

— *any other impurity*: for each impurity, maximum 0.3 per cent;
— *total*: maximum 3.0 per cent;
— *reporting threshold*: 0.1 per cent.

Sulfate

31.5 per cent to 35.0 per cent (dried substance).

Dissolve 0.12 g in 100 mL of *water R* and adjust the solution to pH 11 using *concentrated ammonia R*. Add 30.0 mL of *0.1 M barium chloride* and about 0.5 mg of *phthalein purple R*. Titrate with *0.1 M sodium edetate* adding 50 mL of *ethanol (96 per cent) R* when the colour of the solution begins to change and continue the titration until the violet-blue colour disappears.

1 mL of *0.1 M barium chloride* is equivalent to 9.606 mg of SO_4.

Loss on drying (*2.2.32*)

Maximum 15.0 per cent, determined on 0.500 g by drying at 110 °C under high vacuum for 3 h.

Sulfated ash (*2.4.14*)

Maximum 1.0 per cent, determined on 0.5 g.

ASSAY

Liquid chromatography (*2.2.29*). *Perform weighing steps as quickly as possible and immediately after opening the sample container.*

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference-solution Dissolve the contents of a vial of *netilmicin sulfate for LC assay CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 50 °C.

Mobile phase Solution in *carbon dioxide-free water R* containing 0.15 g/L of *sodium octanesulfonate R*, 20 g/L of *anhydrous sodium sulfate R1*, 20 mL/L of *2-methyl-2-propanol R* and 50 mL/L of *0.2 M potassium dihydrogen phosphate R*, adjusted to pH 3.0 with *dilute phosphoric acid R*; degas.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 205 nm.

Injection 10 µL.

Run time 1.3 times the retention time of netilmicin.

Retention time Netilmicin = about 4 min.

Calculate the percentage content of $C_{42}H_{92}N_{10}O_{34}S_5$ taking into account the assigned content of *netilmicin sulfate for LC assay CRS*.

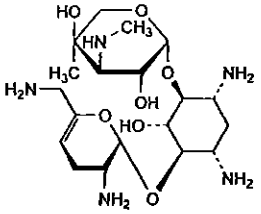
STORAGE

In an airtight container, protected from light. If the substance is sterile, the container is also sterile and tamper-evident.

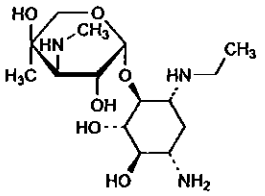
IMPURITIES

Specified impurities *A, B, E, F.*

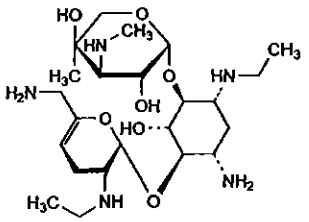
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C, D.*



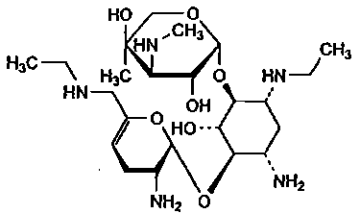
A. 2-deoxy-4-*O*-[3-deoxy-4-*C*-methyl-3-(methylamino)-β-L-arabinopyranosyl]-6-*O*-(2,6-diamino-2,3,4,6-tetradeoxy-α-D-*glycero*-hex-4-enopyranosyl)-L-streptamine (sisomicin),



B. 2-deoxy-6-*O*-[3-deoxy-4-*C*-methyl-3-(methylamino)-β-L-arabinopyranosyl]-1-*N*-ethyl-D-streptamine (1-*N*-ethylgaramine),



C. 4-*O*-[6-amino-2,3,4,6-tetradeoxy-2-(ethylamino)-α-D-*glycero*-hex-4-enopyranosyl]-2-deoxy-6-*O*-[3-deoxy-4-*C*-methyl-3-(methylamino)-β-L-arabinopyranosyl]-1-*N*-ethyl-D-streptamine (2′-*N*-ethylnetilmicin),



D. 4-*O*-[2-amino-2,3,4,6-tetradeoxy-6-(ethylamino)-α-D-*glycero*-hex-4-enopyranosyl]-2-deoxy-6-*O*-[3-deoxy-4-*C*-methyl-3-(methylamino)-β-L-arabinopyranosyl]-1-*N*-ethyl-D-streptamine (6′-*N*-ethylnetilmicin),

E. unknown structure,

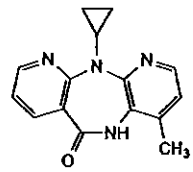
F. unknown structure.

Ph Eur

Nevirapine

Anhydrous Nevirapine

(Ph. Eur. monograph 2255)



$C_{15}H_{14}N_4O$ 266.3 *129618-40-2*

Action and use
Non-nucleoside reverse transcriptase inhibitor; antiviral (HIV).

Ph Eur

DEFINITION
11-Cyclopropyl-4-methyl-5,11-dihydro-6*H*-dipyrido [3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one.

Content
97.5 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Practically insoluble in water, sparingly soluble or slightly soluble in methylene chloride, slightly soluble in methanol.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *anhydrous nevirapine CRS.*
B. Loss on drying (see Tests).

TESTS

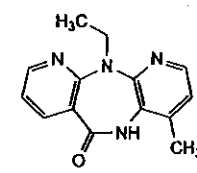
Related substances
Liquid chromatography (*2.2.29*).
Test solution (a) Dissolve 24.0 mg of the substance to be examined in a mixture of 4 mL of *acetonitrile R* and 80 mL of the mobile phase and sonicate until dissolution is complete. Dilute to 100.0 mL with the mobile phase.
Test solution (b) Dilute 3.0 mL of test solution (a) to 25.0 mL with the mobile phase.
Reference solution (a) Dilute 1.0 mL of test solution (a) to 100.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 50.0 mL with the mobile phase.
Reference solution (b) Add 2.0 mL of the mobile phase to a vial of *nevirapine for peak identification CRS* (containing impurities A, B and C), mix and sonicate for 1 min.
Reference solution (c) Dissolve 24.0 mg of *anhydrous nevirapine CRS* in a mixture of 4 mL of *acetonitrile R* and 80 mL of the mobile phase and sonicate until complete dissolution. Dilute to 100.0 mL with the mobile phase. Dilute 3.0 mL of this solution to 25.0 mL with the mobile phase.
Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped amidohexadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 35 °C.
Mobile phase Mix 20 volumes of *acetonitrile R* and 80 volumes of a 2.88 g/L solution of *ammonium dihydrogen phosphate R*, previously adjusted to pH 5.0 using *dilute sodium hydroxide solution R*.
Flow rate 1.0 mL/min.
Detection Spectrophotometer at 220 nm.
Injection 50 µL of test solution (a) and reference solutions (a) and (b).
Run time 10 times the retention time of nevirapine.
Identification of impurities Use the chromatogram supplied with *nevirapine for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B and C.
Relative retention With reference to nevirapine (retention time = about 8 min): impurity B = 0.7; impurity A = 1.5; impurity C = 2.8.
System suitability Reference solution (b):
— *resolution*: minimum 5 between the peaks due to impurity B and nevirapine.
Limits:
— *impurities A, B, C*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *total*: not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.6 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

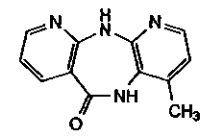
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.
Injection 25 µL of test solution (b) and reference solution (c).
Calculate the percentage content of $C_{15}H_{14}N_4O$ from the declared content of *anhydrous nevirapine CRS*.

IMPURITIES
Specified impurities *A, B, C.*



A. 11-ethyl-4-methyl-5,11-dihydro-6*H*-dipyrido[3,2-b:2′,3′-*e*][1,4]diazepin-6-one,



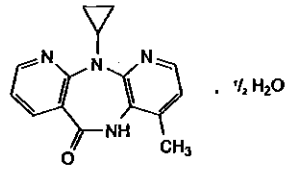
B. 4-methyl-5,11-dihydro-6*H*-dipyrido[3,2-b:2′,3′-*e*][1,4]diazepin-6-one,

C. 4-methyl-11-propyl-5,11-dihydro-6*H*-dipyrido[3,2-b:2′,3′-*e*][1,4]diazepin-6-one.

Ph Eur

Nevirapine Hemihydrate

(Ph. Eur. monograph 2479)



$C_{15}H_{14}N_4O,½H_2O$ 275.3 *220988-26-1*

Action and use
Non-nucleoside reverse transcriptase inhibitor; antiviral (HIV).

Ph Eur

DEFINITION
11-Cyclopropyl-4-methyl-5,11-dihydro-6*H*-dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one hemihydrate.

Content
97.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white powder.

Solubility
Practically insoluble in water, slightly soluble in methanol and in methylene chloride.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *nevirapine hemihydrate CRS.*

B. Water (see Tests).

TESTS
Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in *methanol R* and sonicate until dissolution is complete. Dilute to 50.0 mL with *methanol R*.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *methanol R*. Dilute 1.0 mL of this solution to 10.0 mL with *methanol R*.

Reference solution (b) Add 1 mL of *methanol R* to a vial of *nevirapine for peak identification CRS* (containing impurities A, B and C), mix and sonicate for 1 min.

Reference solution (c) Dissolve 20.0 mg of *anhydrous nevirapine CRS* in *methanol R* and sonicate until dissolution is complete. Dilute to 50.0 mL with *methanol R*.

Column:
— *size*: *l* = 50 mm, Ø = 2.1 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography compatible with 100 per cent aqueous mobile phases R* (1.8 µm);
— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: dissolve 0.77 g of *ammonium acetate R* in 900 mL of *water for chromatography R*, adjust to pH 5.6 with *acetic acid R* and dilute to 1000 mL with *water for chromatography R*;
— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 – 1.35	90	10
1.35 – 3.85	90 → 67	10 → 33
3.85 – 6.70	67 → 60	33 → 40
6.70 – 7.65	60	40

Flow rate 0.7 mL/min.

Detection Spectrophotometer at 282 nm.

Injection 2.0 µL of the test solution and reference solutions (a) and (b).

Identification of impurities Use the chromatogram supplied with *nevirapine for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B and C.

Relative retention With reference to nevirapine (retention time = about 3 min): impurity B = about 0.9; impurity A = about 1.2; impurity C = about 1.3.

System suitability:
— *resolution*: minimum 5.0 between the peaks due to impurity B and nevirapine and minimum 5.0 between the peaks due to nevirapine and impurity A in the chromatogram obtained with reference solution (b);
— *symmetry factor*: maximum 1.7 for the peak due to nevirapine in the chromatogram obtained with reference solution (a).

Calculation of percentage contents:
— for each impurity, use the concentration of nevirapine hemihydrate in reference solution (a).

Limits:
— *impurities A, B, C*: for each impurity, maximum 0.2 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.6 per cent;
— *reporting threshold*: 0.05 per cent.

Water (*2.5.12*)
3.1 per cent to 3.9 per cent, determined on 0.300 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

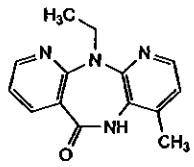
Injection 2.0 µL of the test solution and reference solution (c).

Calculate the percentage content of $C_{15}H_{14}N_4O$ taking into account the assigned content of *anhydrous nevirapine CRS*.

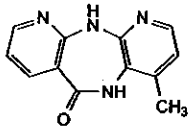
IMPURITIES
Specified impurities *A, B, C.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests

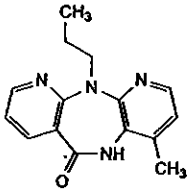
in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) D.



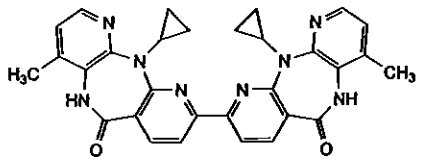
A. 11-ethyl-4-methyl-5,11-dihydro-6*H*-dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one,



B. 4-methyl-5,11-dihydro-6*H*-dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one,



C. 4-methyl-11-propyl-5,11-dihydro-6*H*-dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one,

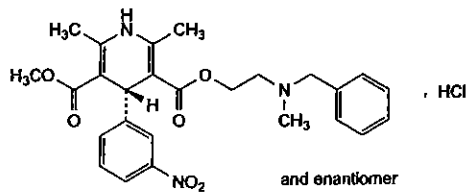


D. 11,11′-dicyclopropyl-4,4′-dimethyl-5,5′,11,11′-tetrahydro-6*H*,6′*H*-9,9′-bidipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepine-6,6′-dione.

Ph Eur

Nicardipine Hydrochloride

(Ph. Eur. monograph 2776)



$C_{26}H_{30}ClN_3O_6$ 516.0 *54527-84-3*

Action and use
Calcium channel blocker.

Ph Eur

DEFINITION

2-[Benzyl(methyl)amino]ethyl methyl (4*RS*)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate hydrochloride.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
Pale yellow or pale greenish-yellow, crystalline powder.

Solubility
Slightly soluble in water, soluble in methanol, sparingly soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *nicardipine hydrochloride CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *methanol R*, evaporate to dryness and record new spectra using the residues.

B. Dissolve 30 mg in 10 mL of *water R* and shake vigorously. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Related substances
Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 50.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 20.0 mL with the mobile phase.

Reference solution (b) Dissolve 4 mg of *nicardipine for system suitability CRS* (containing impurities A, B and C) in 2 mL of the mobile phase.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 30 °C.

Mobile phase Mix 35 volumes of *acetonitrile R* and 65 volumes of a 1.5 g/L solution of *perchloric acid R*.

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µL.

Run time 4 times the retention time of nicardipine.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B and C.

Relative retention With reference to nicardipine (retention time = about 8 min): impurity B = about 0.5; impurity A = about 0.8; impurity C = about 2.1.

System suitability Reference solution (b):
— *resolution*: minimum 1.5 between the peaks due to impurity A and nicardipine.

Calculation of percentage contents:
— for each impurity, use the concentration of nicardipine hydrochloride in reference solution (a).

Limits:
- *impurity B*: maximum 0.5 per cent;
- *impurities A, C*: for each impurity, maximum 0.15 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 1.0 per cent;
- *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

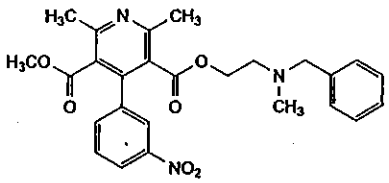
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.400 g in 50 mL of *ethanol (96 per cent) R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

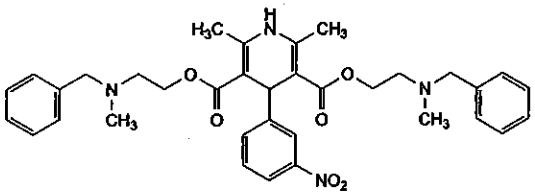
1 mL of *0.1 M sodium hydroxide* is equivalent to 51.60 mg of $C_{26}H_{30}ClN_3O_6$.

STORAGE
In an airtight container, protected from light.

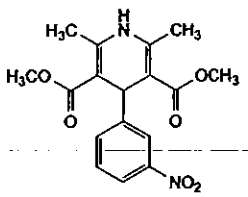
IMPURITIES
Specified impurities *A, B, C.*



A. 2-[benzyl(methyl)amino]ethyl methyl 2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate,



B. bis[2-[benzyl(methyl)amino]ethyl] 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate,

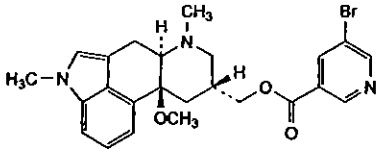


C. dimethyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate.

Ph Eur

Nicergoline

(Ph. Eur. monograph 1998)



$C_{24}H_{26}BrN_3O_3$ 484.4 *27848-84-6*

Action and use
Ergot derivative.

Ph Eur

DEFINITION
[(6a*R*,9*R*,10a*S*)-10a-Methoxy-4,7-dimethyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinolin-9-yl] methyl 5-bromopyridine-3-carboxylate.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS
Appearance
Fine to granular, white or yellowish powder.

Solubility
Practically insoluble in water, freely soluble in methylene chloride, soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION
First identification: A, C.

Second identification: A, B, D.

A. Specific optical rotation (*2.2.7*): + 4.8 to + 5.8 (anhydrous substance).

Dissolve 0.50 g in *ethanol (96 per cent) R* and dilute to 10.0 mL with the same solvent.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 50.0 mg in *ethanol (96 per cent) R* and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of the solution to 50.0 mL with *ethanol (96 per cent) R*.

Spectral range 220-350 nm.

Absorption maximum At 288 nm.

Absorption minimum At 251 nm.

Specific absorbance at the absorption maximum 175 to 185 (anhydrous substance).

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *nicergoline CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *ethanol (96 per cent) R*, evaporate to dryness and record new spectra using the residues.

D. Dissolve 2 mg in 2 mL of *sulfuric acid R*. A blue colour develops.

TESTS
Appearance of solution
The solution is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than intensity 5 of the range of reference solutions of the most appropriate colour (*2.2.2, Method II*).

Dissolve 0.5 g in *ethanol (96 per cent) R* and dilute to 10 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in *acetonitrile R* and dilute to 50.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *acetonitrile R*. Dilute 2.0 mL of this solution to 10.0 mL with *acetonitrile R*.

Reference solution (b) Dissolve 2 mg of *nicergoline for system suitability CRS* (containing impurities A, B, C, D, F and H) in *acetonitrile R* and dilute to 2 mL with the same solvent.

Reference solution (c) Dissolve 5.0 mg of *nicergoline impurity D CRS* in *acetonitrile R* and dilute to 100.0 mL with the same solvent. Dilute 2.0 mL of the solution to 50.0 mL with *acetonitrile R*.

Reference solution (d) Dissolve the contents of a vial of *nicergoline for peak identification CRS* (containing impurity I) in 1 mL of *acetonitrile R*.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped ethylene-bridged octadecylsilyl silica gel for chromatography (hybrid material) R* (3.5 µm);
- *temperature*: 40 °C.

Mobile phase:
- *solution A*: dissolve 34.02 g of *potassium dihydrogen phosphate R* in 930 mL of *water for chromatography R* and dilute to 1000 mL with *water for chromatography R* (buffer solution); dissolve 21.21 g of *tetrabutylammonium hydrogen sulfate R* in 225 mL of the buffer solution and dilute to 250.0 mL with the same solution; adjust to pH 7.5 with a 300 g/L solution of *potassium hydroxide R*;
- *mobile phase A*: mix 2.0 mL of solution A with 300 mL of *acetonitrile R* and 700 mL of *water for chromatography R*;
- *mobile phase B*: mix 2.0 mL of solution A with 300 mL of *water for chromatography R* and 700 mL of *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	100	0
3 - 30	100 → 70	0 → 30
30 - 40	70 → 0	30 → 100
40 - 50	0	100

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 288 nm.

Injection 10 µL.

Identification of impurities Use the chromatogram supplied with *nicergoline for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C, F and H; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity D; use the chromatogram obtained with reference solution (d) to identify the peak due to impurity I.

Relative retention With reference to nicergoline (retention time = about 34 min): impurity D = about 0.06; impurity C = about 0.1; impurity B = about 0.6; impurity H = about 0.8; impurity A = about 0.96; impurity F = about 1.1; impurity I = about 1.2.

System suitability Reference solution (b):
- *resolution*: minimum 2.0 between the peaks due to impurity A and nicergoline.

Calculation of percentage contents:
- for impurity D, use the concentration of impurity D in reference solution (c);
- for impurities other than D, use the concentration of nicergoline in reference solution (a).

Limits:
- *impurity B*: maximum 0.8 per cent;
- *impurity A*: maximum 0.5 per cent;
- *impurity H*: maximum 0.3 per cent;
- *impurities C, D, F, I*: for each impurity, maximum 0.2 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 1.2 per cent;
- *reporting threshold*: 0.05 per cent.

Water (*2.5.32*)

Maximum 0.5 per cent, determined on 0.100 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

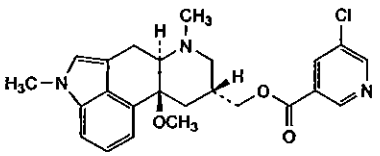
Dissolve 0.400 g in 50 mL of *acetone R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Titrate to the 1st point of inflexion.

1 mL of *0.1 M perchloric acid* is equivalent to 48.44 mg of $C_{24}H_{26}BrN_3O_3$.

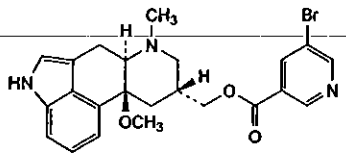
IMPURITIES

Specified impurities *A, B, C, D, F, H, I.*

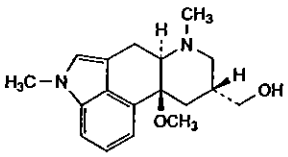
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *E, G, J.*



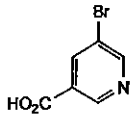
A. [(6a*R*,9*R*,10a*S*)-10a-methoxy-4,7-dimethyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinolin-9-yl] methyl 5-chloropyridine-3-carboxylate (chloronicergoline),



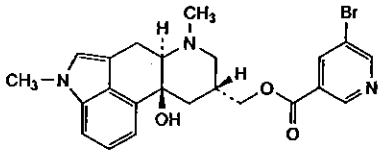
B. [(6a*R*,9*R*,10a*S*)-10a-methoxy-7-methyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinolin-9-yl] methyl 5-bromopyridine-3-carboxylate (1-desmethylnicergoline),



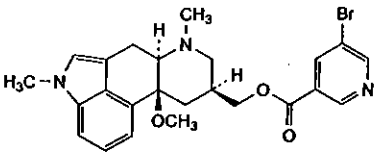
C. [(6a*R*,9*R*,10a*S*)-10a-methoxy-4,7-dimethyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinolin-9-yl] methanol,



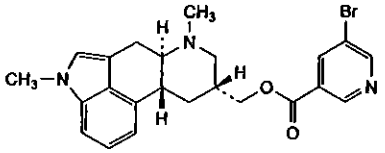
D. 5-bromopyridine-3-carboxylic acid,



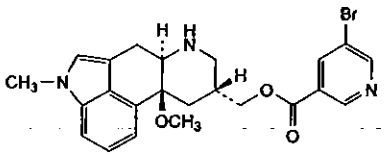
E. [(6a*R*,9*R*,10a*S*)-10a-hydroxy-4,7-dimethyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinolin-9-yl] methyl 5-bromopyridine-3-carboxylate,



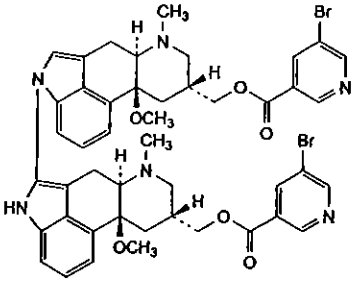
F. [(6a*R*,9*S*,10a*S*)-10a-methoxy-4,7-dimethyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinolin-9-yl] methyl 5-bromopyridine-3-carboxylate (isonicergoline),



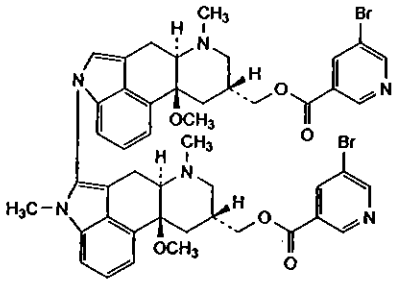
G. [(6a*R*,9*R*,10a*R*)-4,7-dimethyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinolin-9-yl]methyl 5-bromopyridine-3-carboxylate,



H. [(6a*R*,9*R*,10a*S*)-10a-methoxy-4-methyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinolin-9-yl] methyl 5-bromopyridine-3-carboxylate (6-desmethylnicergoline),



I. [(6a*R*,6a′*R*,9*R*,9′*R*,10a*S*,10a′*S*)-9′-[[[(5-bromopyridin-3-yl)carbonyl]oxy]methyl]-10a,10a′-dimethoxy-7,7′-dimethyl-4′,6′,6a,6a′,7,7′,8,8′,9,9′,10,10′,10a,10a′-tetradecahydro-6*H*-4,5′-biindolo[4,3-*fg*]quinoline-9-yl] methyl 5-bromopyridine-3-carboxylate,



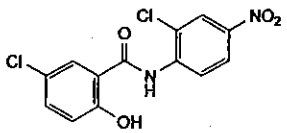
J. [(6a*R*,6a′*R*,9*R*,9′*R*,10a*S*,10a′*S*)-9′-[[[(5-bromopyridin-3-yl)carbonyl]oxy]methyl]-10a,10a′-dimethoxy-4′,7,7′-trimethyl-4′,6′,6a,6a′,7,7′,8,8′,9,9′,10,10′,10a,10a′-tetradecahydro-6*H*-4,5′-biindolo[4,3-*fg*]quinoline-9-yl] methyl 5-bromopyridine-3-carboxylate.

Ph Eur

Niclosamide

Anhydrous Niclosamide

(Ph. Eur. monograph 0679)



$C_{13}H_8Cl_2N_2O_4$ 327.1 *50-65-7*

Action and use
Anthelminthic.

Preparation
Niclosamide Tablets

Ph Eur

DEFINITION
5-Chloro-*N*-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide.

Content
98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
Yellowish-white or yellowish, fine crystals.

Solubility
Practically insoluble in water, sparingly soluble in acetone, slightly soluble in anhydrous ethanol.

IDENTIFICATION

First identification: B, E.

Second identification: A, C, D, E.

A. Melting point (*2.2.14*): 227 °C to 232 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs prepared using about 0.5 mg of substance and 0.3 g of *potassium bromide R*.

Comparison *anhydrous niclosamide CRS*.

C. To 50 mg add 5 mL of *1 M hydrochloric acid* and 0.1 g of *zinc powder R*, heat in a water-bath for 10 min, cool and filter. To the filtrate add 1 mL of a 5 g/L solution of *sodium nitrite R* and allow to stand for 3 min; add 2 mL of a 20 g/L solution of *ammonium sulfamate R*, shake, allow to stand for 3 min and add 2 mL of a 5 g/L solution of *naphthylethylenediamine dihydrochloride R*. A violet colour is produced.

D. Heat the substance on a copper wire in a non-luminous flame. The flame becomes green.

E. Loss on drying (see Tests).

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50 mg of the substance to be examined in *methanol R*, heating gently, cool and dilute to 50.0 mL with the same solvent.

Reference solution Dilute 1.0 mL of the test solution to 100.0 mL with *acetonitrile R*. Dilute 1.0 mL of this solution to 20.0 mL with *acetonitrile R*.

Column:

— *size*: *l* = 0.125 m, Ø = 4 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mixture of equal volumes of *acetonitrile R* and a solution containing 2 g/L of *potassium dihydrogen phosphate R*, 1 g/L of *disodium hydrogen phosphate dodecahydrate R* and 2 g/L of *tetrabutylammonium hydrogen sulfate R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µL.

Run time Twice the retention time of niclosamide.

Limits:

— *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.005 per cent).

5-Chlorosalicylic acid

Maximum 60 ppm.

Test solution To 1.0 g add 15 mL of *water R*, boil for 2 min, cool, filter through a membrane filter (nominal pore size 0.45 µm), wash the filter and dilute the combined filtrate and washings to 20.0 mL with *water R*.

Reference solution Dissolve 30 mg of *5-chlorosalicylic acid R* in 20 mL of *methanol R* and dilute to 100.0 mL with *water R*. Dilute 1.0 mL of this solution to 100.0 mL with *water R*.

To 10.0 mL of the test solution and to 10.0 mL of the reference solution add separately 0.1 mL of *ferric chloride solution R2*. Any violet colour in the test solution is not more intense than that in the reference solution.

2-Chloro-4-nitroaniline

Maximum 100 ppm.

Test solution To 0.250 g add 5 mL of *methanol R*, heat to boiling, cool, add 45 mL of *1 M hydrochloric acid*, heat again to boiling, cool, filter and dilute the filtrate to 50.0 mL with *1 M hydrochloric acid*.

Reference solution Dissolve 50 mg of *2-chloro-4-nitroaniline R* in *methanol R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of the solution to 100.0 mL with *methanol R*. Dilute 2.0 mL of this solution to 20.0 mL with *1 M hydrochloric acid*.

To 10.0 mL of the test solution and to 10.0 mL of the reference solution add separately 0.5 mL of a 5 g/L solution of *sodium nitrite R* and allow to stand for 3 min. Add 1 mL of a 20 g/L solution of *ammonium sulfamate R*, shake, allow to stand for 3 min and add 1 mL of a 5 g/L solution of *naphthylethylenediamine dihydrochloride R*. Any pinkish-violet colour in the test solution is not more intense than that in the reference solution.

Chlorides (*2.4.4*)

Maximum 500 ppm.

To 2 g add a mixture of 1.2 mL of *acetic acid R* and 40 mL of *water R*, boil for 2 min, cool and filter. Dilute 2 mL of the filtrate to 15 mL with *water R*.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.3000 g in 80 mL of a mixture of equal volumes of *acetone R* and *methanol R*. Titrate with 0.1 M tetrabutylammonium hydroxide, determining the end-point potentiometrically (*2.2.20*).

1 mL of 0.1 M tetrabutylammonium hydroxide is equivalent to 32.71 mg of $C_{13}H_8Cl_2N_2O_4$.

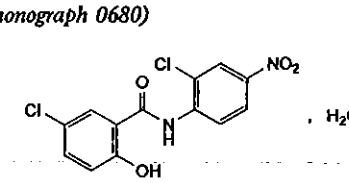
STORAGE

In an airtight container, protected from light.

Ph Eur

Niclosamide Monohydrate

(Ph. Eur. monograph 0680)



$C_{13}H_8Cl_2N_2O_4,H_2O$ 345.1

Action and use

Anthelminthic.

Preparation

Niclosamide Tablets

Ph Eur

DEFINITION

5-Chloro-*N*-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide monohydrate.

Content
98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
Yellowish, fine crystals.

Solubility
Practically insoluble in water, sparingly soluble in acetone, slightly soluble in anhydrous ethanol.

IDENTIFICATION

First identification: B, E.

Second identification: A, C, D, E.

A. Melting point (*2.2.14*): 227 °C to 232 °C, determined after drying at 100-105 °C for 4 h.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Dry the substance to be examined at 100-105 °C for 4 h and examine as discs prepared using about 0.5 mg of substance and 0.3 g of *potassium bromide R*.

Comparison *anhydrous niclosamide CRS*.

C. To 50 mg add 5 mL of *1 M hydrochloric acid* and 0.1 g of *zinc powder R*, heat in a water-bath for 10 min, cool and filter. To the filtrate add 1 mL of a 5 g/L solution of *sodium nitrite R* and allow to stand for 3 min; add 2 mL of a 20 g/L solution of *ammonium sulfamate R*, shake, allow to stand for 3 min and add 2 mL of a 5 g/L solution of *naphthylethylenediamine dihydrochloride R*. A violet colour is produced.

D. Heat the substance on a copper wire in a non-luminous flame. The flame becomes green.

E. Loss on drying (see Tests).

TESTS

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 50 mg of the substance to be examined in *methanol R*, heating gently, cool and dilute to 50.0 mL with the same solvent.

Reference solution Dilute 1.0 mL of the test solution to 100.0 mL with *acetonitrile R*. Dilute 1.0 mL of this solution to 20.0 mL with *acetonitrile R*.

Column:
— *size*: l = 0.125 m, Ø = 4 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mixture of equal volumes of *acetonitrile R* and a solution containing 2 g/L of *potassium dihydrogen phosphate R*, 1 g/L of *disodium hydrogen phosphate dodecahydrate R* and 2 g/L of *tetrabutylammonium hydrogen sulfate R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µL.

Run time Twice the retention time of niclosamide.

Limits:
— *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent);
— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.005 per cent).

5-Chlorosalicylic acid
Maximum 60 ppm.

Test solution To 1.0 g add 15 mL of *water R*, boil for 2 min, cool, filter through a membrane filter (nominal pore size 0.45 µm), wash the filter and dilute the combined filtrate and washings to 20.0 mL with *water R*.

Reference solution Dissolve 30 mg of *5-chlorosalicylic acid R* in 20 mL of *methanol R* and dilute to 100.0 mL with *water R*. Dilute 1.0 mL of this solution to 100.0 mL with *water R*.

To 10.0 mL of the test solution and to 10.0 mL of the reference solution add separately 0.1 mL of *ferric chloride solution R2*. Any violet colour produced in the test solution is not more intense than that in the reference solution.

2-Chloro-4-nitroaniline
Maximum 100 ppm.

Test solution To 0.250 g add 5 mL of *methanol R*, heat to boiling, cool, add 45 mL of *1 M hydrochloric acid*, heat again to boiling, cool, filter and dilute the filtrate to 50.0 mL with *1 M hydrochloric acid*.

Reference solution Dissolve 50 mg of *2-chloro-4-nitroaniline R* in *methanol R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of the solution to 100.0 mL with *methanol R*. Dilute 2.0 mL of this solution to 20.0 mL with *1 M hydrochloric acid*.

To 10.0 mL of the test solution and to 10.0 mL of the reference solution add separately 0.5 mL of a 5 g/L solution of *sodium nitrite R* and allow to stand for 3 min. Add 1 mL of a 20 g/L solution of *ammonium sulfamate R*, shake, allow to stand for 3 min and add 1 mL of a 5 g/L solution of *naphthylethylenediamine dihydrochloride R*. Any pinkish-violet colour produced in the test solution is not more intense than that in the reference solution.

Chlorides (*2.4.4*)
Maximum 500 ppm.

To 2 g add a mixture of 1.2 mL of *acetic acid R* and 40 mL of *water R*, boil for 2 min, cool and filter. Dilute 2 mL of the filtrate to 15 mL with *water R*.

Loss on drying (*2.2.32*)
4.5 per cent to 6.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.3000 g in 80 mL of a mixture of equal volumes of *acetone R* and *methanol R*. Titrate with 0.1 M tetrabutylammonium hydroxide, determining the end-point potentiometrically (*2.2.20*).

1 mL of 0.1 M tetrabutylammonium hydroxide is equivalent to 32.71 mg of $C_{13}H_8Cl_2N_2O_4$.

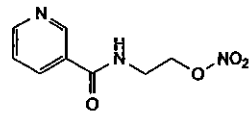
STORAGE

Protected from light.

Ph Eur

Nicorandil

(Ph. Eur. monograph 2332)



$C_8H_9N_3O_4$ 211.2 *65141-46-0*

Action and use
Potassium channel opener.

Preparation
Nicorandil Tablets

Ph Eur

DEFINITION

2-[(Pyridin-3-ylcarbonyl)amino]ethyl nitrate.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Sparingly soluble in water, freely soluble in anhydrous ethanol, practically insoluble in heptane.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *nicorandil CRS*.

TESTS

pH *(2.2.3)*
5.5 to 7.0.

Dissolve 0.25 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent.

Related substances
Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use.*

Test solution Dissolve 50 mg of the substance to be examined in the mobile phase and dilute to 25.0 mL with the mobile phase.

Reference solution (a) Dissolve 2 mg of *nicorandil impurity A CRS* in the mobile phase using sonication and dilute to 25.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 20.0 mL with the test solution.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography compatible with 100 per cent aqueous mobile phases R* (5 µm).

Mobile phase *trifluoroacetic acid R, triethylamine R, tetrahydrofuran R, water R* (3:5:10:982 *V/V/V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µL.

Run time 1.5 times the retention time of nicorandil.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity A.

Relative retention With reference to nicorandil (retention time = about 19 min): impurity A = about 0.9.

System suitability Reference solution (a):
— *resolution*: minimum 2.5 between the peaks due to impurity A and nicorandil.

Calculation of percentage contents:
— for each impurity, use the concentration of nicorandil in reference solution (b).

Limits:
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.2 per cent;
— *reporting threshold*: 0.05 per cent.

Sulfates *(2.4.13)*
Maximum 100 ppm.

Dissolve 5.000 g in 30 mL of *ethanol (50 per cent V/V) R*, add 1 mL of *dilute hydrochloric acid R* and dilute to 50.0 mL with *distilled water R*.

Water *(2.5.32)*
Maximum 0.5 per cent.

Dissolve 0.300 g in a suitable solvent and dilute to 1.0 mL with the same solvent. Inject 0.400 mL of the solution through the septum.

Sulfated ash *(2.4.14)*
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

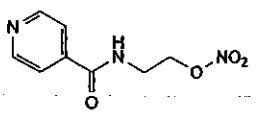
1 mL of *0.1 M perchloric acid* is equivalent to 21.12 mg of $C_8H_9N_3O_4$.

STORAGE

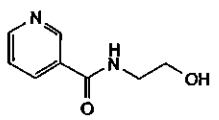
At a temperature of 2 °C to 8 °C.

IMPURITIES

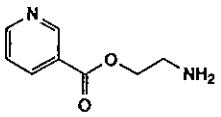
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D.*



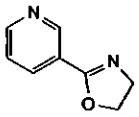
A. 2-[(pyridin-4-ylcarbonyl)amino]ethyl nitrate,



B. *N*-(2-hydroxyethyl)pyridine-3-carboxamide,



C. 2-aminoethyl pyridine-3-carboxylate,

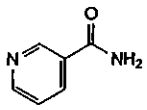


D. 3-(4,5-dihydro-1,3-oxazol-2-yl)pyridine.

Ph Eur

Nicotinamide

(Ph. Eur. monograph 0047)



$C_6H_6N_2O$ 122.1 *98-92-0*

Action and use
Component of vitamin B.

Preparations
Nicotinamide Tablets
Vitamins B and C Injection

Ph Eur

DEFINITION
Pyridine-3-carboxamide.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder or colourless crystals.

Solubility
Freely soluble in water and in anhydrous ethanol, slightly soluble in methylene chloride.

IDENTIFICATION
First identification: B.

Second identification: A, C.

A. Melting point (*2.2.14*): 128 °C to 131 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *nicotinamide CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 5 mg of the substance to be examined in a mixture of equal volumes of *ethanol (96 per cent) R* and *water R* and dilute to 5.0 mL with the same mixture of solvents.

Reference solution Dissolve 5 mg of *nicotinamide CRS* in a mixture of equal volumes of *ethanol (96 per cent) R* and *water R* and dilute to 5.0 mL with the same mixture of solvents.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *water R, ethanol (96 per cent) R, methylene chloride R* (4:45:48 *V/V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the spot in the chromatogram obtained with the reference solution.

TESTS
Solution S
Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_7 (*2.2.2, Method II*).

pH (*2.2.3*)
6.0 to 7.5 for solution S.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.150 g of the substance to be examined in mobile phase A and dilute to 150.0 mL with mobile phase A.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

Reference solution (b) Dissolve 5 mg of *isonicotinamide R* (impurity D) in the test solution and dilute to 100.0 mL with the test solution. Dilute 1.0 mL of this solution to 25.0 mL with the test solution.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
— *mobile phase A*: mix 5.0 mL of *dilute acetic acid R* and 900 mL of *water for chromatography R*, then add 30 mL of *dilute ammonia R3* and 15 mL of *acetonitrile R*; dilute to 1 L with *water for chromatography R*;
— *mobile phase B*: *acetonitrile R*, mobile phase A (50:50 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	98	2
2 - 16	98 → 0	2 → 100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 264 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity D.

Relative retention With reference to nicotinamide (retention time = about 7 min): impurity D = about 0.9.

System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the peaks due to impurity D and nicotinamide.

Calculation of percentage contents:
— for each impurity, use the concentration of nicotinamide in reference solution (a).

Limits:
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.2 per cent;
— *reporting threshold*: 0.05 per cent.

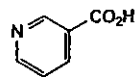
Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* for 18 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

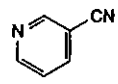
ASSAY
Dissolve 0.100 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 12.21 mg of $C_6H_6N_2O$.

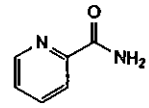
IMPURITIES
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, E.*



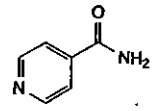
A. pyridine-3-carboxylic acid (nicotinic acid),



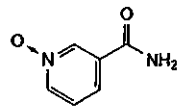
B. pyridine-3-carbonitrile,



C. pyridine-2-carboxamide (picolinamide),



D. pyridine-4-carboxamide (isonicotinamide),

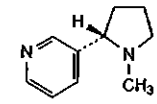


E. pyridine-3-carboxamide 1-oxide (nicotinamide *N*-oxide).

Ph Eur

Nicotine

(Ph. Eur. monograph 1452)



$C_{10}H_{14}N_2$ 162.2 *54-11-5*

Action and use
Aid to smoking cessation.

Preparations
Nicotine Inhalation Cartridges
Nicotine Nasal Spray
Nicotine Sublingual Tablets
Nicotine Transdermal Patches

Ph Eur

DEFINITION
3-[(2*S*)-1-Methylpyrrolidin-2-yl]pyridine.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS
Appearance
Colourless or brownish viscous liquid, volatile, hygroscopic.

Solubility
Soluble in water, miscible with anhydrous ethanol.

IDENTIFICATION
A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *Ph. Eur. reference spectrum of nicotine.*

TESTS
Appearance of solution
Dissolve 1.0 g in *water R* and dilute to 10 mL with the same solvent. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5, BY_5 or R_5 (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*)
−140 to −152.

Dissolve 1.00 g in *anhydrous ethanol R* and dilute to 50.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 20.0 mg of the substance to be examined in *water R* and dilute to 25.0 mL with the same solvent.

Reference solution (a) Dissolve the contents of a vial of *nicotine for system suitability CRS* (containing impurities A, B, C, D, E, F and G) in 1.0 mL of *water R*.

Reference solution (b) Dilute 1.0 mL of the test solution to 10.0 mL with *water R*. Dilute 1.0 mL of this solution to 100.0 mL with *water R*.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer R* (5 µm).

Mobile phase:
— *mobile phase A*: to 900 mL of *water R*, add 25 mL of a 60 g/L solution of *acetic acid R*, then add 6 mL of

concentrated ammonia R1. Adjust to pH 10.0 with *dilute ammonia R2* or *dilute acetic acid R* and dilute to 1000 mL with *water R*;
— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	100	0
3 - 3.01	100 → 95	0 → 5
3.01 - 28	95 → 74	5 → 26
28 - 32	74 → 60	26 → 40

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram supplied with *nicotine for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D, E, F and G.

Relative retention With reference to nicotine (retention time = about 17.8 min): impurity E = about 0.3; impurity C = about 0.55; impurity F = about 0.7; impurity A = about 0.8; impurity D = about 0.86; impurity G = about 0.9; impurity B = about 1.6.

System suitability Reference solution (a):
— *resolution*: minimum 2.5 between the peaks due to impurity G and nicotine.

Limits:
— *impurities A, B, C, D, E, F, G*: for each impurity, not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than 8 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.8 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

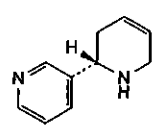
Water (*2.5.12*)
Maximum 0.5 per cent, determined on 1.00 g.

ASSAY
Dissolve 60.0 mg in 30 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (*2.2.20*).

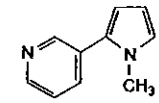
1 mL of *0.1 M perchloric acid* is equivalent to 8.11 mg of $C_{10}H_{14}N_2$.

STORAGE
Under nitrogen, in an airtight container, protected from light.

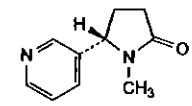
IMPURITIES
Specified impurities *A, B, C, D, E, F, G.*



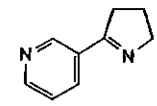
A. (2*S*)-1,2,3,6-tetrahydro-2,3′-bipyridyl (anatabine),



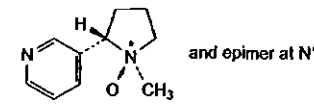
B. 3-(1-methyl-1*H*-pyrrol-2-yl)pyridine (β-nicotyrine),



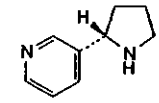
C. (5*S*)-1-methyl-5-(pyridin-3-yl)pyrrolidin-2-one (cotinine),



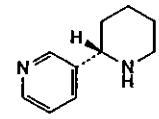
D. 3-(4,5-dihydro-3*H*-pyrrol-2-yl)pyridine (myosmine),



E. (1*RS*,2*S*)-1-methyl-2-(pyridin-3-yl)pyrrolidine 1-oxide (nicotine *N′*-oxide),



F. 3-[(2*S*)-pyrrolidin-2-yl]pyridine (nornicotine),

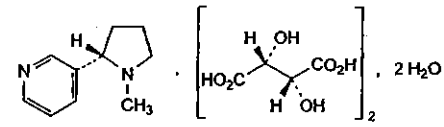


G. 3-[(2*S*)-piperidin-2-yl]pyridine (anabasine).

Ph Eur

Nicotine Ditartrate Dihydrate

(Ph. Eur. monograph 2599)



$C_{18}H_{26}N_2O_{12},2H_2O$ 498.4 *6019-06-3*

Action and use
Aid to smoking cessation.

Ph Eur

DEFINITION
3-[(2*S*)-1-Methylpyrrolidin-2-yl]pyridine bis[(2*R*,3*R*)-2,3-dihydroxybutanedioate] dihydrate.

Content
98.5 per cent to 101.5 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white powder.

Solubility
Soluble in water and in ethanol (96 per cent).

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *nicotine ditartrate dihydrate CRS.*

TESTS
pH (*2.2.3*)
3.0 to 3.4.
Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Specific optical rotation (*2.2.7*)
+ 21.0 to + 23.0.
Dissolve 0.25 g in *water R* and dilute to 25.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*
Test solution Dissolve 60 mg of the substance to be examined in *water R* and dilute to 25.0 mL with the same solvent.
Reference solution (a) Dissolve the contents of a vial of *nicotine for system suitability CRS* (containing impurities A, B, C, D, E, F and G) in 1.0 mL of *water R.*
Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *water R.* Dilute 1.0 mL of this solution to 10.0 mL with *water R.*
Column:
— *size: l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase: end-capped polar-embedded octadecylsilyl amorphous organosilica polymer R* (5 µm).
Mobile phase:
— *mobile phase A*: to 900 mL of *water R* add 25 mL of a 60 g/L solution of *acetic acid R* and 6 mL of *concentrated ammonia R1*; adjust to pH 10.0 with *dilute ammonia R2* or *dilute acetic acid R* and dilute to 1000.0 mL with *water R;*
— *mobile phase B*: *acetonitrile R;*

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	100	0
3 - 3.01	100 → 95	0 → 5
3.01 - 28	95 → 74	5 → 26
28 - 32	74 → 60	26 → 40

Flow rate 1.0 mL/min.
Detection Spectrophotometer at 254 nm.
Injection 20 µL.
Identification of impurities Use the chromatogram supplied with *nicotine for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D, E, F and G.
Relative retention With reference to nicotine (retention time = about 17.8 min): impurity E = about 0.3; impurity C = about 0.55; impurity F = about 0.7; impurity A = about 0.8; impurity D = about 0.86; impurity G = about 0.9; impurity B = about 1.6.
System suitability Reference solution (a):
— *resolution*: minimum 2.5 between the peaks due to impurity G and nicotine.
Limits:
— *impurities A, B, C, D, E, F, G*: for each impurity, not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than 8 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.8 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

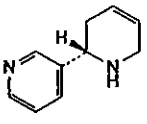
Water (*2.5.12*)
6.5 per cent to 8.0 per cent, determined on 0.100 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

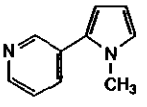
ASSAY
Dissolve 0.180 g in 50 mL of *anhydrous acetic acid R.* Titrate with *0.1 M perchloric acid,* determining the end-point potentiometrically (*2.2.20*).
1 mL of *0.1 M perchloric acid* is equivalent to 23.12 mg of $C_{18}H_{26}N_2O_{12}$.

STORAGE
Protected from light.

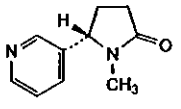
IMPURITIES
Specified impurities *A, B, C, D, E, F, G.*



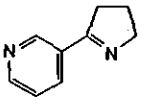
A. (2*S*)-1,2,3,6-tetrahydro-2,3′-bipyridyl (anatabine),



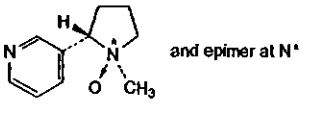
B. 3-(1-methyl-1*H*-pyrrol-2-yl)pyridine (β-nicotyrine),



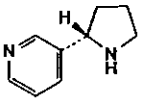
C. (5*S*)-1-methyl-5-(pyridin-3-yl)pyrrolidin-2-one (cotinine),



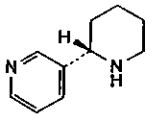
D. 3-(4,5-dihydro-3*H*-pyrrol-2-yl)pyridine (myosmine),



E. (1*RS*,2*S*)-1-methyl-2-(pyridin-3-yl)pyrrolidine 1-oxide (nicotine *N*′-oxide),



F. 3-[(2*S*)-pyrrolidin-2-yl]pyridine (nornicotine),



G. 3-[(2*S*)-piperidin-2-yl]pyridine (anabasine).

Ph Eur

Nicotine Resinate

(Ph. Eur. monograph 1792)

96055-45-7

Action and use
Aid to smoking cessation.

Preparation
Nicotine Resinate Medicated Chewing Gum

Ph Eur

DEFINITION
Complex of nicotine (3-[(2*S*)-1-methylpyrrolidin-2-yl]pyridine) with a weak cationic exchange resin.

Content
95.0 per cent to 115.0 per cent of the declared content of nicotine ($C_{10}H_{14}N_2$) stated on the label (dried substance).

It may contain glycerol.

CHARACTERS
Appearance
White or slightly yellowish powder, hygroscopic.

Solubility
Practically insoluble in water.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Shake a quantity of the substance to be examined equivalent to 100 mg of nicotine with a mixture of 10 mL of *dilute ammonia R2*, 10 mL of *water R*, 5 mL of *strong sodium hydroxide solution R* and 20 mL of *hexane R* for 5 min. Transfer the upper layer to a beaker and evaporate to produce an oily residue. Record the spectrum of the oily residue as a thin film between *sodium chloride R* plates.

Comparison *Ph. Eur. reference spectrum of nicotine.*

B. Nicotine release (see Tests).

TESTS
Nicotine release
Minimum 70 per cent of the content determined under Assay in 10 min.

Transfer an accurately weighed quantity of the substance to be examined, equivalent to about 4 mg of nicotine, to a glass-stoppered test-tube, add 10.0 mL of a 9 g/L solution of *sodium chloride R* previously heated to 37 °C and shake vigorously for 10 min. Immediately filter the liquid through a dry filter paper discarding the 1st millilitre of filtrate. Transfer 1.0 mL of the filtrate to a 20 mL volumetric flask, dilute to 20 mL with *0.1 M hydrochloric acid* and mix. Determine the absorbance (*2.2.25*) at the minima at about 236 nm and 282 nm and at the maximum at 259 nm using 1.0 mL of a 9 g/L solution of *sodium chloride R* diluted to 20 mL with *0.1 M hydrochloric acid* as compensation liquid.

Calculate the percentage of nicotine release using the following expression:

$$\frac{20 \times 10^6 \times (A_{259} - 0.5A_{236} - 0.5A_{282})}{323 \times C \times m}$$

323	=	specific absorbance of nicotine at 259 nm;
C	=	percentage of nicotine in the substance to be examined determined in the assay;
m	=	mass of the substance to be examined, in milligrams;
A_{236}, A_{259}, A_{282}	=	absorbances of the solution at the wavelength indicated by the subscript.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Weigh a quantity of the substance to be examined, equivalent to 30.0 mg of nicotine, into a glass-stoppered test-tube, add 10.0 mL of *dilute ammonia R2* and shake vigorously for 10 min. Centrifuge for 20 min at about 3000 r/min. To 5.0 mL of the clear solution, add 5 mL of a 60 g/L solution of *acetic acid R* and dilute to 25.0 mL with *water R*.

Reference solution (a) Dissolve the contents of a vial of *nicotine for system suitability CRS* (containing impurities A, B, C, D, E, F and G) in 1.0 mL of *water R*.

Reference solution (b) Dilute 1.0 mL of the test solution to 10.0 mL with *water R*. Dilute 1.0 mL of this solution to 100.0 mL with *water R*.

Reference solution (c) Dissolve 46.0 mg of *nicotine ditartrate CRS* in *water R* and dilute to 25.0 mL with the same solvent.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer R* (5 µm).

Mobile phase:
— *mobile phase A*: to 900 mL of *water R*, add 25 mL of a 60 g/L solution of *acetic acid R*, then add 6 mL of *concentrated ammonia R1*; adjust to pH 10.0 with *dilute ammonia R2* or *dilute acetic acid R* and dilute to 1 L with *water R*;
— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	100	0
3 - 3.01	100 → 95	0 → 5
3.01 - 28	95 → 74	5 → 26
28 - 32	74 → 60	26 → 40

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram supplied with *nicotine for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D, E, F and G.

Relative retention With reference to nicotine (retention time = about 18 min): impurity E = about 0.3; impurity C = about 0.55; impurity F = about 0.7; impurity A = about 0.8; impurity D = about 0.86; impurity G = about 0.9; impurity B = about 1.6.

System suitability Reference solution (a):
— *resolution*: minimum 2.5 between the peaks due to impurity G and nicotine.

Limits:
- — *impurities A, B, C, D, E, F, G*: for each impurity, not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
- — *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- — *total*: not more than 8 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.8 per cent);
- — *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 7.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

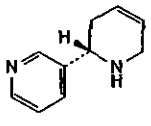
Injection Test solution and reference solution (c).

Calculate the percentage content of nicotine ($C_{10}H_{14}N_2$) taking into account the assigned content of $C_{10}H_{14}N_2$ in *nicotine ditartrate CRS*.

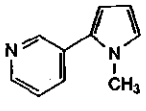
STORAGE
In an airtight container, protected from light.

LABELLING
The label states the content of nicotine.

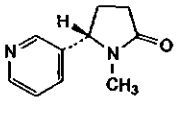
IMPURITIES
Specified impurities *A, B, C, D, E, F, G.*



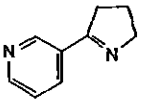
A. (2*S*)-1,2,3,6-tetrahydro-2,3′-bipyridine (anatabine),



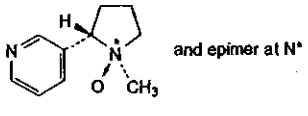
B. 3-(1-methyl-1*H*-pyrrol-2-yl)pyridine (β-nicotyrine),



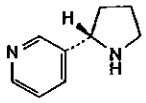
C. (5*S*)-1-methyl-5-(pyridin-3-yl)pyrrolidin-2-one (cotinine),



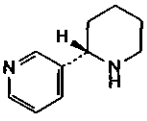
D. 3-(4,5-dihydro-3*H*-pyrrol-2-yl)pyridine (myosmine),



E. (1*RS*,2*S*)-1-methyl-2-(pyridin-3-yl)pyrrolidine 1-oxide (nicotine *N*-oxide),



F. 3-[(2*S*)-pyrrolidin-2-yl]pyridine (nornicotine),

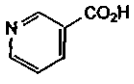


G. 3-[(2*S*)-piperidin-2-yl]pyridine (anabasine).

Ph Eur

Nicotinic Acid

(Ph. Eur. monograph 0459)



$C_6H_5NO_2$ 123.1 *59-67-6*

Action and use
Component of vitamin B.

Preparation
Nicotinic Acid Tablets

Ph Eur

DEFINITION
Pyridine-3-carboxylic acid.

Content
99.5 per cent to 100.5 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Sparingly soluble in water, soluble in boiling water and in boiling ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides and carbonates.

IDENTIFICATION
First identification: A, B.

Second identification: A, C.

A. Melting point (*2.2.14*): 234 °C to 240 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *nicotinic acid CRS.*

C. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Solvent mixture Dissolve 6.8 g of *potassium dihydrogen phosphate R* in 900 mL of *water R*, adjust to pH 7.0 with *dilute sodium hydroxide solution R* and dilute to 1000 mL with *water R*.

Test solution Dissolve 50 mg in the solvent mixture and dilute to 100.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 25.0 mL with the solvent mixture.

Spectral range 237-262 nm.

Absorption maximum At 262 nm.

Absorption minimum At 237 nm.

Absorbance ratio A_{237}/A_{262} = 0.46 to 0.50.

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.120 g of the substance to be examined in 200 µL of *dilute ammonia R1* and dilute to 10.0 mL with mobile phase A.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

Reference solution (b) Dissolve the contents of a vial of *nicotinic acid impurity mixture CRS* (impurities A and B) in 1.0 mL of mobile phase A.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography compatible with 100 per cent aqueous mobile phases R* (4 µm);
- *temperature*: 15 °C.

Mobile phase:
- *mobile phase A*: dilute 2 mL of *acetic acid R* in 950 mL of *water for chromatography R*, adjust to pH 5.6 with *dilute ammonia R1* and dilute to 1000 mL with *water for chromatography R*;
- *mobile phase B*: *acetonitrile R*, *methanol R* (50:50 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	100	0
10 - 30	100→20	0→80
30 - 35	20	80

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 250 nm.

Injection 10 µL.

Identification of impurities Use the chromatogram supplied with *nicotinic acid impurity mixture CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and B.

Relative retention With reference to nicotinic acid (retention time = about 6 min): impurity A = about 2.7; impurity B = about 2.8.

System suitability Reference solution (b):
- *resolution*: minimum 1.5 between the peaks due to impurities A and B.

Limits:
- *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent);
- *total*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent);
- *disregard limit*: 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.03 per cent).

Chlorides (*2.4.4*)

Maximum 200 ppm.

Dissolve 0.25 g in *water R*, heating on a water-bath, and dilute to 15 mL with the same solvent.

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 1 h.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 50 mL of *water R*. Add 0.25 mL of *phenolphthalein solution R*. Titrate with *0.1 M sodium hydroxide* until a pink colour is obtained. Carry out a blank titration.

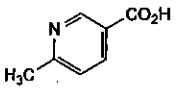
1 mL of *0.1 M sodium hydroxide* is equivalent to 12.31 mg of $C_6H_5NO_2$.

STORAGE

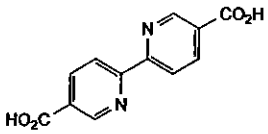
Protected from light.

IMPURITIES

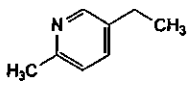
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, E, F, G, H, I.*



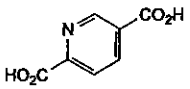
A. 6-methylpyridine-3-carboxylic acid (6-methylnicotinic acid),



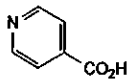
B. 2,2′-bipyridine-5,5′-dicarboxylic acid (6,6′-dinicotinic acid),



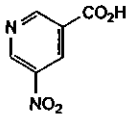
C. 5-ethyl-2-methylpyridine,



D. pyridine-2,5-dicarboxylic acid,

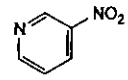


E. pyridine-4-carboxylic acid (isonicotinic acid),

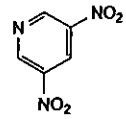


F. 5-nitropyridine-3-carboxylic acid (5-nitronicotinic acid),

G. pyridine,



H. 3-nitropyridine,

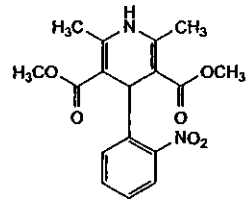


I. 3,5-dinitropyridine.

Ph Eur

Nifedipine

(Ph. Eur. monograph 0627)



$C_{17}H_{18}N_2O_6$ 346.3 *21829-25-4*

Action and use
Calcium channel blocker.

Preparations
Nifedipine Capsules
Nifedipine Oral Suspension
Nifedipine Prolonged-release Capsules
Nifedipine Prolonged-release Tablets

Ph Eur

DEFINITION
Dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS
Appearance
Yellow, crystalline powder.

Solubility
Practically insoluble in water, freely soluble in acetone, sparingly soluble in ethanol.

When exposed to daylight and to artificial light of certain wavelengths, it readily converts to a nitrosophenylpyridine derivative. Exposure to ultraviolet light leads to the formation of a nitrophenylpyridine derivative.

Prepare solutions immediately before use in the dark or under long-wavelength light (> 420 nm) and protect them from light.

IDENTIFICATION
First identification: B.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 171 °C to 175 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *nifedipine CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 10 mg of *nifedipine CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *ethyl acetate R, cyclohexane R* (40:60 *V/V*).

Application 5 µL.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, appearance at 254 nm and size to the principal spot in the chromatogram obtained with the reference solution.

D. To 25 mg in a test tube, add 10 mL of a mixture of 1.5 volumes of *hydrochloric acid R*, 3.5 volumes of *water R* and 5 volumes of *alcohol R* and dissolve with gentle heating. Add 0.5 g of *zinc R* in granules and allow to stand for 5 min with occasional swirling. Filter into a second test tube, add 5 mL of a 10 g/L solution of *sodium nitrite R* to the filtrate and allow to stand for 2 min. Add 2 mL of a 50 g/L solution of *ammonium sulfamate R*, shake vigorously with care and add 2 mL of a 5 g/L solution of *naphthylethylenediamine dihydrochloride R*. An intense red colour develops which persists for not less than 5 min.

TESTS
Impurity D and other basic impurities
Transfer 4 g to a 250 mL conical flask and dissolve in 160 mL of *glacial acetic acid R* using an ultrasonic bath. Titrate with *0.1 M perchloric acid* using 0.25 mL of *naphtholbenzein solution R* as indicator until the colour changes from brownish-yellow to green. Not more than 0.48 mL of *0.1 M perchloric acid* is required (0.14 per cent).

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.200 g of the substance to be examined in 20 mL of *methanol R* and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 10 mg of *nifedipine impurity A CRS* in *methanol R* and dilute to 25.0 mL with the same solvent.

Reference solution (b) Dissolve 10 mg of *nifedipine impurity B CRS* in *methanol R* and dilute to 25.0 mL with the same solvent.

Reference solution (c) Mix 1.0 mL of reference solution (a), 1.0 mL of reference solution (b) and 0.1 mL of the test solution and dilute to 20.0 mL with the mobile phase. Dilute 2.0 mL of this solution to 10.0 mL with the mobile phase.

Column:
— *size: l* = 0.15 m, Ø = 4.6 mm,
— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R, methanol R, water R* (9:36:55 *V/V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 235 nm.

Injection 20 µL; inject the test solution and reference solution (c).

Run time Twice the retention time of nifedipine.

Elution order Impurity A, impurity B, nifedipine.

Retention time Nifedipine = about 15.5 min.

System suitability Reference solution (c):
— *resolution*: minimum 1.5 between the peaks due to impurity A and impurity B and minimum 1.5 between the peaks due to impurity B and nifedipine.

Limits:
— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.1 per cent),
— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.1 per cent),
— *any other impurity*: not more than the area of the peak due to nifedipine in the chromatogram obtained with reference solution (c) (0.1 per cent),
— *total*: not more than 0.3 per cent,
— *disregard limit*: 0.1 times the area of the peak due to nifedipine in the chromatogram obtained with reference solution (c) (0.01 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

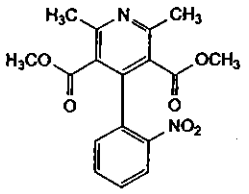
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.1300 g in a mixture of 25 mL of *2-methyl-2-propanol R* and 25 mL of *perchloric acid solution R*. Titrate with *0.1 M cerium sulfate* using 0.1 mL of *ferroin R* as indicator, until the pink colour disappears. Titrate slowly towards the end of the titration. Carry out a blank titration.

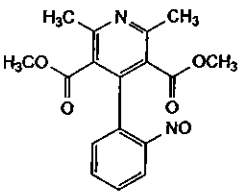
1 mL of *0.1 M cerium sulfate* is equivalent to 17.32 mg of $C_{17}H_{18}N_2O_6$.

STORAGE
Protected from light.

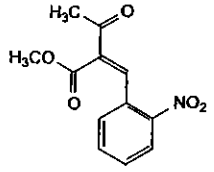
IMPURITIES
Specified impurities *A, B, C, D.*



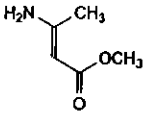
A. dimethyl 2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate (nitrophenylpyridine analogue),



B. dimethyl 2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate (nitrosophenylpyridine analogue),



C. methyl 2-(2-nitrobenzylidene)-3-oxobutanoate,

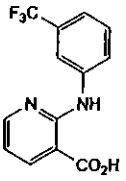


D. methyl 3-aminobut-2-enoate.

Ph Eur

Niflumic Acid

(Ph. Eur. monograph 2115)



$C_{13}H_9F_3N_2O_2$ 282.2 *4394-00-7*

Ph Eur

DEFINITION
2-[[3-(Trifluoromethyl)phenyl]amino]pyridine-3-carboxylic acid.

Content
98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS
Appearance
Pale yellow, crystalline powder.

Solubility
Practically insoluble in water, freely soluble in acetone, soluble in ethanol (96 per cent) and in methanol.

mp
About 204 °C.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *niflumic acid CRS.*

TESTS
Impurity C
Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.50 g of the substance to be examined in 5 mL of *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution Dissolve 25 mg of *3-trifluoromethylaniline R* (impurity C) in 20 mL of *methanol R* and dilute to 100 mL with the same solvent. Dilute 1.0 mL of this solution to 100 mL with *methanol R*.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *acetic acid R, ethyl acetate R, toluene R* (5:25:90 *V/V/V*).

Application 10 µL.

Development Over 3/4 of the plate.

Drying In air, until the solvents have evaporated.

Detection Spray with *4-dimethylaminocinnamaldehyde solution R* and heat at 60 °C for 10 min.

Limit:
— *impurity C*: any spot due to impurity C is not more intense than the principal spot in the chromatogram obtained with the reference solution (50 ppm).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in 10 mL of *acetonitrile R* and dilute to 20.0 mL with *water R*.

Reference solution Dissolve 5.0 mg of *niflumic acid impurity A CRS*, 5.0 mg of *niflumic acid impurity B CRS* and 6.0 mg of *niflumic acid impurity E CRS* in 20 mL of *acetonitrile R*, add 5.0 mL of the test solution and dilute to 50.0 mL with *water R*. Dilute 1.0 mL of this solution to 100.0 mL with a mixture of equal volumes of *acetonitrile R* and *water R*.

Column:
- *size*: *l* = 0.125 m, Ø = 4.0 mm;
- *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 25 °C.

Mobile phase *phosphoric acid R, acetonitrile R, water R* (2.5:500:500 *V/V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 267 nm.

Injection 10 µL.

Run time 4 times the retention time of niflumic acid.

Relative retention With reference to niflumic acid (retention time = about 5.5 min): impurity A = about 0.25; impurity B = about 0.57; impurity E = about 0.64.

System suitability Reference solution:
- *resolution*: minimum 1.5 between the peaks due to impurities B and E.

Limits:
- *impurity B*: not more than 4 times the area of the corresponding peak in the chromatogram obtained with the reference solution (0.4 per cent);
- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.1 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the peak due to niflumic acid in the chromatogram obtained with the reference solution (0.10 per cent);
- *sum of impurities other than B*: not more than twice the area of the peak due to niflumic acid in the chromatogram obtained with the reference solution (0.2 per cent);
- *disregard limit*: 0.5 times the area of the peak due to niflumic acid in the chromatogram obtained with the reference solution (0.05 per cent).

Chlorides (*2.4.4*)

Maximum 200 ppm.

Dissolve 0.5 g in a mixture of 1 mL of *nitric acid R* and 10 mL of *methanol R*, and dilute to 20 mL with *water R*. To 10 mL of this solution add 5 mL of *water R*.

Phosphates (*2.4.11*)

Maximum 100 ppm.

Dilute 1.0 mL of the test solution prepared as described in general chapter *2.4.8* (method C) to 100 mL with *water R*.

Loss on drying (*2.2.32*)

Maximum 0.3 per cent, determined on 2.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

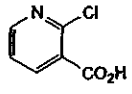
Dissolve 0.200 g in a mixture of 10 mL of *water R* and 40 mL of *ethanol (96 per cent) R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M sodium hydroxide* is equivalent to 28.22 mg of $C_{13}H_9F_3N_2O_2$.

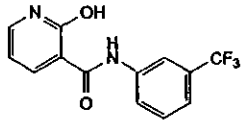
IMPURITIES

Specified impurities *A, B, C.*

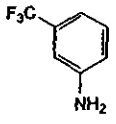
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *E, F.*



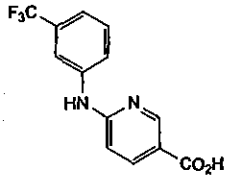
A. 2-chloropyridine-3-carboxylic acid,



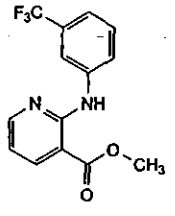
B. 2-hydroxy-*N*-[3-(trifluoromethyl)phenyl]pyridine-3-carboxamide,



C. 3-(trifluoromethyl)aniline,



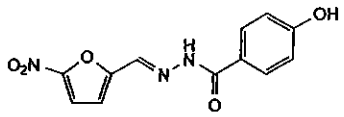
E. 6-[[3-(trifluoromethyl)phenyl]amino]pyridine-3-carboxylic acid,



F. methyl 2-[[3-(trifluoromethyl)phenyl]amino]pyridine-3-carboxylate.

Nifuroxazide

(Ph. Eur. monograph 1999)



$C_{12}H_9N_3O_5$ 275.2 *965-52-6*

Action and use
Antibacterial.

Ph Eur

DEFINITION
(*E*)-4-Hydroxy-*N'*-[(5-nitrofuran-2-yl) methylidene]benzohydrazide.

Content
98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance
Bright yellow, crystalline powder.

Solubility
Practically insoluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *nifuroxazide CRS.*

TESTS

Specific absorbance (*2.2.25*)
940 to 1000 at the absorption maximum at 367 nm.
Protected from light, dissolve 10.0 mg in 10 mL of *ethylene glycol monomethyl ether R* and dilute to 100.0 mL with *methanol R*. Dilute 5.0 mL of this solution to 100.0 mL with *methanol R*.

Impurity A
Maximum 0.05 per cent.
Test solution (a) Dissolve 1.0 g of the substance to be examined in *dimethyl sulfoxide R* and dilute to 10.0 mL with the same solvent.
Test solution (b) To 5.5 mL of test solution (a) add 50.0 mL of *water R* while stirring. Allow to stand for 15 min and filter.
Reference solution To 0.5 mL of test solution (a) add 5.0 mL of a 50 mg/L solution of *4-hydroxybenzohydrazide R* (impurity A) in *dimethyl sulfoxide R*. Add 50.0 mL of *water R* while stirring. Allow to stand for 15 min and filter.
Add 0.5 mL of *phosphomolybdotungstic reagent R* and 10.0 mL of *sodium carbonate solution R* separately to 10.0 mL of test solution (b) and to 10.0 mL of the reference solution. Allow to stand for 1 h. Examine the 2 solutions at 750 nm.
The absorbance (*2.2.25*) of the solution obtained with test solution (b) is not greater than that obtained with the reference solution.

Related substances
Liquid chromatography (*2.2.29*). *Use amber volumetric flasks, unless otherwise specified.*
Solvent mixture *acetonitrile R, water R* (40:60 *V/V*).
Test solution Dissolve 10.0 mg of the substance to be examined in the solvent mixture, using sonication for not more than 5 min, and dilute to 100.0 mL with the solvent mixture.
Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.
Reference solution (b) In order to prepare impurity E *in situ*, dissolve 5 mg of the substance to be examined in the solvent mixture in a colourless volumetric flask, using sonication for 5 min, and dilute to 50 mL with the solvent mixture. Allow to stand in ambient light for 1 h.
Reference solution (c) Dissolve 5.0 mg of *methyl parahydroxybenzoate CRS* (impurity B) in the solvent mixture and dilute to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 100.0 mL with the solvent mixture.
Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: spherical *octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 10 °C.
Mobile phase:
— *mobile phase A*: *tetrahydrofuran R, water R* (5:95 *V/V*);
— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	67	33
10 - 30	67 → 43	33 → 57

Flow rate 1.0 mL/min.
Detection Spectrophotometer at 280 nm.
Injection 50 µL.
Relative retention With reference to nifuroxazide (retention time = about 8 min): impurity A (keto-enol tautomers) = about 0.36 and 0.39; impurity E = about 0.9; impurity B = about 1.2; impurity C = about 2.6; impurity D = about 3.4.
System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the peaks due to impurity E and nifuroxazide.
Limits:
— *impurity E*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
— *impurities B, C, D*: for each impurity, not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent), and not more than 1 such peak has an area greater than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *sum of impurities other than E*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);
— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent); disregard the peaks due to impurity A.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

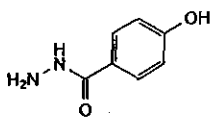
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.200 g, with heating if necessary, in 30 mL of *dimethylformamide R* and add 20 mL of *water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

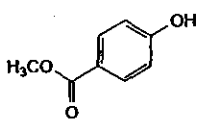
1 mL of *0.1 M sodium hydroxide* is equivalent to 27.52 mg of $C_{12}H_9N_3O_5$.

STORAGE
Protected from light.

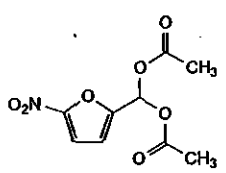
IMPURITIES
Specified impurities *A, B, C, D, E.*



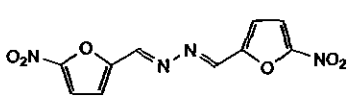
A. 4-hydroxybenzohydrazide (*p*-hydroxybenzohydrazide),



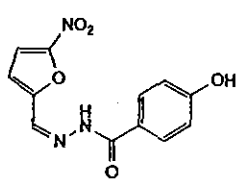
B. methyl 4-hydroxybenzoate (methyl parahydroxybenzoate),



C. (5-nitrofuran-2-yl)methylidene diacetate,



D. (*E,E*)-*N,N'*-bis[(5-nitrofuran-2-yl)methylidene]hydrazine (5-nitrofurfural azine),

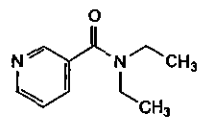


E. (*Z*)-4-hydroxy-*N'*-[(5-nitrofuran-2-yl) methylidene]benzohydrazide.

Ph Eur

Nikethamide

(Ph. Eur. monograph 0233)



$C_{10}H_{14}N_2O$ 178.2 *59-26-7*

Action and use
Central nervous system stimulant.

Ph Eur

DEFINITION
Nikethamide contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of *N,N*-diethylpyridine-3-carboxamide, calculated with reference to the anhydrous substance.

CHARACTERS
An oily liquid or a crystalline mass, colourless or slightly yellowish, miscible with water and with alcohol.

IDENTIFICATION
First identification: A, B.

Second identification: A, C, D.

A. Dissolve 0.15 g in *0.01 M hydrochloric acid* and dilute to 100.0 mL with the same acid. Dilute 1.0 mL of this solution to 100.0 mL with *0.01 M hydrochloric acid*. Examined between 230 nm and 350 nm (*2.2.25*) in a 2 cm cell, the solution shows a single absorption maximum, at 263 nm. The specific absorbance at the maximum is about 285.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *nikethamide CRS*.

C. Heat 0.1 g with 1 mL of *dilute sodium hydroxide solution R*. Diethylamine is evolved progressively and is recognisable by its characteristic odour and by its turning *red litmus paper R* blue.

D. Dilute 1 mL of solution S (see Tests) to 250 mL with *water R*. To 2 mL of this solution add 2 mL of *cyanogen bromide solution R*. Add 3 mL of a 25 g/L solution of *aniline R* and shake. A yellow colour develops.

TESTS

Solution S
Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Appearance
The substance to be examined, in liquid form or liquefied by slight heating, is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5 (*2.2.2, Method II*).

pH (*2.2.3*)
The pH of solution S is 6.0 to 7.8.

Refractive index (*2.2.6*)
1.524 to 1.526.

Related substances
Examine by thin-layer chromatography (*2.2.27*), using *silica gel GF_{254} R* as the coating substance.

Test solution Dissolve 0.4 g of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dissolve 40 mg of *ethylnicotinamide CRS* in *methanol R* and dilute to 100 mL with the same solvent.

Reference solution (b) Dilute 1 mL of reference solution (a) to 10 mL with *methanol R*.

Apply separately to the plate 10 µL of each solution. Develop over a path of 15 cm using a mixture of 25 volumes of *propanol R* and 75 volumes of *chloroform R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with the test solution, any spot corresponding to ethylnicotinamide is not more intense than the spot in the chromatogram obtained with reference solution (a) (1.0 per cent) and any spot, apart from the principal spot and the spot corresponding to ethylnicotinamide, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.1 per cent).

Water (*2.5.12*)
Not more than 0.3 per cent, determined on 2.00 g by the semi-micro determination of water.

Sulfated ash (*2.4.14*)
Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

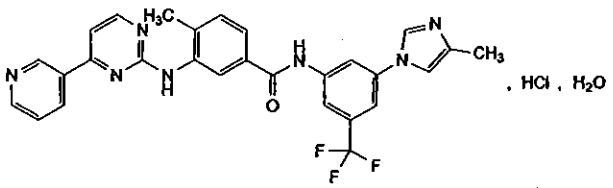
Dissolve 0.150 g in a mixture of 5 mL of *acetic anhydride R* and 20 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 17.82 mg of $C_{10}H_{14}N_2O$.

Ph Eur

Nilotinib Hydrochloride Monohydrate

(Ph. Eur. monograph 2993)



$C_{28}H_{23}ClF_3N_7O,H_2O$ 584.0 *923288-90-8*

Action and use
Tyrosine kinase (BCR-ABL) inhibitor; treatment of chronic myeloid leukaemia.

Ph Eur

DEFINITION

4-Methyl-*N*-[3-(4-methyl-1*H*-imidazol-1-yl)-5-(trifluoromethyl)phenyl]-3-[[4-(pyridin-3-yl)pyrimidin-2-yl]amino]benzamide hydrochloride monohydrate.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or slightly yellowish or slightly greenish-yellow, hygroscopic, crystalline powder.

Solubility
Practically insoluble in water, slightly soluble in anhydrous ethanol, very slightly soluble in heptane.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *nilotinib hydrochloride monohydrate CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *anhydrous ethanol R*, evaporate to dryness and record new spectra using the residues.

B. It gives reaction (b) of chlorides (*2.3.1*).

C. Water (see Tests).

TESTS

Impurity A
Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Solvent mixture *dimethyl sulfoxide R, water R* (20:80 *V/V*).

Test solution Dissolve 0.300 g of the substance to be examined in 2 mL of *dimethyl sulfoxide R*, add 7 mL of *water R*, allow to equilibrate at room temperature without shaking to avoid foam formation, and then dilute to 10.0 mL with *water R*. Shake well, allow the substance to be examined to precipitate for about 2 h in the dark and filter the supernatant through a 0.45 µm filter.

Reference solution (a) Dissolve 7.5 mg of *nilotinib impurity A CRS* in *dimethyl sulfoxide R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of the solution to 100.0 mL with *dimethyl sulfoxide R*. Dilute 2.0 mL of this solution to 10.0 mL with *water R*.

Reference solution (b) Dissolve 7.5 mg of *nilotinib impurity B CRS* and 7.5 mg of *nilotinib impurity C CRS* in *dimethyl sulfoxide R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of the solution to 100.0 mL with *dimethyl sulfoxide R*. Dilute 2.0 mL of this solution to 10.0 mL with *water R*.

Reference solution (c) Dilute 2.0 mL of reference solution (b) to 10.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.15 m, Ø = 3.0 mm;
— *stationary phase*: *encapsulated polar-embedded octadecylsilyl silica gel for chromatography R* (3 µm);
— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: 1.36 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*;
— *mobile phase B*: mobile phase A, *acetonitrile R1* (20:80 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	85	15
2 - 10	85 → 80	15 → 20
10 - 12	80 → 75	20 → 25
12 - 18	75 → 10	25 → 90
18 - 19	10	90

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 207 nm.

Injection 20 µL of the test solution and reference solution (a).

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity A.

Relative retention With reference to nilotinib (retention time = about 17 min): impurity A = about 0.3.

System suitability:
— *repeatability*: maximum relative standard deviation of 5.0 per cent determined on 6 injections of reference solution (a).

Calculation of content:
— for impurity A, use the concentration of impurity A in reference solution (a).

Limit:
— *impurity A*: maximum 3 ppm.

Impurities B and C

Liquid chromatography (*2.2.29*) as described in the test for impurity A with the following modifications.

Detection Spectrophotometer at 225 nm.

Injection Test solution and reference solutions (b) and (c).

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B and C.

Relative retention With reference to nilotinib (retention time = about 17 min): impurity C = about 0.2; impurity B = about 0.6.

System suitability:
— *signal-to-noise ratio*: minimum 10 for the peaks due to impurities B and C in the chromatogram obtained with reference solution (c);
— *repeatability*: maximum relative standard deviation of 5.0 per cent for the areas of the peaks due to impurities B and C, determined on 6 injections of reference solution (b).

Calculation of contents:
— for impurities B and C, use the concentration of the corresponding impurity in reference solution (b).

Limits:
— *impurities B, C*: for each impurity, maximum 2 ppm.

Related substances

Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Solvent mixture *anhydrous ethanol R, water R* (50:50 *V/V*).

Test solution Dissolve 20.0 mg of the substance to be examined in the solvent mixture and dilute to 200.0 mL with the solvent mixture.

Reference solution (a) Dissolve 20.0 mg of *nilotinib hydrochloride monohydrate CRS* in the solvent mixture and dilute to 200.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 2 mg of *nilotinib for system suitability CRS* (containing impurities E, F and G) in the solvent mixture and dilute to 20 mL with the solvent mixture.

Column:
— *size*: *l* = 0.15 m, Ø = 3.0 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography compatible with 100 per cent aqueous mobile phases R* (3 µm);
— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: 1.36 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*;
— *mobile phase B*: mobile phase A, *acetonitrile for chromatography R* (20:80 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	90	10
2 - 16	90 → 10	10 → 90
16 - 17	10	90

Flow rate 0.8 mL/min.

Detection Spectrophotometer at 240 nm.

Injection 5 µL of the test solution and reference solutions (b) and (c).

Identification of impurities Use the chromatogram supplied with *nilotinib for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities E, F and G.

Relative retention With reference to nilotinib (retention time = about 12 min): impurity E = about 1.03; impurity F = about 1.07; impurity G = about 1.09.

System suitability Reference solution (c):
— *resolution*: minimum 1.5 between the peaks due to impurities F and G;
— *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to nilotinib.

Calculation of percentage contents:
— for each impurity, use the concentration of nilotinib hydrochloride monohydrate in reference solution (b).

Limits:
— *impurity F*: maximum 0.2 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.40 per cent;
— *reporting threshold*: 0.05 per cent.

Water (*2.5.12*)

3.0 per cent to 5.0 per cent, determined on 0.150 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{28}H_{23}ClF_3N_7O$ taking into account the assigned content of *nilotinib hydrochloride monohydrate CRS*.

STORAGE

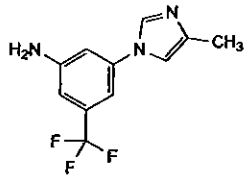
In an airtight container.

IMPURITIES

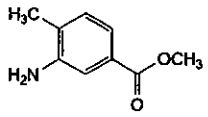
Specified impurities *A, B, C, F.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for

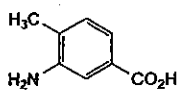
demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *D, E, G, H.*



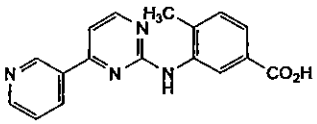
A. 3-(4-methyl-1*H*-imidazol-1-yl)-5-(trifluoromethyl)aniline,



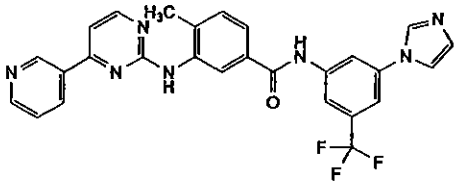
B. methyl 3-amino-4-methylbenzoate,



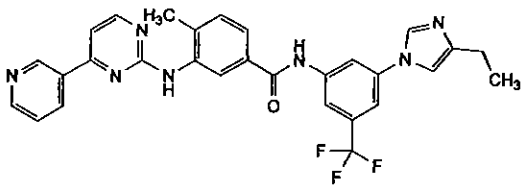
C. 3-amino-4-methylbenzoic acid,



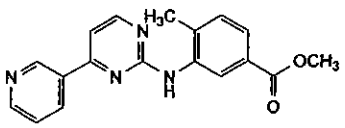
D. 4-methyl-3-[[4-(pyridin-3-yl)pyrimidin-2-yl]amino]benzoic acid,



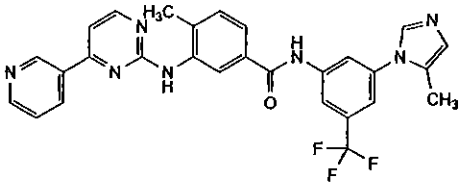
E. *N*-[3-(1*H*-imidazol-1-yl)-5-(trifluoromethyl)phenyl]-4-methyl-3-[[4-(pyridin-3-yl)pyrimidin-2-yl]amino] benzamide,



F. *N*-[3-(4-ethyl-1*H*-imidazol-1-yl)-5-(trifluoromethyl)phenyl]-4-methyl-3-[[4-(pyridin-3-yl) pyrimidin-2-yl]amino]benzamide,



G. methyl 4-methyl-3-[[4-(pyridin-3-yl)pyrimidin-2-yl]amino] benzoate,

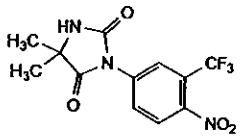


H. 4-methyl-*N*-[3-(5-methyl-1*H*-imidazol-1-yl)-5-(trifluoromethyl)phenyl]-3-[[4-(pyridin-3-yl)pyrimidin-2-yl]amino]benzamide.

Ph Eur

Nilutamide

(Ph. Eur. monograph 2256)



$C_{12}H_{10}F_3N_3O_4$ 317.2 *63612-50-0*

Action and use
Cytotoxic.

Ph Eur

DEFINITION
5,5-Dimethyl-3-[4-nitro-3-(trifluoromethyl) phenyl]imidazolidine-2,4-dione.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Very slightly soluble in water, freely soluble in acetone, soluble in anhydrous ethanol.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *nilutamide CRS.*

TESTS

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Solvent mixture *acetonitrile for chromatography R, water R* (35:65 *V/V*).

Test solution Dissolve 0.10 g of the substance to be examined in the solvent mixture and dilute to 100 mL with the solvent mixture.

Reference solution (a) Dilute 20.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 100.0 mL with the solvent mixture.

Reference solution (b) Dissolve 2 mg of the substance to be examined and 2 mg of *nilutamide impurity B CRS* in the solvent mixture and dilute to 50 mL with the solvent mixture.

Column:
— *size:* l = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: spherical *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: 2.0 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 7.5 with *1 M sodium hydroxide*;
— *mobile phase B*: *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 8	55	45
8 - 30	55 → 30	45 → 70

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µL.

Relative retention With reference to nilutamide (retention time = about 5.3 min): impurity B = about 0.9.

System suitability Reference solution (b):
— *resolution*: minimum 3.0 between the peaks due to impurity B and nilutamide.

Limits:
— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)
Maximum 0.5 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY
Liquid chromatography (*2.2.29*). *The solutions are stable for 24 h at room temperature and in daylight.*

Solvent mixture *acetonitrile for chromatography R, water R* (35:65 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Reference solution Dissolve 50.0 mg of *nilutamide CRS* in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: spherical *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 40 volumes of *acetonitrile R* and 60 volumes of a 2.0 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 7.5 with *1 M sodium hydroxide*.

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 267 nm.

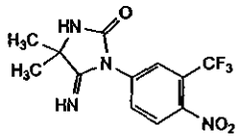
Injection 20 µL.

Retention time About 9 min.

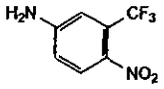
Calculate the percentage content of $C_{12}H_{10}F_3N_3O_4$ from the declared content of *nilutamide CRS*.

STORAGE
Protected from light.

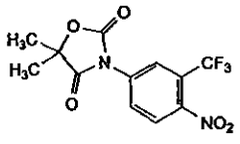
IMPURITIES
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D.*



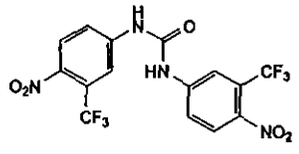
A. 5-imino-4,4-dimethyl-1-[4-nitro-3-(trifluoromethyl)phenyl] imidazolidin-2-one,



B. 4-nitro-3-(trifluoromethyl)aniline (nifeline),



C. 5,5-dimethyl-3-[4-nitro-3-(trifluoromethyl) phenyl]oxazolidine-2,4-dione,

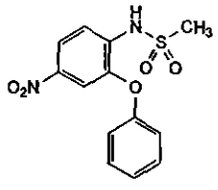


D. 1,3-bis[4-nitro-3-(trifluoromethyl)phenyl]urea.

Ph Eur

Nimesulide

(Ph. Eur. monograph 1548)



$C_{13}H_{12}N_2O_5S$ 308.3 *51803-78-2*

Action and use
Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Ph Eur

DEFINITION
N-(4-Nitro-2-phenoxyphenyl)methanesulfonamide.

Content
98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance
Yellowish, crystalline powder.

Solubility
Practically insoluble in water, freely soluble in acetone, slightly soluble in anhydrous ethanol.

mp
About 149 °C.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *nimesulide CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *acetone R*, evaporate to dryness and record new spectra using the residues.

TESTS

Absorbance (*2.2.25*)
Maximum 0.50 at 450 nm.

Dissolve 1.0 g in *acetone R* and dilute to 10.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 20 mg of the substance to be examined in 8 mL of *acetonitrile R* and dilute to 20.0 mL with *water R*.

Reference solution (a) Dissolve 5 mg of *2-phenoxyaniline R* (impurity C) in 10 mL of *acetonitrile R* and dilute to 25.0 mL with *water R*. Dilute 1.0 mL of the solution to 50.0 mL with the mobile phase. Mix 1.0 mL of this solution with the contents of a vial of *nimesulide impurity D CRS* previously dissolved in 1.0 mL of *acetonitrile R*.

Reference solution (b) Dilute 1.0 mL of the test solution to 10.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 100.0 mL with the mobile phase.

Reference solution (c) Dissolve 4 mg of *nimesulide for peak identification CRS* (containing impurities A, B, E and F) in 4.0 mL of *acetonitrile R* and dilute to 10.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.125 m, Ø = 4.0 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 35 volumes of *acetonitrile R* and 65 volumes of a 1.15 g/L solution of *ammonium dihydrogen phosphate R* previously adjusted to pH 7.0 with *ammonia R*.

Flow rate 1.3 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µL.

Run time 7 times the retention time of nimesulide.

Identification of impurities Use the chromatogram supplied with *nimesulide for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, E and F; use the chromatogram obtained with reference solution (a) to identify the peaks due to impurities C and D.

Relative retention With reference to nimesulide (retention time = about 5 min): impurity A = about 0.3; impurity B = about 2.4; impurity C = about 3.2; impurity D = about 3.7; impurity E = about 4.2; impurity F = about 6.1.

System suitability Reference solution (a):
— *resolution*: minimum 2.0 between the peaks due to impurities C and D.

Limits:
— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity C = 0.7; impurity E = 1.4;
— *impurity E*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
— *impurities A, B, C, D, F*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

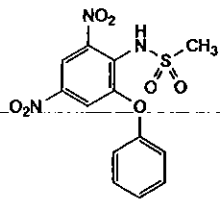
Dissolve 0.240 g in 30 mL of previously neutralised *acetone R* and add 20 mL of *water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M sodium hydroxide* is equivalent to 30.83 mg of $C_{13}H_{12}N_2O_5S$.

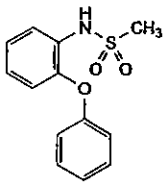
IMPURITIES

Specified impurities *A, B, C, D, E, F.*

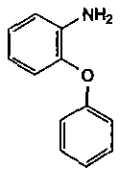
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *G.*



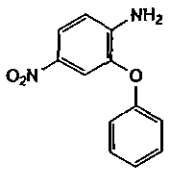
A. *N*-(2,4-dinitro-6-phenoxyphenyl)methanesulfonamide,



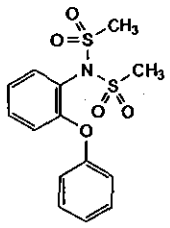
B. *N*-(2-phenoxyphenyl)methanesulfonamide,



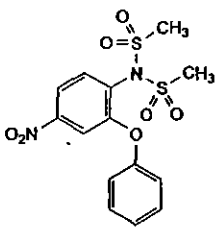
C. 2-phenoxyaniline,



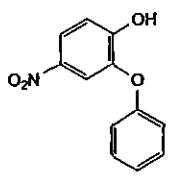
D. 4-nitro-2-phenoxyaniline,



E. *N,N*-bis(methylsulfonyl)-2-phenoxyaniline,



F. *N,N*-bis(methylsulfonyl)-4-nitro-2-phenoxyaniline,

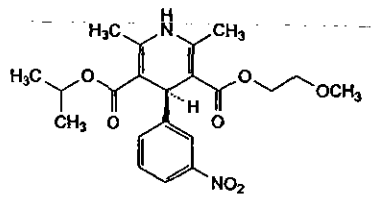


G. 4-nitro-2-phenoxyphenol.

Ph Eur

Nimodipine

(Ph. Eur. monograph 1245)



and enantiomer

$C_{21}H_{26}N_2O_7$ 418.4 *66085-59-4*

Action and use
Calcium channel blocker.

Preparations
Nimodipine Infusion
Nimodipine Tablets

Ph Eur

DEFINITION

2-Methoxyethyl 1-methylethyl (4*RS*)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate.

Content
98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance
Light yellow or yellow, crystalline powder.

Solubility
Practically insoluble in water, freely soluble in ethyl acetate, sparingly soluble in anhydrous ethanol.

It shows polymorphism (*5.9*).

Exposure to ultraviolet light leads to the formation of a nitrophenylpyridine derivative.

IDENTIFICATION

A. Optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *nimodipine CRS.*

If the spectra obtained in the solid state show differences, record new spectra using 20 g/L solutions in *methylene chloride R* and a 0.2 mm cell.

TESTS

Prepare solutions immediately before use either protected from light or under long-wavelength light (> 420 nm).

Solution S
Dissolve 1.0 g in *acetone R* and dilute to 20.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*).

Optical rotation (*2.2.7*)
−0.10° to + 0.10°, determined on solution S.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 40 mg of the substance to be examined in 2.5 mL of *tetrahydrofuran R* and dilute to 25.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 4 mg of *nimodipine for peak identification CRS* (containing impurity C) in 0.25 mL of *tetrahydrofuran R* and dilute to 2.5 mL with the mobile phase.

Reference solution (c) Dilute 0.5 mL of the test solution to 25.0 mL with the mobile phase. Mix 0.5 mL of this solution with 0.5 mL of *nimodipine impurity A CRS* and dilute to 10.0 mL with the mobile phase.

Column:
— *size: l* = 0.125 m, Ø = 4.6 mm;
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature:* 40 °C.

Mobile phase *methanol R, tetrahydrofuran R, water R* (20:20:60 *V/V/V*).

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 235 nm.

Injection 20 µL.

Run time 4.5 times the retention time of nimodipine.

Identification of impurities Use the chromatogram supplied with *nimodipine for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity C; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity A.

Relative retention With reference to nimodipine (retention time = about 7 min): impurity C = about 0.5; impurity A = about 0.9.

System suitability Reference solution (c):

— *peak-to-valley ratio*: minimum 4.0, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to nimodipine.

Limits:

— *impurity C*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *unspecified impurities* : for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying *(2.2.32)*
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash *(2.4.14)*
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Prepare solutions immediately before use either protected from light or under long-wavelength light (> 420 nm).

Dissolve with gentle heating 0.180 g in a mixture of 25 mL of *2-methyl-2-propanol R* and 25 mL of *perchloric acid solution R*. Add 0.1 mL of *ferroin R*. Titrate with *0.1 M cerium sulfate*. Titrate slowly towards the end of the titration. Carry out a blank titration.

1 mL of *0.1 M cerium sulfate* is equivalent to 20.92 mg of $C_{21}H_{26}N_2O_7$.

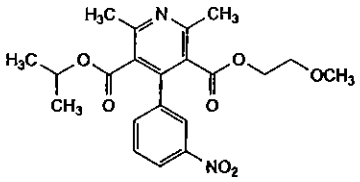
STORAGE

Protected from light.

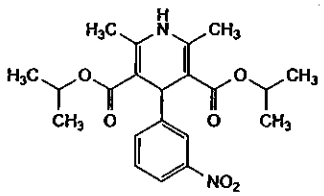
IMPURITIES

Specified impurities C.

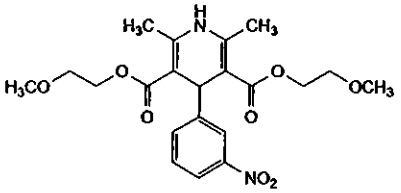
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) A, B.



A. 2-methoxyethyl 1-methylethyl 2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate,



B. bis(1-methylethyl) 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate,

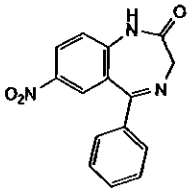


C. bis(2-methoxyethyl) 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate.

Ph Eur

Nitrazepam

(Ph. Eur. monograph 0415)



$C_{15}H_{11}N_3O_3$ 281.3 *146-22-5*

Action and use
Benzodiazepine.

Preparations
Nitrazepam Oral Suspension
Nitrazepam Tablets

Ph Eur

DEFINITION

7-Nitro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or yellow, crystalline powder.

Solubility
Practically insoluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *nitrazepam CRS.*

TESTS

Related substances

Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Test solution Dissolve 50 mg of the substance to be examined in *acetonitrile R* and dilute to 20.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *acetonitrile R*. Dilute 1.0 mL of this solution to 10.0 mL with *acetonitrile R*.

Reference solution (b) Dissolve 2 mg of *clonazepam CRS* in *acetonitrile R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of this solution to 10.0 mL with the test solution.

Column:
- *size:* l = 0.25 m, Ø = 4.0 mm;
- *stationary phase: octylsilyl silica gel for chromatography R* (5 µm);
- *temperature:* 40 °C.

Mobile phase:
- *mobile phase A:* 7.8 g/L solution of *sodium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*;
- *mobile phase B: acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	65	35
3 - 10	65 → 50	35 → 50
10 - 20	50	50

Flow rate 1 mL/min.

Detection Spectrophotometer at 270 nm.

Injection 10 µL.

Relative retention With reference to nitrazepam (retention time = about 9 min): clonazepam = about 1.1.

System suitability Reference solution (b):
- *peak-to-valley ratio:* minimum 4.0, where H_p = height above the baseline of the peak due to clonazepam and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to nitrazepam.

Limits:
- *unspecified impurities:* for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total:* not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *disregard limit:* 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 25 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

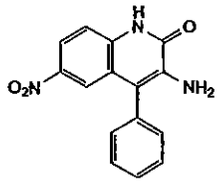
1 mL of *0.1 M perchloric acid* is equivalent to 28.13 mg of $C_{15}H_{11}N_3O_3$.

STORAGE

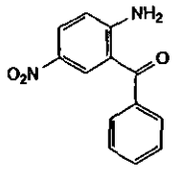
Protected from light.

IMPURITIES

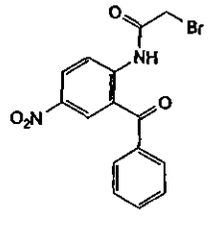
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D.*



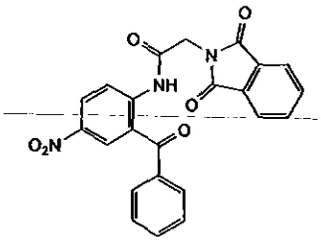
A. 3-amino-6-nitro-4-phenylquinolin-2(1*H*)-one,



B. (2-amino-5-nitrophenyl)phenylmethanone,



C. 2-bromo-*N*-[4-nitro-2-(phenylcarbonyl)phenyl]acetamide,

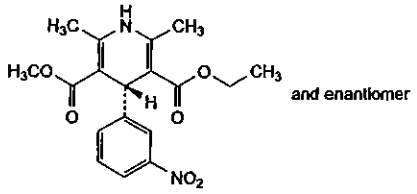


D. 2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N*-[4-nitro-2-(phenylcarbonyl)phenyl]acetamide.

Ph Eur

Nitrendipine

(Ph. Eur. monograph 1246)



and enantiomer

$C_{18}H_{20}N_2O_6$ 360.4 *39562-70-4*

Action and use
Calcium channel blocker.

Ph Eur

DEFINITION

Ethyl methyl (4*RS*)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate.

Content
98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance
Yellow, crystalline powder.

Solubility
Practically insoluble in water, freely soluble in ethyl acetate, sparingly soluble in anhydrous ethanol and in methanol.

It shows polymorphism (*5.9*).

Exposure to ultraviolet light leads to the formation of a nitrophenylpyridine derivative.

Prepare solutions immediately before use either protected from light or under long-wavelength light (> 420 nm).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *nitrendipine CRS.*

If the spectra obtained in the solid state show differences, record new spectra using 20 g/L solutions in *methylene chloride R* and a 0.2 mm cell.

TESTS

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 20 mg of the substance to be examined in 2.5 mL of *tetrahydrofuran R* and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 15.0 mg of *nitrendipine impurity A CRS* in 2.5 mL of *tetrahydrofuran R* and dilute to 10.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 20.0 mL with the mobile phase.

Reference solution (c) Dilute 0.5 mL of the test solution to 20.0 mL with the mobile phase.

Reference solution (d) Mix 1.0 mL of reference solution (b) and 1.0 mL of reference solution (c), then dilute to 25.0 mL with the mobile phase.

Reference solution (e) Dissolve 2 mg of *nitrendipine for peak identification CRS* (containing impurities B and C) in 0.5 mL of *tetrahydrofuran R* and dilute to 1.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.125 m, Ø = 4 mm;
— *stationary phase*: irregular *octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 40 °C.

Mobile phase *acetonitrile R, tetrahydrofuran R, water R* (14:22:64 *V/V/V*).

Flow rate 1 mL/min.

Detection Spectrophotometer at 235 nm.

Injection 10 µL of the test solution and reference solutions (a), (d) and (e).

Run time 5 times the retention time of nitrendipine.

Identification of impurities Use the chromatogram supplied with *nitrendipine for peak identification CRS* and the chromatogram obtained with reference solution (e) to identify the peaks due to impurities B and C; use the chromatogram obtained with reference solution (d) to identify the peak due to impurity A.

Relative retention With reference to nitrendipine (retention time = about 9 min): impurity B = about 0.7; impurity A = about 0.8; impurity C = about 1.4.

System suitability Reference solution (d):
— *resolution*: minimum 2.0 between the peaks due to impurity A and nitrendipine.

Limits:
— *impurities B, C*: for each impurity, not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);
— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.15 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: maximum 0.7 per cent;
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.160 g with gentle heating if necessary in a mixture of 25 mL of *2-methyl-2-propanol R* and 25 mL of *perchloric acid solution R*. Titrate with *0.1 M cerium sulfate*, using 0.1 mL of *ferroin R* as indicator. Titrate slowly towards the end of the titration. Carry out a blank titration.

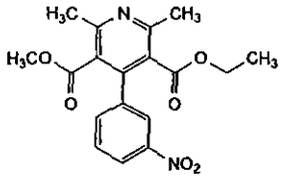
1 mL of *0.1 M cerium sulfate* is equivalent to 18.02 mg of $C_{18}H_{20}N_2O_6$.

STORAGE

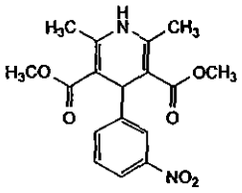
Protected from light.

IMPURITIES

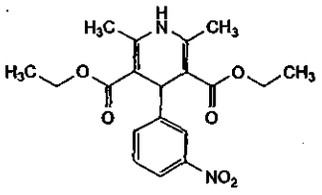
Specified impurities *A, B, C.*



A. ethyl methyl 2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate,



B. dimethyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate,



C. diethyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate.

Ph Eur

Any opalescence is not more intense than that of a standard prepared at the same time in the same manner using 13 mL of *water R*, 0.5 mL of *nitric acid R*, 0.5 mL of *chloride standard solution (5 ppm Cl) R* and 0.3 mL of *silver nitrate solution R2*.

Sulfates (*2.4.13*)
Maximum 10 ppm.

To 15 g add 0.2 g of *sodium carbonate R*. After carbon dioxide has evolved, evaporate to dryness. Dissolve the residue in 15 mL of *distilled water R*.

Iron (*2.4.9*)
Maximum 10 ppm.

Dissolve the residue obtained in the test for sulfated ash in 1 mL of *dilute hydrochloric acid R* and dilute to 20 mL with *water R*. Dilute 1 mL of this solution to 10 mL with *water R*.

Sulfated ash
Maximum 0.01 per cent.

Carefully evaporate 20.00 g to dryness. Moisten the residue with a few drops of *sulfuric acid R* and ignite to dull red.

ASSAY
To 0.750 g add 50 mL of *water R* and titrate with *1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *1 M sodium hydroxide* is equivalent to 63.0 mg of HNO_3.

STORAGE
Protected from light.

Ph Eur

Nitric Acid

(Ph. Eur. monograph 1549)

HNO_3 63.0 *7697-37-2*

Ph Eur

DEFINITION
Content
68.0 per cent *m/m* to 70.0 per cent *m/m*.

CHARACTERS
Appearance
Clear, colourless or almost colourless liquid.

Solubility
Miscible with water.

Relative density
About 1.41.

IDENTIFICATION
A. Dilute 1 mL to 100 mL with *water R*. The solution is strongly acid (*2.2.4*).

B. 0.2 mL of the solution obtained in identification test A gives the reaction of nitrates (*2.3.1*).

TESTS
Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Dilute 2 mL to 10 mL with *water R*.

Chlorides (*2.4.4*)
Maximum 0.5 ppm.

To 5 g add 10 mL of *water R* and 0.3 mL of *silver nitrate solution R2* and allow to stand for 2 min protected from light.

Nitric Oxide

(Ph. Eur. monograph 1550)

NO 30.01 *10102-43-9*

Ph Eur

DEFINITION
Content
Minimum 99.0 per cent *V/V* of NO.

This monograph applies to nitric oxide for medicinal use.

CHARACTERS
Appearance
Colourless gas which turns brown when exposed to air.

Solubility
At 20 °C and at a pressure of 101 kPa, 1 volume dissolves in about 21 volumes of water.

PRODUCTION
Carbon dioxide
Gas chromatography (*2.2.28*).

Gas to be examined The substance to be examined.

Reference gas Mixture containing 3000 ppm *V/V* of *carbon dioxide R1* in *nitrogen R*.

Column:
— *material*: stainless steel;
— *size*: *l* = 3.5 m, Ø = 2 mm;
— *stationary phase*: *ethylvinylbenzene-divinylbenzene copolymer R*;
— *temperature*: 50 °C.

Carrier gas *helium for chromatography R*.

Flow rate 15 mL/min.

Detection Thermal conductivity.

Injection Loop injector.

System suitability:
— the chromatograms obtained show a clear separation of carbon dioxide from nitric oxide.

Limit:
— *carbon dioxide*: not more than the area of the corresponding peak in the chromatogram obtained with the reference gas (3000 ppm *V/V*).

Nitrogen

Gas chromatography (*2.2.28*).

Gas to be examined The substance to be examined.

Reference gas Mixture containing 3000 ppm *V/V* of *nitrogen R* in *helium for chromatography R*.

Column:
— *material*: stainless steel;
— *size*: *l* = 3.5 m, Ø = 2 mm;
— *stationary phase*: *molecular sieve for chromatography R* (0.5 nm);
— *temperature*: 50 °C.

Carrier gas *helium for chromatography R*.

Flow rate 15 mL/min.

Detection Thermal conductivity.

Injection Loop injector.

System suitability:
— the chromatograms obtained show a clear separation of nitrogen from nitric oxide.

Limit:
— *nitrogen*: not more than the area of the corresponding peak in the chromatogram obtained with the reference gas (3000 ppm *V/V*).

Nitrogen dioxide

Maximum 400 ppm *V/V*.

Ultraviolet absorption spectrophotometry analyser.

Gas to be examined The substance to be examined.

Reference gas (a) *nitrogen R1*.

Reference gas (b) Mixture containing 400 ppm *V/V* of *nitrogen dioxide R* in *nitrogen R*.

Apparatus:
— an ultraviolet-visible light source (analytical wavelength about 400 nm);
— a sample gas cell through which the feed gas flows;
— a closed reference gas cell containing *nitrogen R1* in parallel with the sample gas cell;
— a rotating chopper which feeds light alternately through the reference gas cell and the sample gas cell;
— a semiconductor detector which generates a frequency modulated output whose amplitude is a measure of the difference of absorption of the sample gas and the reference gas.

Analysis:
— set the zero of the instrument using reference gas (a) through the sample gas cell at a flow rate of 1 L/min;
— adjust the span while feeding reference gas (b) through the sample gas cell at a flow rate of 1 L/min;
— feed the gas to be examined through the sample gas cell at a flow rate of 1 L/min, read the value from the instrument output and calculate, if necessary, the concentration of nitrogen dioxide.

Nitrous oxide

Gas chromatography (*2.2.28*).

Gas to be examined The substance to be examined.

Reference gas Mixture containing 3000 ppm *V/V* of *nitrous oxide R* in *nitrogen R*.

Column:
— *material*: stainless steel;
— *size*: *l* = 3.5 m, Ø = 2 mm;
— *stationary phase*: *ethylvinylbenzene-divinylbenzene copolymer R*;
— *temperature*: 50 °C.

Carrier gas *helium for chromatography R*.

Flow rate 15 mL/min.

Detection Thermal conductivity.

Injection Loop injector.

System suitability:
— the chromatograms obtained show a clear separation of nitrous oxide from nitric oxide.

Limit:
— *nitrous oxide*: not more than the area of the corresponding peak in the chromatogram obtained with the reference gas (3000 ppm *V/V*).

Water (*2.5.28*)

Maximum 100 ppm *V/V*.

Assay

Determine the content of nitric oxide by difference using the mass balance equation after determining the sum of the impurities described under Production.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of nitric oxide.*

STORAGE

Compressed at a pressure not exceeding 2.5 MPa (25 bar) measured at 15 °C, in suitable containers complying with the legal regulations.

IMPURITIES

Specified impurities *A, B, C, D, E.*

A. CO_2: carbon dioxide,

B. N_2: nitrogen,

C. NO_2: nitrogen dioxide,

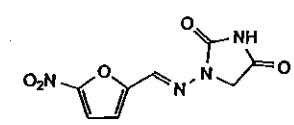
D. N_2O: nitrous oxide,

E. H_2O: water.

Ph Eur

Nitrofurantoin

(Ph. Eur. monograph 0101)



$C_8H_6N_4O_5$ 238.2 *67-20-9*

Action and use

Antibacterial.

Preparations

Nitrofurantoin Oral Suspension

Nitrofurantoin Tablets

Ph Eur

DEFINITION

Nitrofurantoin contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of 1-[[(5-nitrofuran-2-yl)methylene]amino]imidazolidine-2,4-dione, calculated with reference to the dried substance.

CHARACTERS

A yellow, crystalline powder or yellow crystals, very slightly soluble in water and in ethanol (96 per cent), soluble in dimethylformamide.

IDENTIFICATION

A. *Carry out the test protected from bright light.* Use the solution prepared for the assay. Examined between 220 nm and 400 nm (*2.2.25*), the solution shows two absorption maxima, at 266 nm and 367 nm. The ratio of the absorbance at the maximum at 367 nm to that at the maximum at 266 nm is 1.36 to 1.42.

B. Dissolve about 10 mg in 10 mL of *dimethylformamide R*. To 1 mL of the solution add 0.1 mL of 0.5 M alcoholic potassium hydroxide. A brown colour develops.

TESTS

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel HF_{254} R* as the coating substance.

Test solution Dissolve 0.25 g of the substance to be examined in a minimum of *dimethylformamide R* and dilute to 10 mL with *acetone R*.

Reference solution Dilute 1 mL of the test solution to 100 mL with *acetone R*.

Apply separately to the plate 10 µL of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *methanol R* and 90 volumes of *nitromethane R*. Allow the plate to dry in air and heat at 100 °C to 105 °C for 5 min. Examine in ultraviolet light at 254 nm. Spray with *phenylhydrazine hydrochloride solution R*. Heat the plate at 100 °C to 105 °C for a further 10 min. When examined in ultraviolet light and after spraying, any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (1.0 per cent).

Loss on drying (*2.2.32*)

Not more than 1.0 per cent, determined on 1.00 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Carry out the assay protected from bright light Dissolve 0.120 g in 50 mL of *dimethylformamide R* and dilute to 1000.0 mL with *water R*. Dilute 5.0 mL of the solution to 100.0 mL with a solution containing 18 g/L of *sodium acetate R* and 0.14 per cent *V/V* of *glacial acetic acid R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 367 nm, using the sodium acetate solution described above as compensation liquid.

Calculate the content of $C_8H_6N_4O_5$, taking the specific absorbance to be 765.

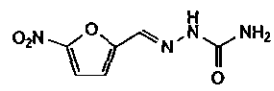
STORAGE

Store protected from light, at a temperature below 25 °C.

Ph Eur

Nitrofurazone

(Nitrofural, Ph. Eur. monograph 1135)



$C_6H_6N_4O_4$ 198.1 *59-87-0*

Action and use

Antibacterial; topical antiprotozoal.

Ph Eur

DEFINITION

2-[(5-Nitrofuran-2-yl)methylene]diazanecarboxamide.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

Yellow or brownish-yellow, crystalline powder.

Solubility

Very slightly soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: B.

Second identification: A, C, D.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*). *Carry out the test protected from bright light.*

Test solution Use the solution prepared for the assay.

Spectral range 220-400 nm.

Absorption maxima At 260 nm and 375 nm.

Absorbance ratio A_{375}/A_{260} = 1.15 to 1.30.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *nitrofural CRS*.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 10 mg of *nitrofural CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel G plate R*.

Mobile phase *methanol R, nitromethane R* (10:90 *V/V*).

Application 5 µL.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with *phenylhydrazine hydrochloride solution R*.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve about 1 mg in 1 mL of *dimethylformamide R* and add 0.1 mL of *alcoholic potassium hydroxide solution R*. A violet-red colour is produced.

TESTS

pH (*2.2.3*)

5.0 to 7.0.

To 1.0 g add 100 mL of *carbon dioxide-free water R*. Shake and filter.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dissolve 10.0 mg of *nitrofural impurity B CRS* in the mobile phase and dilute to 20.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve 10 mg of the substance to be examined and 10 mg of *nitrofurantoin R* in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 5.0 mL of the solution to 100.0 mL with the mobile phase.

Reference solution (c) Dissolve with the aid of ultrasound the contents of a vial of *nitrofural for peak identification CRS* (containing impurities A and B) in 1.0 mL of the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R, water R* (40:60 *V/V*).

Flow rate 1 mL/min.

Detection Spectrophotometer at 310 nm.

Injection 20 µL.

Run time 10 times the retention time of nitrofural.

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A and B.

Relative retention With reference to nitrofural (retention time = about 4 min): nitrofurantoin = about 1.2; impurity B = about 4.0; impurity A = about 7.6.

System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the peaks due to nitrofural and nitrofurantoin.

Limits:
— *impurities A, B*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

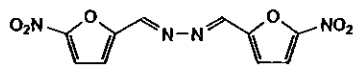
ASSAY
Carry out the assay protected from bright light.

Dissolve 60.0 mg in 20 mL of *dimethylformamide R* and dilute to 500.0 mL with *water R*. Dilute 5.0 mL of the solution to 100.0 mL with *water R*. Prepare a reference solution in the same manner using 60.0 mg of *nitrofural CRS*. Measure the absorbances (*2.2.25*) of the 2 solutions at the absorption maximum at 375 nm.

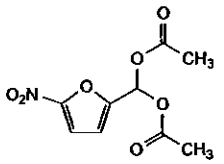
Calculate the content of $C_6H_6N_4O_4$ from the absorbances measured and the concentrations of the solutions.

STORAGE
Protected from light.

IMPURITIES
Specified impurities *A, B.*



A. 1,2-bis[(5-nitrofuran-2-yl)methylidene]diazane,



B. (5-nitrofuran-2-yl)methylene diacetate.

Ph Eur

Nitrogen

(Ph. Eur. monograph 1247)

Nitrogen should be kept in approved metal cylinders, the shoulders of which are painted black and the remainder grey. The cylinder should carry a label stating 'Nitrogen'.

N_2 28.01 *7727-37-9*

Ph Eur

DEFINITION
Content
Minimum 99.5 per cent *V/V* of N_2.

This monograph applies to nitrogen for medicinal use.

CHARACTERS
Appearance
Colourless, odourless gas.

Solubility
At 20 °C and at a pressure of 101 kPa, 1 volume dissolves in about 62 volumes of water and about 10 volumes of ethanol (96 per cent).

PRODUCTION
Carbon dioxide
Maximum 300 ppm *V/V*, determined using an infrared analyser (*2.5.24*).

Gas to be examined The substance to be examined. It must be filtered to avoid stray light phenomena.

Reference gas (a) *Nitrogen R1.*

Reference gas (b) Mixture containing 300 ppm *V/V* of *carbon dioxide R1* in *nitrogen R1.*

Calibrate the apparatus and set the sensitivity using reference gases (a) and (b). Measure the content of carbon dioxide in the gas to be examined.

Carbon monoxide
Maximum 5 ppm *V/V*, determined using an infrared analyser (*2.5.25*).

Gas to be examined The substance to be examined. It must be filtered to avoid stray light phenomena.

Reference gas (a) *Nitrogen R1.*

Reference gas (b) Mixture containing 5 ppm *V/V* of *carbon monoxide R* in *nitrogen R1*.

Calibrate the apparatus and set the sensitivity using reference gases (a) and (b). Measure the content of carbon monoxide in the gas to be examined.

Oxygen

Maximum 50 ppm *V/V*, determined using an oxygen analyser with a detector scale ranging from 0-100 ppm *V/V* and equipped with an electrochemical cell.

The gas to be examined passes through a detection cell containing an aqueous solution of an electrolyte, generally potassium hydroxide. The presence of oxygen in the gas to be examined produces variation in the electric signal recorded at the outlet of the cell that is proportional to the oxygen content.

Calibrate the analyser according to the instructions of the manufacturer. Pass the gas to be examined through the analyser using a suitable pressure regulator and airtight metal tubes and operating at the prescribed flow-rates until constant readings are obtained.

Water (*2.5.28*)

Maximum 67 ppm *V/V*.

Assay

Gas chromatography (*2.2.28*).

Gas to be examined The substance to be examined.

Reference gas (a) Ambient air.

Reference gas (b) *Nitrogen R1*.

Column:
- *material*: stainless steel;
- *size*: *l* = 2 m, Ø = 2 mm;
- *stationary phase*: *molecular sieve for chromatography R* (0.5 nm).

Carrier gas *helium for chromatography R*.

Flow rate 40 mL/min.

Temperature:
- *column*: 50 °C;
- *detection*: 130 °C.

Detection Thermal conductivity.

Injection Loop injector.

Inject reference gas (a). Adjust the injected volumes and operating conditions so that the height of the peak due to nitrogen in the chromatogram obtained with the reference gas is at least 35 per cent of the full scale of the recorder.

System suitability:
- the chromatograms obtained show a clear separation of oxygen and nitrogen.

Calculate the content of N_2 in the gas to be examined.

IDENTIFICATION

First identification: A.

Second identification: B, C.

A. Examine the chromatograms obtained in the assay (see Production).

Results The principal peak in the chromatogram obtained with the substance to be examined is similar in retention time to the principal peak in the chromatogram obtained with reference gas (b).

B. In a 250 mL conical flask replace the air by the substance to be examined. Place a burning or glowing splinter of wood in the flask. The splinter is extinguished.

C. In a suitable test tube, place 0.1 g of *magnesium R* in turnings. Close the tube with a two-hole stopper fitted with a glass tube reaching about 1 cm above the turnings. Pass the substance to be examined through the glass tube for 1 min without heating, then for 15 min while heating the test tube to a red glow. After cooling, add 5 mL of *dilute sodium hydroxide solution R*. The evolving vapours change the colour of moistened *red litmus paper R* blue.

TESTS

Carbon dioxide (*2.1.6*)

Maximum 300 ppm *V/V*, determined using a carbon dioxide detector tube.

Carbon monoxide (*2.1.6*)

Maximum 5 ppm *V/V*, determined using a carbon monoxide detector tube.

Water vapour (*2.1.6*)

Maximum 67 ppm *V/V*, determined using a water vapour detector tube.

STORAGE

As a compressed gas or a liquid in appropriate containers complying with the legal regulations.

IMPURITIES

Specified impurities *A, B, C, D.*

A. CO_2: carbon dioxide,

B. CO: carbon monoxide,

C. O_2: oxygen,

D. H_2O: water.

Ph Eur

Low-Oxygen Nitrogen

(Nitrogen, Low-Oxygen, Ph. Eur. monograph 1685)

N_2 28.01

Ph Eur

DEFINITION

This monograph applies to nitrogen which is used for inerting finished medicinal products which are particularly sensitive to degradation by oxygen. It does not necessarily apply to nitrogen used in earlier production steps.

Content

Minimum 99.5 per cent *V/V* of N_2, calculated by deduction of the sum of impurities found when performing the test for impurities.

CHARACTERS

Colourless and odourless gas.

Solubility

At 20 °C and at a pressure of 101 kPa, 1 volume dissolves in about 62 volumes of water and about 10 volumes of alcohol.

PRODUCTION

Oxygen

Maximum 5 ppm *V/V*, determined using an oxygen analyser with a detector scale ranging from 0 ppm *V/V* to 100 ppm *V/V* and equipped with an electrochemical cell.

The gas to be examined passes through a detection cell containing an aqueous solution of an electrolyte, generally potassium hydroxide. The presence of oxygen in the gas to be examined produces variation in the electric signal recorded at the outlet of the cell that is proportional to the oxygen content.

Calibrate the analyser according to the manufacturer's instructions. Pass the gas to be examined through the analyser using a suitable pressure regulator and airtight metal tubes and operating at the prescribed flow rates until constant readings are obtained.

Impurities

Gas chromatography (*2.2.28*).

Gas to be examined The substance to be examined.

Reference gas (a) Use ambient air.

Reference gas (b) Use *nitrogen R1*.

Column:
- *material*: stainless steel,
- *size*: *l* = 2 m, Ø = 2 mm,
- *stationary phase*: appropriate molecular sieve for chromatography (0.5 nm).

Carrier gas *helium for chromatography R*.

Flow rate 40 mL/min.

Temperature:
- *column*: 50 °C,
- *detector*: 130 °C.

Detection Thermal conductivity.

System suitability Reference gas (a): adjust the injected volumes and operating conditions so that the height of the peak due to nitrogen in the chromatogram obtained is at least 35 per cent of the full scale of the recorder:
- the chromatogram obtained shows a clear separation of oxygen and nitrogen.

Limit:
- *total*: not more than 0.5 per cent of the sum of the areas of all the peaks (0.5 per cent *V/V*).

IDENTIFICATION

First identification: A.

Second identification: B, C.

A. Examine the chromatograms obtained in the test for impurities (see Production).

Results The principal peak in the chromatogram obtained with the gas to be examined is similar in retention time to the principal peak in the chromatogram obtained with reference gas (b).

B. In a 250 mL conical flask replace the air by the gas to be examined. Place a burning or glowing splinter of wood in the flask. The splinter is extinguished.

C. In a suitable test tube, place 0.1 g of *magnesium R* in turnings. Close the tube with a two-hole stopper fitted with a glass tube reaching about 1 cm above the turnings. Pass the gas to be examined through the glass tube for 1 min without heating, then for 15 min while heating the test tube to a red glow. After cooling, add 5 mL of *dilute sodium hydroxide solution R*. The evolving vapours turn the colour of moistened *red litmus paper R* blue.

STORAGE

Where the gas has to be stored, store as a compressed gas or a liquid in appropriate containers complying with the legal regulations.

IMPURITIES

A. O_2: oxygen,

B. Ar: argon.

Ph Eur

Nitrous Oxide

(Ph. Eur. monograph 0416)

Nitrous oxide should be kept in approved metal cylinders which are painted blue and carry a label stating 'Nitrous Oxide'. In addition, 'Nitrous Oxide' or the symbol 'N2O' should be stencilled in paint on the shoulder of the cylinder.

N_2O 44.01 *10024-97-2*

Action and use

General anaesthetic; analgesic.

Ph Eur

DEFINITION

Content

Minimum 98.0 per cent *V/V* of N_2O in the gaseous phase, when sampled at 15 °C.

This monograph applies to nitrous oxide for medicinal use.

CHARACTERS

Appearance

Colourless gas.

Solubility

At 20 °C and at a pressure of 101 kPa, 1 volume dissolves in about 1.5 volumes of water.

PRODUCTION

Nitrous oxide is produced from ammonium nitrate by thermic decomposition.

Examine the gaseous phase.

If the test is performed on a cylinder, keep the cylinder at room temperature for at least 6 h before carrying out the tests. Keep the cylinder in the vertical position with the outlet valve uppermost.

Carbon dioxide

Gas chromatography (*2.2.28*).

Gas to be examined The substance to be examined.

Reference gas A mixture containing 300 ppm *V/V* of *carbon dioxide R1* in *nitrous oxide R*.

Column:
- *material*: stainless steel;
- *size*: *l* = 3.5 m, Ø = 2 mm;
- *stationary phase*: *ethylvinylbenzene-divinylbenzene copolymer R*.

Carrier gas *helium for chromatography R*.

Flow rate 15 mL/min.

Temperature:
- *column*: 40 °C;
- *detector*: 90 °C.

Detection Thermal conductivity.

Injection Loop injector.

Adjust the injected volumes and operating conditions so that the height of the peak due to carbon dioxide in the chromatogram obtained with the reference gas is at least 35 per cent of the full scale of the recorder. The test is not valid unless the chromatograms obtained show a clear separation of carbon dioxide from nitrous oxide.

Limit:
- *carbon dioxide*: not more than the area of the corresponding peak in the chromatogram obtained with the reference gas (300 ppm *V/V*).

Carbon monoxide

Gas chromatography (*2.2.28*). *When the test is carried out on a cylinder, use the first portion of gas to be withdrawn.*

Gas to be examined The substance to be examined.

Reference gas A mixture containing 5 ppm *V/V* of *carbon monoxide R* in *nitrous oxide R.*

Column:
— *material:* stainless steel;
— *size:* *l* = 2 m, Ø = 4 mm;
— *stationary phase:* suitable molecular sieve for chromatography (0.5 nm).

Carrier gas *helium for chromatography R.*

Flow rate 60 mL/min.

Temperature:
— *column:* 50 °C;
— *injection port and detector:* 130 °C.

Detection Flame ionisation with methaniser.

Injection Loop injector.

Adjust the injected volumes and the operating conditions so that the height of the peak due to carbon monoxide in the chromatogram obtained with the reference gas is at least 35 per cent of the full scale of the recorder.

Limit:
— *carbon monoxide:* not more than the area of the corresponding peak in the chromatogram obtained with the reference gas (5 ppm *V/V*).

Nitrogen monoxide and nitrogen dioxide
Maximum 2 ppm *V/V* in total in the gaseous and liquid phases, determined using a chemiluminescence analyser (*2.5.26*).

Gas to be examined The substance to be examined.

Reference gas (a) *Nitrous oxide R.*

Reference gas (b) A mixture containing 2 ppm *V/V* of *nitrogen monoxide R* in *nitrogen R1.*

Calibrate the apparatus and set the sensitivity using reference gases (a) and (b). Measure the content of nitrogen monoxide and nitrogen dioxide, separately examining the samples collected from the gaseous phase and the liquid phase of the gas to be examined.

Multiply the result obtained by the quenching correction factor in order to correct the quenching effect on the analyser response caused by the nitrous oxide matrix effect.

The quenching correction factor is determined by applying a known reference mixture of nitrogen monoxide in nitrous oxide and comparing the actual content with the content indicated by the analyser which has been calibrated with an NO/N_2 reference mixture.

$$\text{Quenching correction factor} = \frac{\text{actual nitrogen monoxide content}}{\text{indicated nitrogen monoxide content}}$$

Water
Maximum 67 ppm *V/V*, determined using an electrolytic hygrometer (*2.5.28*).

Assay
Infrared analyser (*2.5.35*).

Gas to be examined The substance to be examined. It must be filtered to avoid stray light phenomena.

Reference gas (a) *Nitrous oxide R.*

Reference gas (b) A mixture containing 5.0 per cent *V/V* of *nitrogen R1* and 95.0 per cent *V/V* of *nitrous oxide R.*

Calibrate the apparatus and set the sensitivity using reference gases (a) and (b). Measure the content of nitrous oxide in the gas to be examined.

IDENTIFICATION
First identification: A.

Second identification: B, C.

A. It complies with the limits of the assay.

B. Place a glowing splinter of wood in the substance to be examined. The splinter bursts into flame.

C. Introduce the substance to be examined into *alkaline pyrogallol solution R.* A brown colour does not develop.

TESTS
Examine the gaseous phase.

If the test is performed on a cylinder, keep the cylinder of the substance to be examined at room temperature for at least 6 h before carrying out the tests. Keep the cylinder in the vertical position with the outlet valve uppermost.

Carbon dioxide
Maximum 300 ppm *V/V*, determined using a carbon dioxide detector tube (*2.1.6*).

Carbon monoxide
Maximum 5 ppm *V/V*, determined using a carbon monoxide detector tube (*2.1.6*). *When the test is carried out on a cylinder, use the first portion of the gas to be withdrawn.*

Nitrogen monoxide and nitrogen dioxide
Maximum 2 ppm *V/V*, determined using a nitrogen monoxide and nitrogen dioxide detector tube (*2.1.6*).

Water vapour
Maximum 67 ppm *V/V*, determined using a water vapour detector tube (*2.1.6*).

STORAGE
Store liquefied under pressure in suitable containers complying with the legal regulations. The taps and valves are not greased or oiled.

IMPURITIES
Specified impurities *A, B, C, D, E.*

A. CO_2: carbon dioxide,

B. CO: carbon monoxide,

C. NO: nitrogen monoxide,

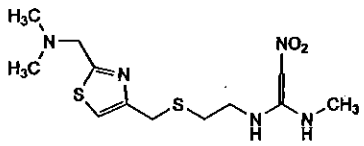
D. NO_2: nitrogen dioxide,

E. H_2O: water.

Ph Eur

Nizatidine

(Ph. Eur. monograph 1453)



$C_{12}H_{21}N_5O_2S_2$ 331.5 *76963-41-2*

Action and use
Histamine H_2 receptor antagonist; treatment of peptic ulcer.

Preparation
Nizatidine Infusion

Ph Eur

DEFINITION
(*EZ*)-*N*-[2-[[[2-[(Dimethylamino)methyl]thiazol-4-yl]methyl]sulfanyl]ethyl]-*N'*-methyl-2-nitroethene-1,1-diamine.

Content
97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
Almost white or slightly brownish, crystalline powder.

Solubility
Sparingly soluble in water, soluble in methanol.

IDENTIFICATION

First identification: C.

Second identification: A, B, D.

A. Melting point (*2.2.14*): 131 °C to 134 °C.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 0.10 g in *methanol R* and dilute to 100.0 mL with the same solvent. Dilute 2.0 mL of the solution to 100.0 mL with *methanol R*.

Spectral range 220-350 nm.

Absorption maxima At 242 nm and 325 nm.

Absorbance ratio A_{325}/A_{242} = 2.2 to 2.5.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *nizatidine CRS*.

D. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 50 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dissolve 50 mg of *nizatidine CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 50 mg of *nizatidine CRS* and 50 mg of *ranitidine hydrochloride CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *water R, concentrated ammonia R1, 2-propanol R, ethyl acetate R* (4:8:30:50 *V/V/V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Expose to iodine vapour until the spots are clearly visible. Examine in daylight.

System suitability Reference solution (b):
— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5 (*2.2.2, Method II*).

Dissolve 0.2 g in a 10 g/L solution of *hydrochloric acid R* and dilute to 20 mL with the same solution.

pH (*2.2.3*)
8.5 to 10.0.

Dissolve 0.2 g in *carbon dioxide-free water R* and dilute to 20 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture Mobile phase B, mobile phase A (15:85 *V/V*).

Test solution (a) Dissolve 50 mg of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Test solution (b) Dissolve 15.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 15.0 mg of *nizatidine CRS* in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (c) Dissolve 5 mg of the substance to be examined and 0.5 mg of *nizatidine impurity F CRS* in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Reference solution (d) Dissolve 5 mg of *2-(dimethylamino) thioacetamide hydrochloride R* (impurity H hydrochloride) in the solvent mixture and dilute to 10.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 20.0 mL with the solvent mixture. Use 1.0 mL of this solution to dissolve 5 mg of *nizatidine for system suitability CRS* (containing impurities A, B, C, D, G, J and K).

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: dissolve 5.9 g of *ammonium acetate R* in 760 mL of *water R*, add 1 mL of *diethylamine R*, and adjust to pH 7.5 with *acetic acid R*;
— *mobile phase B*: *methanol R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	85	15
3 - 20	85 → 50	15 → 50
20 - 45	50	50

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL of test solution (a) and reference solutions (a), (c) and (d).

Identification of impurities Use the chromatogram supplied with *nizatidine for system suitability CRS* and the chromatogram obtained with reference solution (d) to identify the peaks due to impurities A, B, C, D, G, H, J and K; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity F.

Relative retention With reference to nizatidine (retention time = about 18 min): impurity A = about 0.19; impurity K = about 0.21; impurity H = about 0.5; impurity B = about 0.6; impurity C = about 0.66; impurity J = about 0.7; impurity D = about 0.75; impurity F = about 1.03; impurity G = about 1.5.

System suitability:
— *resolution*: minimum 2.0 between the peaks due to nizatidine and impurity F in the chromatogram obtained with reference solution (c); minimum 1.5 between the peaks due to impurities A and K in the chromatogram obtained with reference solution (d).

Limits:
— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the

corresponding correction factor: impurity B = 1.7; impurity D = 2.3; impurity H = 0.5;
- — *impurities A, B, C, D, F, G, H, J, K*: for each impurity, not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
- — *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- — *total*: not more than 15 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent);
- — *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Mobile phase Mobile phase B, mobile phase A (35:65 *V/V*).

Injection Test solution (b) and reference solution (b).

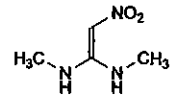
Retention time Nizatidine = 9 min.

Calculate the percentage content of $C_{12}H_{21}N_5O_2S_2$ taking into account the assigned content of *nizatidine CRS*.

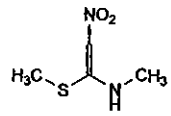
IMPURITIES

Specified impurities *A, B, C, D, F, G, H, J, K.*

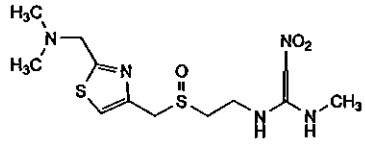
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *E, I.*



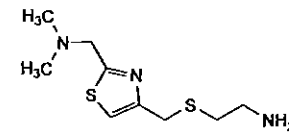
A. *N,N'*-dimethyl-2-nitroethene-1,1-diamine,



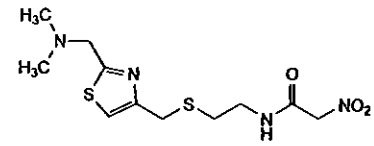
B. (*EZ*)-*N*-methyl-1-(methylsulfanyl)-2-nitroethen-1-amine,



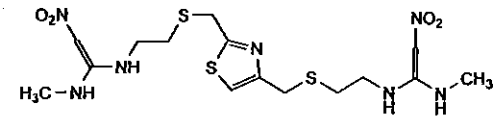
C. (*EZ*)-*N*-[2-[[[2-[(dimethylamino)methyl]thiazol-4-yl]methyl]sulfinyl]ethyl]-*N'*-methyl-2-nitroethene-1,1-diamine,



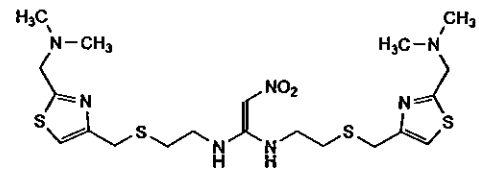
D. 2-[[[2-[(dimethylamino)methyl]thiazol-4-yl]methyl]sulfanyl]ethanamine,



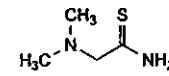
E. *N*-[2-[[[2-[(dimethylamino)methyl]thiazol-4-yl]methyl]sulfanyl]ethyl]-2-nitroacetamide,



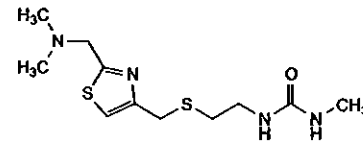
F. (*EZ*)-*N*-methyl-*N'*-[2-[[[4-[[[2-[[(*EZ*)-1-(methylamino)-2-nitroethenyl]amino]ethyl]sulfanyl]methyl]thiazol-2-yl]methyl]sulfanyl]ethyl]-2-nitroethene-1,1-diamine,



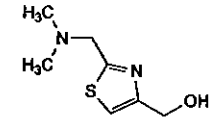
G. *N,N'*-bis[2-[[[2-[(dimethylamino)methyl]thiazol-4-yl]methyl]sulfanyl]ethyl]-2-nitroethene-1,1-diamine,



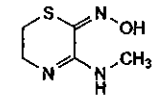
H. 2-(dimethylamino)thioacetamide,



I. *N*-[2-[[[2-[(dimethylamino)methyl]thiazol-4-yl]methyl]sulfanyl]ethyl]-*N'*-methylurea,



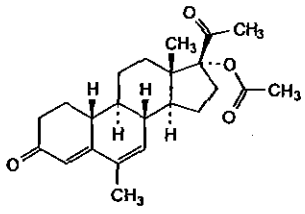
J. [2-[(dimethylamino)methyl]thiazol-4-yl]methanol,



K. 3-(methylamino)-5,6-dihydro-2*H*-1,4-thiazin-2-one oxime.

Nomegestrol Acetate

(Ph. Eur. monograph 1551)



$C_{23}H_{30}O_4$ 370.5 *58652-20-3*

Action and use
Progestogen.

Ph Eur

DEFINITION

6-Methyl-3,20-dioxo-19-norpregna-4,6-dien-17-yl acetate.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, freely soluble in acetone, soluble in ethanol (96 per cent), practically insoluble in heptane.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *nomegestrol acetate for ID and assay CRS.*

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5 (*2.2.2, Method II*).

Dissolve 1.0 g in *methylene chloride R* and dilute to 10 mL with the same solvent.

Specific optical rotation (*2.2.7*)
−64.0 to −60.0 (dried substance).

Dissolve 0.500 g in *anhydrous ethanol R* and dilute to 25.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in *methanol R* and dilute to 50.0 mL with the same solvent.

Reference solution (a) Dissolve 25.0 mg of *nomegestrol acetate for ID and assay CRS* in *methanol R* and dilute to 50.0 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (c) Dissolve 25.0 mg of *nomegestrol acetate impurity A CRS* in *methanol R* and dilute to 100.0 mL with the same solvent. Dilute 0.25 mL of the solution to 25.0 mL with reference solution (a).

Reference solution (d) Dissolve the contents of a vial of *nomegestrol acetate impurity B CRS* in 1 mL of *methanol R.*

Column:
— *size:* l = 0.25 m, Ø = 4.6 mm;
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile for chromatography R, methanol R1, water for chromatography R* (24:38:38 *V/V/V*).

Flow rate 1.3 mL/min.

Detection Spectrophotometer at 245 nm and at 290 nm.

Injection 10 µL of the test solution and reference solutions (b), (c), (d).

Run time 1.5 times the retention time of nomegestrol acetate.

Identification of impurities Use the chromatogram obtained with reference solution (c) at 245 nm to identify the peak due to impurity A; use the chromatogram obtained with reference solution (d) at 290 nm to identify the peak due to impurity B.

Relative retention With reference to nomegestrol acetate (retention time = about 17 min): impurity B = about 0.6; impurity A = about 1.1.

System suitability Reference solution (c) at 245 nm:
— *peak-to-valley ratio*: minimum 5, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to nomegestrol acetate.

Calculation of percentage contents:
— for each impurity at 245 nm, use the concentration of impurity A in reference solution (c);
— for each impurity at 290 nm, use the concentration of nomegestrol acetate in reference solution (b).

Limits:
— *impurity A at 245 nm*: maximum 0.2 per cent;
— *impurity B at 290 nm*: maximum 0.15 per cent;
— *unspecified impurities at 245 nm and at 290 nm*: for each impurity, maximum 0.10 per cent;
— *sum of impurities other than A at 245 nm and 290 nm*: maximum 0.3 per cent;
— *reporting threshold at 245 nm and at 290 nm*: 0.05 per cent.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications:

Detection Spectrophotometer at 290 nm.

Injection Test solution and reference solution (a).

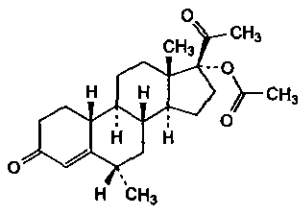
Calculate the percentage content of $C_{23}H_{30}O_4$ taking into account the assigned content of *nomegestrol acetate for ID and assay CRS.*

STORAGE

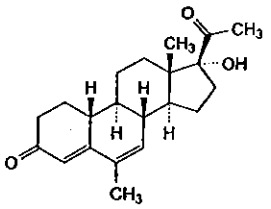
Protected from light.

IMPURITIES

Specified impurities *A, B.*



A. 6α-methyl-3,20-dioxo-19-norpregn-4-en-17-yl acetate,



B. 17-hydroxy-6-methyl-19-norpregna-4,6-diene-3,20-dione (nomegestrol).

Ph Eur

Nonoxinol 9

(Ph. Eur. monograph 1454)

Action and use
Spermatocide.

Ph Eur

DEFINITION
α-(4-Nonylphenyl)-ω-hydroxynona(oxyethylene).

Mixture consisting mainly of monononylphenyl ethers of macrogols corresponding to the formula: $C_9H_{19}C_6H_4$-$[OCH_2\text{-}CH_2]_n$-OH where the average value of *n* is 9. It may contain free macrogols.

CHARACTERS

Appearance
Clear, colourless or light yellow, viscous liquid.

Solubility
Miscible with water, with ethanol (96 per cent) and with vegetable oils.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *nonoxinol 9 CRS.*

Preparation Film between *sodium chloride R* plates.

B. Cloud point (see Tests).

TESTS

Acidity or alkalinity
Boil 1.0 g with 20 mL of *carbon dioxide-free water R* for 1 min, with constant stirring. Cool and filter. To 10 mL of the filtrate, add 0.05 mL of *bromothymol blue solution R1*. Not more than 0.5 mL of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Hydroxyl value (*2.5.3, Method A*)
84 to 94.

Cloud point
52 °C to 58 °C.

Dissolve 1.0 g in 99 g of *water R*. Transfer about 30 mL of this solution into a test-tube, heat on a water-bath and stir continuously until the solution becomes cloudy. Remove the test-tube from the water-bath (ensuring that the temperature does not increase to more than 2 °C) and continue to stir. The cloud point is the temperature at which the solution becomes sufficiently clear that the entire thermometer bulb is plainly seen.

Ethylene oxide and dioxan (*2.4.25*)
Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Water (*2.5.12*)
Maximum 0.5 per cent, determined on 2.00 g.

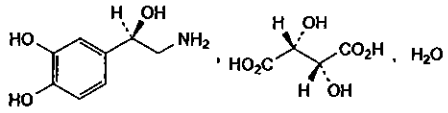
Total ash (*2.4.16*)
Maximum 0.4 per cent, determined on 1.0 g.

STORAGE
In an airtight container.

Ph Eur

Noradrenaline Acid Tartrate / Norepinephrine Acid Tartrate

(Noradrenaline Tartrate, Ph. Eur. monograph 0285)



$C_{12}H_{17}NO_9,H_2O$ 337.3 *108341-18-0*

Action and use
Alpha-adrenoceptor agonist.

Preparation
Noradrenaline Injection/Norepinephrine Injection

Ph Eur

DEFINITION
(1*R*)-2-Amino-1-(3,4-dihydroxyphenyl)ethanol hydrogen (2*R*,3*R*)-2,3-dihydroxybutanedioate monohydrate.

Content
98.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION
A. Dissolve 2 g in 20 mL of a 5 g/L solution of *sodium metabisulfite R* and make alkaline by addition of *ammonia R*. Keep in iced water for 1 h and filter. Reserve the filtrate for identification test C. Wash the precipitate with 3 quantities, each of 2 mL, of *water R*, then with 5 mL of *ethanol (96 per cent) R* and finally with 5 mL of *methylene chloride R* and dry *in vacuo* for 3 h. The specific optical rotation (*2.2.7*) of the precipitate (noradrenaline base) is −48 to −44, determined using a 20.0 g/L solution in *0.5 M hydrochloric acid*.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs of noradrenaline base prepared as described in identification test A.

Comparison Use noradrenaline base prepared as described in identification test A from a suitable amount of *noradrenaline tartrate CRS*.

C. 0.2 mL of the filtrate obtained in identification test A gives reaction (b) of tartrates (*2.3.1*).

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

Dissolve 0.2 g in *water R* and dilute to 10 mL with the same solvent. Examine the solution immediately.

Related substances

Liquid chromatography (*2.2.29*). *Protect the solutions from air. Remove oxygen from the mobile phases with nitrogen R immediately before use. Fill up the flasks.*

Test solution Dissolve 0.20 g of the substance to be examined in mobile phase A and dilute to 50 mL with mobile phase A.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

Reference solution (b) Dissolve 10 mg of the substance to be examined in 5 mL of *0.1 M hydrochloric acid*. To 1 mL of this solution add 0.1 mL of *strong hydrogen peroxide solution R* and expose to UV light at 254 nm for 90 min. Dilute to 10 mL with mobile phase A. The degradation of noradrenaline produces 2 peaks, one with a relative retention of about 1.2 (unidentified compound) and the other with a relative retention of about 1.5 (impurity B). Use this solution to identify the peak due to impurity B.

Reference solution (c) Dissolve 7.5 mg of *noradrenaline impurity D CRS* and 5 mg of *noradrenaline impurity E CRS* in mobile phase A and dilute to 100 mL with mobile phase A.

Reference solution (d) Dissolve 5 mg of *noradrenaline impurity F CRS* in mobile phase A and dilute to 10 mL with mobile phase A. To 1 mL of this solution, add 1 mL of reference solution (c) and dilute to 20 mL with mobile phase A.

Column:
- *size*: *l* = 0.10 m, Ø = 4.6 mm;
- *stationary phase*: end-capped monolithic octadecylsilyl silica gel for chromatography R;
- *temperature*: 25 °C.

Mobile phase:
- *mobile phase A*: dissolve 0.50 g of *sodium heptanesulfonate R* in *water for chromatography R* and dilute to 1000 mL with the same solvent; adjust to pH 2.2 with *phosphoric acid R*;
- *mobile phase B*: dissolve 0.25 g of *sodium heptanesulfonate R* in *water for chromatography R* and dilute to 500 mL with the same solvent; add 500 mL of *acetonitrile for chromatography R* and adjust the apparent pH to 2.4 with *phosphoric acid R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Flow rate (mL/min)
0 - 2.0	98	2	1.5
2.0 - 17.0	98 → 70	2 → 30	1.5
17.0 - 24.0	70 → 50	30 → 50	1.5
24.0 - 24.1	50 → 0	50 → 100	1.5 → 4.0
24.1 - 28.0	0	100	4.0
28.0 - 28.1	0 → 98	100 → 2	4.0
28.1 - 30.0	98	2	4.0 → 1.5

Detection Spectrophotometer at 280 nm, except for impurity F: spectrophotometer at 254 nm.

Injection 20 µL of the test solution and reference solutions (a), (b) and (d).

Relative retention With reference to noradrenaline (retention time = about 3 min): impurity B = about 1.5; impurity D = about 2.8; impurity E = about 3.0; impurity F = about 6.9.

System suitability Reference solution (d):
- *resolution*: minimum 1.5 between the peaks due to impurities D and E.

Limits:
- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.3; impurity E = 0.3; impurity F = 1.5;
- *impurity F at 254 nm*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *impurities B, D, E at 280 nm*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *unspecified impurities at 280 nm*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *sum of the impurities at 280 nm and impurity F at 254 nm*: maximum 0.3 per cent;
- *disregard limit at 280 nm*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)

4.5 per cent to 5.8 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 50 mL of *anhydrous acetic acid R*, heating gently if necessary. Titrate with *0.1 M perchloric acid* using 0.1 mL of *crystal violet solution R* as indicator, until a bluish-green colour is obtained.

1 mL of *0.1 M perchloric acid* is equivalent to 31.93 mg of $C_{12}H_{17}NO_9$.

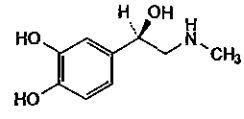
STORAGE

In an airtight container or preferably in a sealed tube under vacuum or under an inert gas, protected from light.

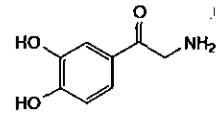
IMPURITIES

Specified impurities *B, D, E, F.*

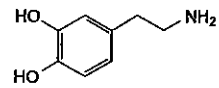
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, C, G.*



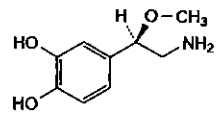
A. 4-[(1*R*)-1-hydroxy-2-(methylamino)ethyl]benzene-1,2-diol (adrenaline),



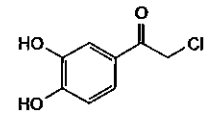
B. 2-amino-1-(3,4-dihydroxyphenyl)ethanone (noradrenalone),



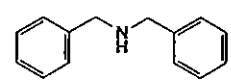
C. 4-(2-aminoethyl)benzene-1,2-diol (dopamine),



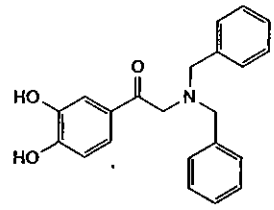
D. 4[(1*R*)-2-amino-1-methoxyethyl]benzene-1,2-diol (noradrenaline methyl ether),



E. 2-chloro-1-(3,4-dihydroxyphenyl)ethanone,



F. *N*-benzyl-1-phenylmethanamine,

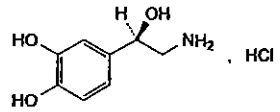


G. 2-(dibenzylamino)-1-(3,4-dihydroxyphenyl)ethanone.

Ph Eur

Noradrenaline Hydrochloride / Norepinephrine Hydrochloride

(Noradrenaline Hydrochloride, Ph. Eur. monograph 0732)



$C_8H_{12}ClNO_3$ 205.6 *329-56-6*

Action and use
Alpha-adrenoceptor agonist.

Ph Eur

DEFINITION
(1*R*)-2-Amino-1-(3,4-dihydroxyphenyl)ethanol hydrochloride.

Content
98.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or brownish-white, crystalline powder.

Solubility
Very soluble in water, slightly soluble in ethanol (96 per cent).

It becomes coloured on exposure to air and light.

IDENTIFICATION
A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs of noradrenaline base prepared as follows. Dissolve 2 g in 20 mL of a 5 g/L solution of *sodium metabisulfite R* and make alkaline by addition of *ammonia R*. Keep in iced water for 1 h and filter. Wash the precipitate with 3 quantities, each of 2 mL, of *water R*, then with 5 mL of *ethanol (96 per cent) R* and finally with 5 mL of *methylene chloride R* and dry *in vacuo* for 3 h.

Comparison Use noradrenaline base prepared as above from a suitable amount of *noradrenaline tartrate CRS*.

C. 0.2 mL of solution S (see Tests) gives reaction (a) of chlorides (*2.3.1*).

TESTS
Solution S
Dissolve 0.500 g in *carbon dioxide-free water R* and dilute to 25.0 mL with the same solvent.

Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than a mixture of 0.2 mL of blue primary solution, 0.4 mL of yellow primary solution, 0.4 mL of red primary solution and 9 mL of a 13.7 per cent *V/V* solution of *dilute hydrochloric acid R* (*2.2.2, Method II*).

Dissolve 0.2 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent. Examine the solution immediately.

pH (*2.2.3*)
3.5 to 4.5 for solution S.

Specific optical rotation (*2.2.7*)
−37 to −41 (anhydrous substance), determined on solution S.

Related substances
Liquid chromatography (*2.2.29*). *Protect the solutions from air. Remove oxygen from the mobile phases with nitrogen R immediately before use. Fill up the flasks.*

Test solution Dissolve 0.125 g of the substance to be examined in mobile phase A and dilute to 50 mL with mobile phase A.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

Reference solution (b) Dissolve 10 mg of the substance to be examined in 5 mL of *0.1 M hydrochloric acid*. To 1 mL of this solution add 0.1 mL of *strong hydrogen peroxide solution R* and expose to UV light at 254 nm for 90 min. Dilute to 10 mL with mobile phase A. The degradation of noradrenaline produces 2 peaks, one with a relative retention of about 1.2 (unidentified compound) and the other with a relative retention of about 1.5 (impurity B). Use this solution to identify the peak due to impurity B.

Reference solution (c) Dissolve 7.5 mg of *noradrenaline impurity D CRS* and 5 mg of *noradrenaline impurity E CRS* in mobile phase A and dilute to 100 mL with mobile phase A.

Reference solution (d) Dissolve 5 mg of *noradrenaline impurity F CRS* in mobile phase A and dilute to 10 mL with mobile phase A. To 1 mL of this solution, add 1 mL of reference solution (c) and dilute to 20 mL with mobile phase A.

Column:
— *size*: *l* = 0.10 m, Ø = 4.6 mm;

— *stationary phase*: end-capped monolithic octadecylsilyl silica gel for chromatography R;
— *temperature*: 25 °C.

Mobile phase:
— *mobile phase A*: dissolve 0.50 g of *sodium heptanesulfonate R* in *water for chromatography R* and dilute to 1000 mL with the same solvent; adjust to pH 2.2 with *phosphoric acid R*;
— *mobile phase B*: dissolve 0.25 g of *sodium heptanesulfonate R* in *water for chromatography R* and dilute to 500 mL with the same solvent; add 500 mL of *acetonitrile for chromatography R* and adjust the apparent pH to 2.4 with *phosphoric acid R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Flow rate (mL/min)
0 - 2.0	98	2	1.5
2.0 - 17.0	98 → 70	2 → 30	1.5
17.0 - 24.0	70 → 50	30 → 50	1.5
24.0 - 24.1	50 → 0	50 → 100	1.5 → 4.0
24.1 - 28.0	0	100	4.0
28.0 - 28.1	0 → 98	100 → 2	4.0
28.1 - 30.0	98	2	4.0 → 1.5

Detection Spectrophotometer at 280 nm, except for impurity F: spectrophotometer at 254 nm.

Injection 20 µL of the test solution and reference solutions (a), (b) and (d).

Relative retention With reference to noradrenaline (retention time = about 3 min): impurity B = about 1.5; impurity D = about 2.8; impurity E = about 3.0; impurity F = about 6.9.

System suitability Reference solution (d):
— *resolution*: minimum 1.5 between the peaks due to impurities D and E.

Limits:
— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.3; impurity E = 0.3; impurity F = 1.5;
— *impurity D at 280 nm*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *impurity F at 254 nm*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *impurities B, E at 280 nm*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *unspecified impurities at 280 nm*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *sum of impurities other than D at 280 nm*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *sum of the impurities at 280 nm and impurity F at 254 nm*: maximum 0.7 per cent;
— *disregard limit at 280 nm*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)
Maximum 0.5 per cent, determined on 1.000 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

In order to avoid overheating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.

Dissolve 0.180 g in 50 mL of *acetic anhydride R* and add 10 mL of *anhydrous formic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 20.56 mg of $C_8H_{12}ClNO_3$.

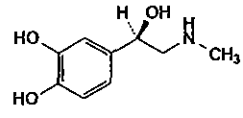
STORAGE

In an airtight container, or preferably in a sealed tube under vacuum or under an inert gas, protected from light.

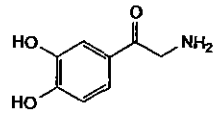
IMPURITIES

Specified impurities *B, D, E, F.*

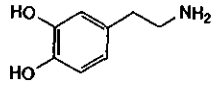
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, C, G.*



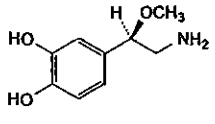
A. 4-[(1*R*)-1-hydroxy-2-(methylamino)ethyl]benzene-1,2-diol (adrenaline),



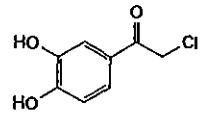
B. 2-amino-1-(3,4-dihydroxyphenyl)ethanone (noradrenalone),



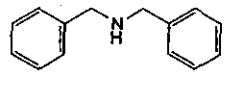
C. 4-(2-aminoethyl)benzene-1,2-diol (dopamine),



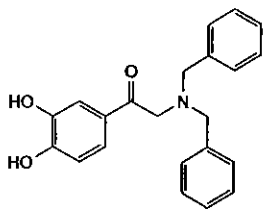
D. 4-[(1*R*)-2-amino-1-methoxyethyl]benzene-1,2-diol (noradrenaline methyl ether),



E. 2-chloro-1-(3,4-dihydroxyphenyl)ethanone,



F. *N*-benzyl-1-phenylmethanamine,

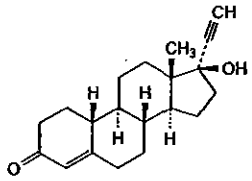


G. 2-(dibenzylamino)-1-(3,4-dihydroxyphenyl)ethanone.

Ph Eur

Norethisterone

(Ph. Eur. monograph 0234)



$C_{20}H_{26}O_2$ 298.4 *68-22-4*

Action and use
Progestogen.

Preparations
Norethisterone Tablets
Estradiol and Norethisterone Tablets

Ph Eur

DEFINITION
17-Hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one.

Content
98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or yellowish-white, crystalline powder.

Solubility
Practically insoluble in water, soluble in methylene chloride, sparingly soluble in acetone and in anhydrous ethanol.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *norethisterone CRS*.

TESTS

Specific optical rotation (*2.2.7*)
−32.0 to −37.0 (dried substance).
Dissolve 0.250 g in *acetone R* and dilute to 25.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).
Test solution Dissolve 25.0 mg of the substance to be examined in a mixture of 40 volumes of *water R* and 60 volumes of *acetonitrile R1* and dilute to 10.0 mL with the same mixture of solvents.
Reference solution (a) Dissolve 5 mg of *norethisterone for system suitability CRS* (containing impurities A, B, C, D, E, F, G and H) in a mixture of 40 volumes of *water R* and 60 volumes of *acetonitrile R1* and dilute to 2.0 mL with the same mixture of solvents.
Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with a mixture of 40 volumes of *water R* and 60 volumes of *acetonitrile R1*. Dilute 1.0 mL of this solution to 10.0 mL with a mixture of 40 volumes of *water R* and 60 volumes of *acetonitrile R1*.
Column:
— *size*: l = 0.15 m, Ø = 4.6 mm,
— *stationary phase*: spherical *end-capped octylsilyl silica gel for chromatography R* (5 µm).
Mobile phase:
— *mobile phase A*: *water R*;
— *mobile phase B*: *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	63	37
20 - 25	63 → 20	37 → 80
25 - 35	20	80

Flow rate 1.0 mL/min.
Detection Variable wavelength spectrophotometer capable of operating at 254 nm and at 210 nm.
Injection 20 µL.
Identification of impurities Use the chromatogram obtained with reference solution (a) and the chromatogram supplied with *norethisterone for system suitability CRS* to identify the peaks due to the impurities A, B, C, D, E, F, G and H.
Relative retention at 254 nm With reference to norethisterone (retention time = about 10 min): impurity H = about 0.3; impurity A = about 0.8; impurity B = about 0.9; impurity G = about 1.5; impurity C (at 210 nm) = about 1.6; impurity D (at 210 nm) = about 1.7; impurity E = about 2.3; impurity F = about 2.4.
System suitability Reference solution (a) at 254 nm:
— *resolution*: baseline separation between the peaks due to impurity B and norethisterone;
— *peak-to-valley ratio*: minimum 1.2, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity B.
Limits Spectrophotometer at 254 nm:
— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 2.5; impurity E = 0.7; impurity F = 1.4; impurity H = 1.7;
— *impurities E, G, H*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
— *impurities A, B, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

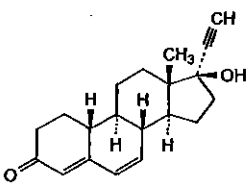
Limits Spectrophotometer at 210 nm:
— *impurities C, D*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

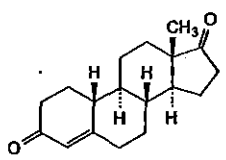
ASSAY
Dissolve 0.200 g in 40 mL of *tetrahydrofuran R*. Add 10 mL of a 100 g/L solution of *silver nitrate R* and titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Rinse the electrode with *acetone R* after each titration.

1 mL of *0.1 M sodium hydroxide* is equivalent to 29.84 mg of $C_{20}H_{26}O_2$.

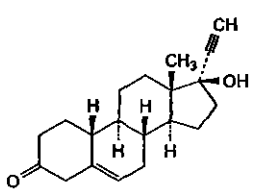
IMPURITIES
Specified impurities *A, B, C, D, E, F, G, H.*



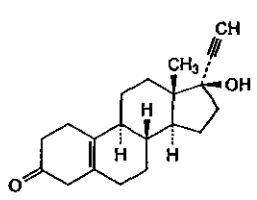
A. 17-hydroxy-19-nor-17α-pregna-4,6-dien-20-yn-3-one,



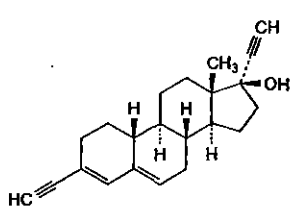
B. estr-4-ene-3,17-dione (norandrostenedione),



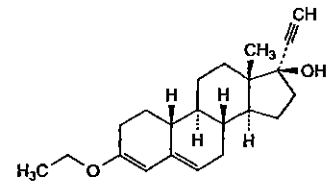
C. 17-hydroxy-19-nor-17α-pregn-5-en-20-yn-3-one,



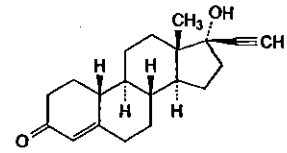
D. 17-hydroxy-19-nor-17α-pregn-5(10)-en-20-yn-3-one,



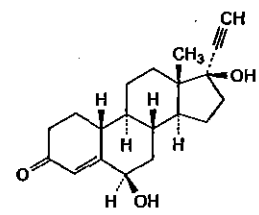
E. 3-ethynyl-19-nor-17α-pregna-3,5-dien-20-yn-17-ol,



F. 3-ethoxy-19-nor-17α-pregna-3,5-dien-20-yn-17-ol,



G. 17-hydroxy-19-norpregn-4-en-20-yn-3-one (17-*epi*-norethisterone),

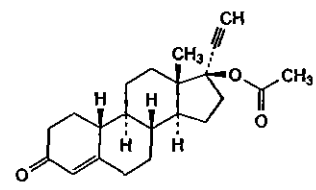


H. 6β,17-dihydroxy-19-nor-17α-pregn-4-en-20-yn-3-one (6β-hydroxynorethisterone).

Ph Eur

Norethisterone Acetate

(Ph. Eur. monograph 0850)



$C_{22}H_{28}O_3$ 340.5 *51-98-9*

Action and use
Progestogen.

Preparation
Estradiol and Norethisterone Acetate Tablets

Ph Eur

DEFINITION
3-Oxo-19-nor-17α-pregn-4-en-20-yn-17-yl acetate.

Content
97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS
Appearance
White or yellowish-white, crystalline powder.

Solubility
Practically insoluble in water, freely soluble in methylene chloride, soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *norethisterone acetate CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *methylene chloride R*, evaporate to dryness on a water-bath and record new spectra using the residues.

TESTS

Specific optical rotation (*2.2.7*)

−35 to −30 (dried substance).

Dissolve 0.500 g in *anhydrous ethanol R* and dilute to 25.0 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 25.0 mg of the substance to be examined in mobile phase A and dilute to 10.0 mL with mobile phase A.

Test solution (b) Dilute 1.0 mL of test solution (a) to 10.0 mL with mobile phase A.

Reference solution (a) Dissolve the contents of a vial of *norethisterone acetate for system suitability CRS* (containing impurities B, C, D, E, F, G and H) in 1.0 mL of mobile phase A.

Reference solution (b) Dilute 1.0 mL of test solution (a) to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

Reference solution (c) Dissolve 25.0 mg of *norethisterone acetate CRS* in mobile phase A and dilute to 10.0 mL with mobile phase A. Dilute 1.0 mL of the solution to 10.0 mL with mobile phase A.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A: water for chromatography R, acetonitrile R1* (40:60 *V/V*);

— *mobile phase B: water for chromatography R, acetonitrile R1* (10:90 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 17	100	0
17 - 20	100 → 0	0 → 100
20 - 39	0	100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 254 nm and, for impurities B and C, at 210 nm.

Injection 20 µL of test solution (a) and reference solutions (a) and (b).

Identification of impurities Use the chromatograms supplied with *norethisterone acetate for system suitability CRS* and the chromatograms obtained with reference solution (a) to identify the peaks due to impurities B, C, D + G, E, F and H.

Relative retention With reference to norethisterone acetate (retention time = about 12 min): impurity F = about 0.4; impurities D and G = about 0.6; impurity E = about 0.8; impurity C = about 1.5; impurity B = about 1.6; impurity H = about 2.8.

System suitability Reference solution (a) at 210 nm:

— *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity B.

Calculation of percentage contents:

— *correction factors*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity C = 1.3; impurity F = 1.7;

— for each impurity, use the concentration of norethisterone acetate in reference solution (b).

Limits:

— *impurities B, C at 210 nm*: for each impurity, maximum 0.3 per cent;

— *impurities F, H*: for each impurity, maximum 0.3 per cent;

— *impurity E*: maximum 0.2 per cent;

— *sum of impurities D and G*: maximum 0.2 per cent;

— *unspecified impurities*: for each impurity, maximum 0.10 per cent;

— *total*: maximum 1.0 per cent;

— *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

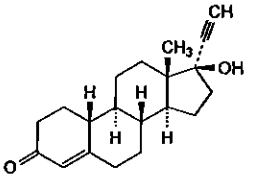
Injection Test solution (b) and reference solution (c).

Calculate the percentage content of $C_{22}H_{28}O_3$ taking into account the assigned content of *norethisterone acetate CRS.*

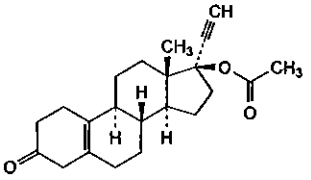
IMPURITIES

Specified impurities *B, C, D, E, F, G, H.*

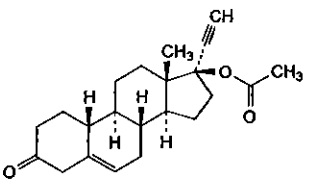
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, I, J.*



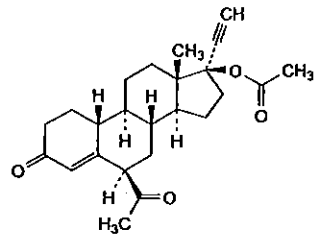
A. 17-hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one (norethisterone),



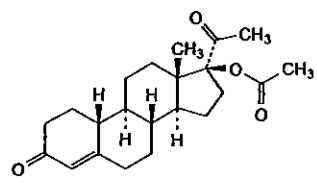
B. 3-oxo-19-nor-17α-pregn-5(10)-en-20-yn-17-yl acetate,



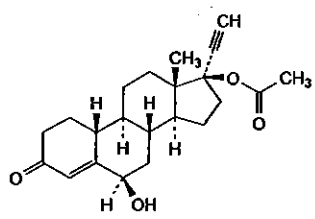
C. 3-oxo-19-nor-17α-pregn-5-en-20-yn-17-yl acetate,



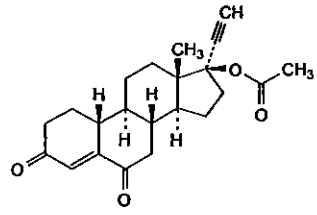
D. 6β-acetyl-3-oxo-19-nor-17α-pregn-4-en-20-yn-17-yl acetate,



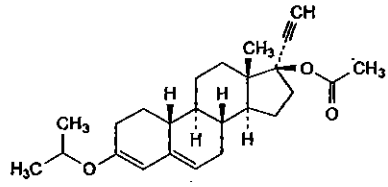
E. 3,20-dioxo-19-nor-17α-pregn-4-en-17-yl acetate,



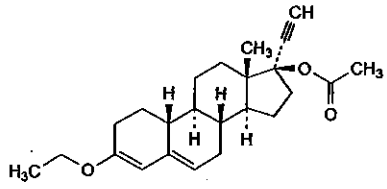
F. 6β-hydroxy-3-oxo-19-nor-17α-pregn-4-en-20-yn-17-yl acetate,



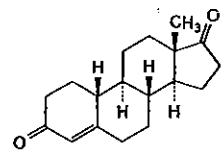
G. 3,6-dioxo-19-nor-17α-pregn-4-en-20-yn-17-yl acetate,



H. 3-(propan-2-yloxy)-19-nor-17α-pregna-3,5-dien-20-yn-17-yl acetate,



I. 3-ethoxy-19-nor-17α-pregna-3,5-dien-20-yn-17-yl acetate,

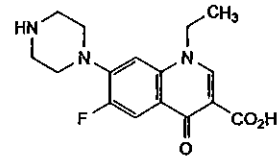


J. estr-4-ene-3,17-dione (norandrostenedione).

Ph Eur

Norfloxacin

(Ph. Eur. monograph 1248)



$C_{16}H_{18}FN_3O_3$ 319.3 *70458-96-7*

Action and use
Fluoroquinolone antibacterial.

Preparation
Norfloxacin Tablets

Ph Eur

DEFINITION
1-Ethyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or pale yellow, hygroscopic, photosensitive, crystalline powder.

Solubility
Very slightly soluble in water, slightly soluble in acetone and in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *norfloxacin CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methylene chloride R*, evaporate to dryness in a water-bath at 45 °C, dry in an oven at 105 °C for about 1 h and record new spectra using the residues.

TESTS
Appearance of solution
Dissolve 0.5 g in a previously filtered 4 g/L solution of *sodium hydroxide R* in *methanol R* and dilute to 50 mL with the same solution. The solution is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution B_7 (*2.2.2, Method II*).

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R, water R* adjusted to pH 2.0 with *phosphoric acid R* (5:95 *V/V*).

Test solution Dissolve 20.0 mg of the substance to be examined in 25 mL of the solvent mixture using sonication, and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 4 mg of *norfloxacin for system suitability CRS* (containing impurities A, E and H) in 5 mL of the solvent mixture using sonication, and dilute to 10 mL with the solvent mixture.

Reference solution (c) Dissolve 4 mg of *norfloxacin for peak identification CRS* (containing impurity K) in 5 mL of the solvent mixture using sonication, and dilute to 10 mL with the solvent mixture.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped amidohexadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 60 °C.

Mobile phase:
- *mobile phase A*: *water for chromatography R* adjusted to pH 2.0 with *phosphoric acid R*;
- *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	95	5
5 - 7	95 → 93	5 → 7
7 - 10	93 → 87	7 → 13
10 - 15	87 → 47	13 → 53
15 - 20	47 → 10	53 → 90

Flow rate 1.4 mL/min.

Detection Spectrophotometer at 265 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram supplied with *norfloxacin for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, E and H; use the chromatogram supplied with *norfloxacin for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peak due to impurity K.

Relative retention With reference to norfloxacin (retention time = about 11 min): impurity K = about 0.6; impurity E = about 0.97; impurity A = about 1.5; impurity H = about 1.6.

System suitability Reference solution (b):
- *resolution*: minimum 3.0 between the peaks due to impurities A and H;
- *peak-to-valley ratio*: minimum 5.0, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to norfloxacin.

Limits:
- *impurities E, K*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)
Maximum 1.0 per cent, determined on 0.700 g. Use as the solvent a mixture of 10 mL of *anhydrous methanol R* and 20 mL of *formamide R*.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Dissolve 0.240 g in 80 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 31.93 mg of $C_{16}H_{18}FN_3O_3$.

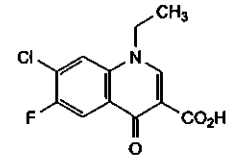
STORAGE

In an airtight container, protected from light.

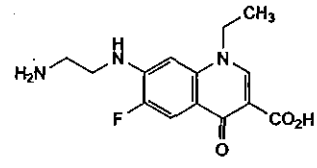
IMPURITIES

Specified impurities *E, K.*

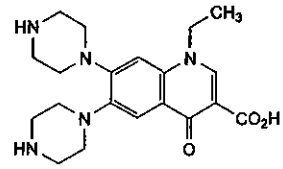
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, F, G, H, I, J.*



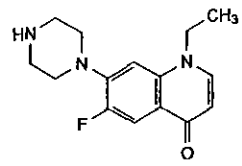
A. 7-chloro-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid,



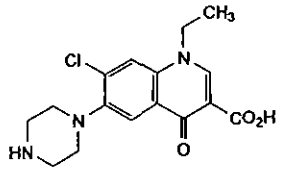
B. 7-[(2-aminoethyl)amino]-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid,



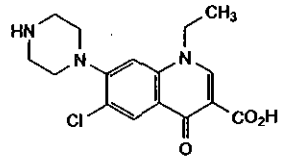
C. 1-ethyl-4-oxo-6,7-di(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid,



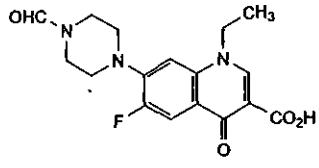
D. 1-ethyl-6-fluoro-7-(piperazin-1-yl)quinolin-4(1*H*)-one,



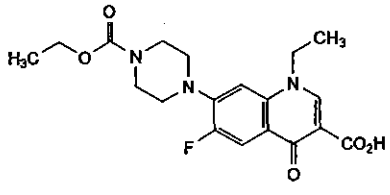
E. 7-chloro-1-ethyl-4-oxo-6-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid,



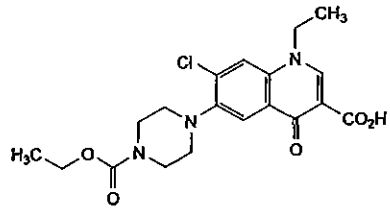
F. 6-chloro-1-ethyl-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid,



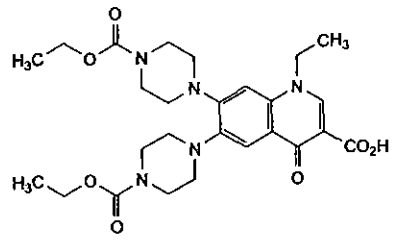
G. 1-ethyl-6-fluoro-7-(4-formylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid,



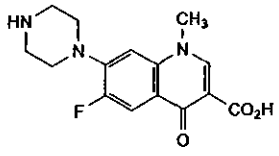
H. 7-[4-(ethoxycarbonyl)piperazin-1-yl]-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid,



I. 7-chloro-6-[4-(ethoxycarbonyl)piperazin-1-yl]-1-ethyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid,



J. 6,7-bis[4-(ethoxycarbonyl)piperazin-1-yl]-1-ethyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid,



K. 6-fluoro-1-methyl-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid.

Ph Eur

Norflurane

(Ph. Eur. monograph 2257)

$C_2H_2F_4$ 102.0 *811-97-2*

Action and use
General anaesthetic.

Ph Eur

DEFINITION

1,1,1,2-Tetrafluoroethane (HFC 134a).

CHARACTERS

Appearance
Clear, colourless gas, liquid under pressure.

Solubility
At 20 °C and at a pressure of 101 kPa, slightly soluble in water, freely soluble in ethanol (96 per cent).

Relative density
About 1.23 at 20 °C, for the gas in the liquid phase.

bp
About −26 °C.

It is a hygroscopic, non-flammable gas.

IDENTIFICATION

Carry out either test A or test B.

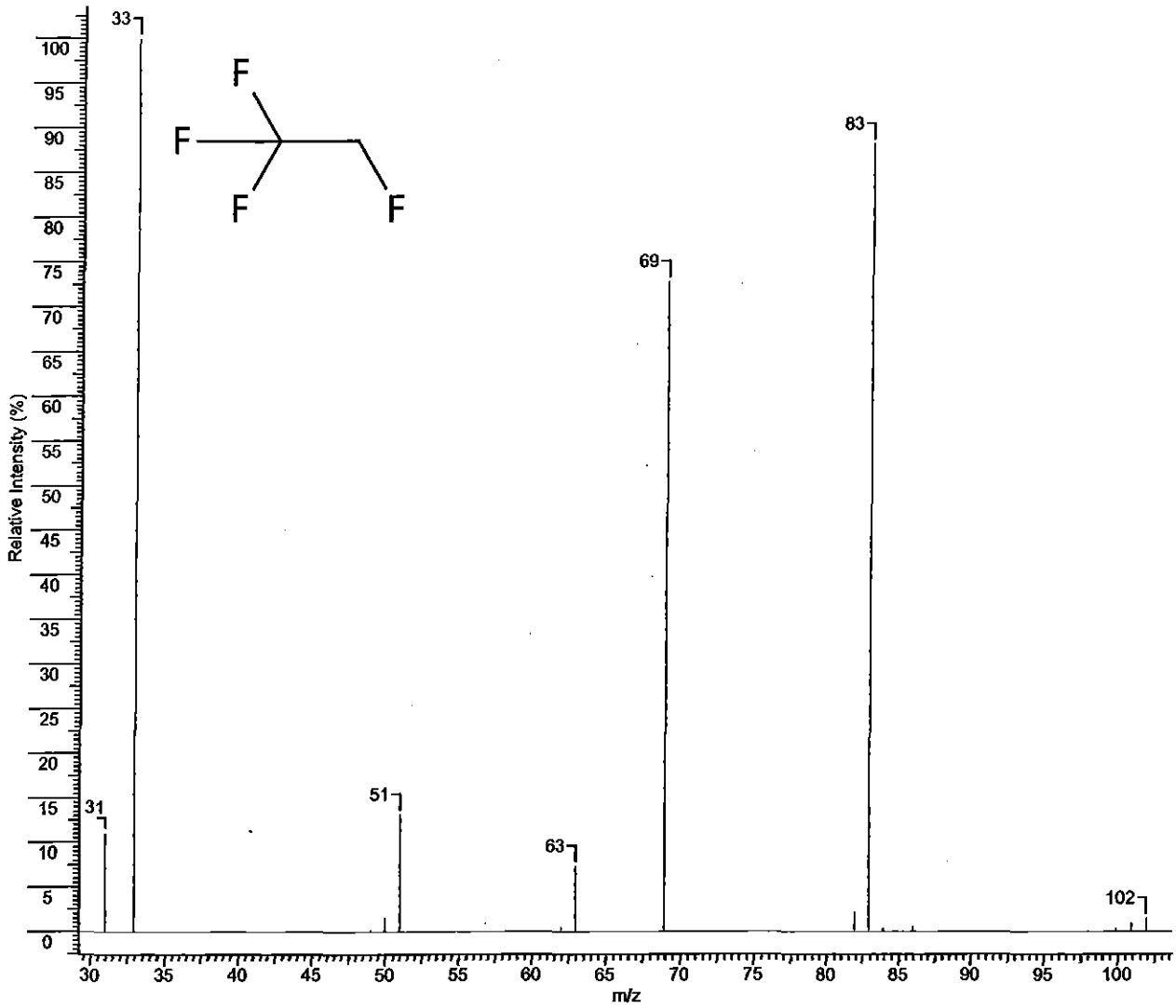
A. Infrared absorption spectrophotometry (*2.2.24*). *Carry out the test at atmospheric pressure.*

Preparation Dilute the gas to be examined in *nitrogen R* (approximately 20:80 *V/V* depending on the sensitivity of the spectrophotometer).

Comparison *Ph. Eur. reference spectrum of norflurane.*

B. Mass spectrometry (*2.2.43*).

Results The mass spectrum obtained with the gas to be examined is similar to the mass spectrum of norflurane shown in Figure 2257.-1.



m/z	RI (%)	Ion	*m/z*	RI (%)	Ion
31	11.1	$[CF]^+$	82	2.1	$[CF_2\text{-}CHF]^{+\cdot}$
33	100.0	$[CH_2F]^+$	83	88.2	$[CF_2\text{-}CH_2F]^+$
50	1.5	$[CF_2]^{+\cdot}$	100	0.3	$[CF_3\text{-}CF]^{+\cdot}$
51	13.2	$[CHF_2]^+$	101	0.9	$[CF_3\text{-}CHF]^+$
63	7.4	$[CF\text{=}CHF]^+$	102	1.5	$[CF_3\text{-}CH_2F]^{+\cdot}$
69	72.9	$[CF_3]^+$			

Figure 2257.-1. – *Mass spectrum of norflurane*

TESTS

Acidity

Maximum 0.1 ppm, expressed as HCl.

Transfer 200 mL of deionised *water R* previously neutralised to *bromocresol purple solution R* to a glass washing bottle fitted with a distribution tube with a sintered-glass disc. Pass 750 g of the gas to be examined through the water, at a rate of about 60 L/h. Titrate with *0.02 M sodium hydroxide* using *bromocresol purple solution R* as indicator until the colour changes from yellow to bluish-violet. Carry out a blank titration using deionised *water R*.

1 mL of *0.02 M sodium hydroxide* is equivalent to 0.729 mg of HCl.

Non-volatile matter

Maximum 50 ppm.

Carry out the test using a glass double-wall vessel (see Figure 2257.-2).

Dry the removable part in an oven at 105 ± 2 °C for 30 min. Allow to cool in a desiccator and weigh to the nearest 0.1 mg. Connect it to the vessel.

Weigh the gas cylinder to the nearest 1 g. Fill the vessel with about 500 mL of liquefied gas and weigh the gas cylinder again. Determine the mass of the sample by weight difference. Using a suitable heating device such as a water-bath, heat the removable part such that the sample evaporates in about 2 h. Dry the removable part in an oven at 105 ± 2 °C for 30 min. Allow to cool in a desiccator and

weigh to the nearest 0.1 mg. Determine the mass of the residue by weight difference.

Calculate the content of non-volatile matter in the gas using the following expression:

$$\frac{10^3 \times m}{M}$$

m = mass of residue, in milligrams;
M = mass of sample, in grams.

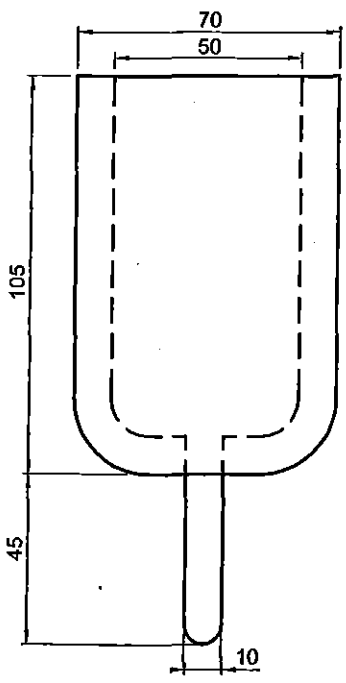


Figure 2257.-2. – *Glass double-wall vessel*
Dimensions in millimetres

Non-condensable gases

Maximum 1.5 per cent *V/V*.

Gas chromatography (*2.2.28*).

Gas to be examined The sample is taken from the vapour phase maintaining the cylinder in an upright position. Evacuate the gas loop using a multiway tap and fill cautiously with the gas to be examined.

Reference gases Mixtures of ambient air in *helium for chromatography R* covering a concentration range of 0.5 per cent to 2.0 per cent.

Column:
— *material*: stainless steel;
— *size*: *l* = 5 m, Ø = 2 mm;
— *stationary phase*: *oxypropionitrilsilyl silica gel for chromatography R* (150-180 µm).

Carrier gas *helium for chromatography R.*

Flow rate 21 mL/min.

Temperature:
— *column*: 80 °C;
— *injection port*: 150 °C;
— *detector*: 180 °C.

Detection Thermal conductivity.

Injection 150 µL loop injector.

Run time 10 min.

Relative retention With reference to norflurane (retention time = about 4 min): non-condensable gases = about 0.4.

Determine the concentration (*V/V*) of non-condensable gases in the gas to be examined using the calibration curve obtained with the reference gases.

Related substances

Gas chromatography (*2.2.28*), equipped with a gas valve sampling system and a cryogenic unit, coupled with mass spectrometry (*2.2.43*).

Gas to be examined Connect the cylinder to the gas valve sampling system and sample from the liquid phase. Then evacuate the loop including the transfer line using a multiway tap and a vacuum pump. Open the valves of the cylinder and fill the loop cautiously with the gas to be examined.

Reference gas (a) Prepare a mixture in *helium for chromatography R* of the impurities expected in the gas to be examined (see Table 2257.-1) at a concentration of 2-6 ppm each, always including impurity G.

Reference gas (b) Prepare a mixture of FC 1318my/c (impurity S) and FC 1318my/t (impurity T) in *helium for chromatography R* at a combined concentration of approximately 20 ppm.

Reference gas (c) Prepare a mixture of CFC 114 (impurity L) and HCC 40 (impurity W) in *helium for chromatography R* at a concentration of approximately 1 ppm each.

Reference gas (d) Prepare a 5-fold dilution of reference gas (a) in *helium for chromatography R.*

Column:
— *material*: fused silica;
— *size*: *l* = 60 m, Ø = 0.18 mm;
— *stationary phase*: *cyanopropyl(3)phenyl(3)methyl(94) polysiloxane R* (film thickness 1 µm).

Carrier gas *helium for chromatography R.*

Flow rate 1.1 mL/min.

Split ratio 1:75.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 2	−25
	2 - 7.2	−25 → −12
	7.2 - 14	−12 → 15
	14 - 18.7	15 → 250
	18.7 - 21.2	250
Injection port		150

Detection Mass spectrometer; the following settings were found to be suitable and are given as examples; if the detector has different setting parameters, adjust the detector settings so as to comply with the system suitability criteria:
— *ionisation mode*: electron impact (70 eV);
— *trap current*: 0.2 mA;
— *mass range*: 30-300 Da;
— *scan rate*: ≤ 0.2 s/scan;
— *temperature*: ion source: 160 °C; transfer line: 200 °C.

Injection 500 µL.

Identification of impurities Use the reconstructed ion chromatogram obtained with reference gas (a) and the information supplied in Table 2257.-1.

System suitability:
— *resolution*: minimum 1.4 between the peaks due to FC 1318my/c (impurity S) and FC 1318my/t (impurity T) in the chromatogram obtained with reference gas (b);

Table 2257.-1. – *Norflurane impurities: quantification ions and relative retentions*

Impurity	Code	Structure	M_r	Quantification ion *(m/z)*	Relative retention
P	CFC 13	$CClF_3$	104	69	0.71
QQ	HFC 23	CHF_3	70	51	*0.73*
JJ	HFC 1132a	$CH_2=CF_2$	64	64	*0.73*
N	CFC 115	$CClF_2$-CF_3	154	85	0.77
D	HFC 143a	CH_3-CF_3	84	65/69*	*0.81*
SS	HFC 32	CH_2F_2	52	51/33*	*0.81*
II	HFC 1123	$CHF=CF_2$	82	63	*0.82*
MM	HFC 125	CHF_2-CF_3	120	101	*0.84*
T	FC 1318my/t	CF_3-CF=CF-CF_3	200	131	*0.84*
S	FC 1318my/c	CF_3-CF=CF-CF_3	200	131	0.87
Q	CFC 217ba	CF_3-$CClF$-CF_3	204	85	0.93
KK	HFC 1234yf	$CH_2=CF$-CF_3	114	114	0.96
RR	HFC 245cb	CH_3-CF_2-CF_3	134	65	0.98
Norflurane	HFC 134a	CH_2F-CF_3	102	83	1
R	FC 115B1	$CBrF_2$-CF_3	198	119	1.03
H	HFC 1225ye/c	CHF=CF-CF_3	132	113	*1.10*
E	HFC 152a	CH_3-CHF_2	66	65	*1.11*
PP	HFC 161	CH_3-CH_2F	48	33/47*	*1.11*
I	HFC 1243zf	$CH_2=CH$-CF_3	96	96	*1.11*
LL	HFC 1234ze	$CHF=CH$-CF_3	114	114	1.14
A	CFC 12	CCl_2F_2	120	85	1.17
C	HFC 134	CHF_2-CHF_2	102	51/83*	1.21
NN	HFC 1336mzz/c	CF_3-CH=CH-CF_3	164	95	*1.30*
GG	HCFC 22	$CHClF_2$	86	51	*1.32*
L	CFC 114	$CClF_2$-$CClF_2$	170	85	*1.63*
M	CFC 114a	CCl_2F-CF_3	170	101/103*	*1.64*
W	HCC 40	CH_3Cl	50	52	1.67
G	HCFC 1122	$CHCl=CF_2$	98	98	1.72
DD	HCFC 124a	CHF_2-$CClF_2$	136	101	1.77
B	HCFC 124	$CHClF$-CF_3	136	67	1.87
HH	HCFC 31	CH_2ClF	68	68	1.97
Y	HCFC 1122a/c	$CHF=CClF$	98	98	2.03
O	CFC 12B1	$CBrClF_2$	164	85	2.08
AA	HCFC 1131/t	$CHCl=CHF$	80	80	2.19
OO	HFC 152	CH_2F-CH_2F	66	33	2.41
FF	HCFC 133a	CH_2Cl-CF_3	118	118	2.47
F	CFC 1112a	$CCl_2=CF_2$	132	132	2.74
Z	HCFC 1131/c	$CHCl=CHF$	80	80	2.84
J	CFC 11	CCl_3F	136	101	2.97
CC	HCFC 123a	$CHClF$-$CClF_2$	152	67	3.15
BB	HCFC 123	$CHCl_2$-CF_3	152	83	*3.18*

Impurity	Code	Structure	M_r	Quantification ion *(m/z)*	Relative retention
K	CFC 113	CCl_2F-$CClF_2$	186	151	*3.18*
X	HCFC 1121/t	CHCl=CClF	114	114	3.25
V	HCC 30	CH_2Cl_2	84	49	3.29
EE	HCFC 132b	$CClF_2$-CH_2Cl	134	99	3.32
U	HCC 1120	$CHCl=CCl_2$	130	95	3.59

* Depending on the actual chromatographic resolution and potentially overlapping compounds, it may be necessary to select a different quantification ion.

— *signal-to-noise ratio*: minimum 10 each for CFC 114 (impurity L) and HCC 40 (impurity W) in the chromatogram obtained with reference gas (c).

Selectivity – co-elution Several impurities elute with a resolution of less than 1; close inspection of the individual ion traces is necessary to detect and quantify potential co-eluting compounds; the relative retentions of these compounds are indicated in italics in Table 2257.-1.

Before assessing the presence of individual impurities in the reconstructed ion chromatogram, a suitable background subtraction may be necessary. Two potential sources of background have to be taken into account: use of cryo-cooling (liquid carbon dioxide, *m/z* 44) and possible bleeding of the column (siloxanes, several ions at *m/z* greater than 200).

If a peak is observed at a retention time where co-elution is possible, the identification and quantification are applied to each of the co-eluting compounds.

Calculation of parts per million contents:
— for impurities A, B, C, D, E, F, G, H and I, use the concentration of the corresponding impurity in reference gas (a);
— for the other impurities, use the concentration of impurity G in reference gas (a);
— for the reporting threshold, use the concentration of impurity G in reference gas (d).

Limits:

Saturated impurities
— *HFC 134 (impurity C)*: maximum 1000 ppm;
— *HFC 152a (impurity E)*: maximum 500 ppm;
— *CFC 12 (impurity A), HCFC 124 (impurity B)*: for each impurity, maximum 100 ppm;
— *HFC 143a (impurity D)*: maximum 50 ppm.

Unsaturated impurities
— *CFC 1112a (impurity F), HCFC 1122 (impurity G), HFC 1225yelc (impurity H), HFC 1243zf (impurity I)*: for each impurity, maximum 5 ppm.

Other detectable impurities
— *other saturated or unsaturated impurities or unknow impurities*: for each impurity, maximum 5 ppm;
— *sum of other detectable impurities*: maximum 10 ppm.

Total
Maximum 1000 ppm.

Reporting threshold
1 ppm.

Water *(2.5.32)*
Maximum 10 ppm, determined on 30.0 g. *Take care to avoid uptake of water by the gas to be examined during the test.*

Transfer the gas to be examined in liquid phase from the inverted steel cylinder to an evacuated sample can.
To transfer the sample, connect a metal tube with fittings at one end to the cylinder valve, and at the other end to the needle valve on the sample can.

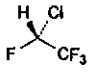
IMPURITIES

Specified impurities *A, B, C, D, E, F, G, H, I.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *J, K, L, M, N, O, P, Q, R, S, T, U, V, W, X, Y, Z, AA, BB, CC, DD, EE, FF, GG, HH, II, JJ, KK, LL, MM, NN, OO, PP, QQ, RR, SS.*

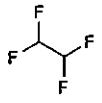
CCl_2F_2

A. dichlorodifluoromethane (CFC 12 [75-71-8]),



and enantiomer

B. (2*RS*)-2-chloro-1,1,1,2-tetrafluoroethane (HCFC 124 [2837-89-0]),

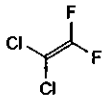


C. 1,1,2,2-tetrafluoroethane (HFC 134 [359-35-3]),

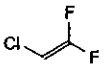
H_3C-CF_3

D. 1,1,1-trifluoroethane (HFC 143a [420-46-2]),

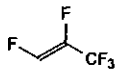
E. 1,1-difluoroethane (HFC 152a [75-37-6]),



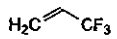
F. 1,1-dichloro-2,2-difluoroethene (CFC 1112a [79-35-6]),



G. 1-chloro-2,2-difluoroethene (HCFC 1122 [359-10-4]),

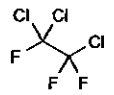


H. (1*Z*)-1,2,3,3,3-pentafluoroprop-1-ene (HFC 1225ye/c [5528-43-8]),

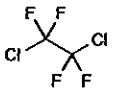


I. 3,3,3-trifluoroprop-1-ene (HFC 1243zf [677-21-4]),

J. trichlorofluoromethane (CFC 11 [75-69-4]),

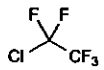


K. 1,1,2-trichloro-1,2,2-trifluoroethane (CFC 113 [76-13-1]),



L. 1,2-dichloro-1,1,2,2-tetrafluoroethane (CFC 114 [76-14-2]),

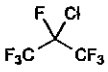
M. 1,1-dichloro-1,2,2,2-tetrafluoroethane (CFC 114a [374-07-2]),



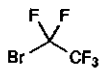
N. 1-chloro-1,1,2,2,2-pentafluoroethane (CFC 115 [76-15-3]),

O. bromochlorodifluoromethane (CFC 12B1 [353-59-3]),

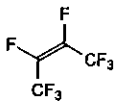
P. chlorotrifluoromethane (CFC 13 [75-72-9]),



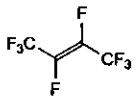
Q. 2-chloro-1,1,1,2,3,3,3-heptafluoropropane (CFC 217ba [76-18-6]),



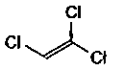
R. 1-bromo-1,1,2,2,2-pentafluoroethane (FC 115B1 [354-55-2]),



S. (2*Z*)-1,1,1,2,3,4,4,4-octafluorobut-2-ene (FC 1318my/c [1516-65-0]),



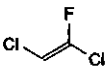
T. (2*E*)-1,1,1,2,3,4,4,4-octafluorobut-2-ene (FC 1318my/t [1516-64-9]),



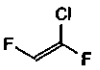
U. 1,1,2-trichloroethene (trichloroethylene, HCC 1120 [79-01-6]),

V. dichloromethane (methylene chloride, HCC 30 [75-09-2]),

W. chloromethane (methyl chloride, HCC 40 [74-87-3]),

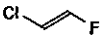


X. (*E*)-1,2-dichloro-1-fluoroethene (HCFC 1121/t),

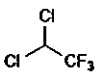


Y. (*Z*)-1-chloro-1,2-difluoroethene (HCFC 1122a/c [359-04-6]),

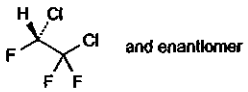
Z. (*Z*)-1-chloro-2-fluoroethene (HCFC 1131/c [2268-31-7]),



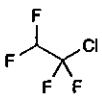
AA. (*E*)-1-chloro-2-fluoroethene (HCFC 1131/t [2268-32-8]),



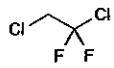
BB. 1,1-dichloro-2,2,2-trifluoroethane (HCFC 123 [306-83-2]),



CC. (2*RS*)-1,2-dichloro-1,1,2-trifluoroethane (HCFC 123a [354-23-4]),



DD. 1-chloro-1,1,2,2-tetrafluoroethane (HCFC 124a [354-25-6]),

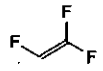


EE. 1,2-dichloro-1,1-difluoroethane (HCFC 132b [1649-08-7]),

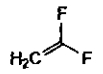
FF. 2-chloro-1,1,1-trifluoroethane (HCFC 133a [75-88-7]),

GG. chlorodifluoromethane (HCFC 22 [75-45-6]),

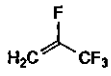
HH. chlorofluoromethane (HCFC 31 [593-70-4]),



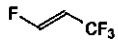
II. 1,1,2-trifluoroethene (HFC 1123 [359-11-5]),



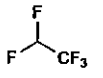
JJ. 1,1-difluoroethene (HFC 1132a [75-38-7]),



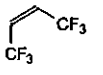
KK. 2,3,3,3-tetrafluoroprop-1-ene (HFC 1234yf [754-12-1]),



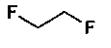
LL. (1*E*)-1,3,3,3-tetrafluoroprop-1-ene (HFC 1234ze [1645-83-6]),



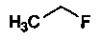
MM. 1,1,1,2,2-pentafluoroethane (HFC 125 [354-33-6]),



NN. (2*Z*)-1,1,1,4,4,4-hexafluorobut-2-ene (HFC 1336mzz/c [692-49-9]),

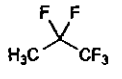


OO. 1,2-difluoroethane (HFC 152 [624-72-6]),



PP. fluoroethane (HFC 161 [353-36-6]),

QQ. trifluoromethane (HFC 23 [75-46-7]),



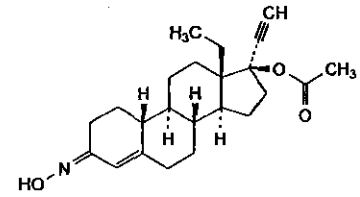
RR. 1,1,1,2,2-pentafluoropropane (HFC 245cb [1814-88-6]),

SS. difluoromethane (HFC 32 [75-10-5]).

Ph Eur

Norgestimate

(Ph. Eur. monograph 1732)



$C_{23}H_{31}NO_3$ 369.5 *35189-28-7*

Action and use
Progestogen.

Ph Eur

DEFINITION

(3*EZ*)-13β-Ethyl-3-(hydroxyimino)-18,19-dinor-17α-pregn-4-en-20-yn-17-yl acetate.

Content
98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Practically insoluble in water, freely soluble in methylene chloride, soluble in acetone.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *norgestimate CRS.*

TESTS

Specific optical rotation (*2.2.7*)
+ 42.0 to + 50.0 (dried substance).

Dissolve 0.200 g in *methylene chloride R* and dilute to 20.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture *water R, methanol R* (1:4 *V/V*).

Test solution Dissolve 25.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dilute 2.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 20.0 mL with the solvent mixture.

Reference solution (b) Dissolve 2 mg of *norgestimate for system suitability CRS* (containing impurity A) in 4 mL of the solvent mixture.

Column:
— *size:* *l* = 0.10 m, Ø = 4.6 mm;
— *stationary phase:* spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature:* 40 °C.

Mobile phase *acetonitrile R, tetrahydrofuran for chromatography R, water R* (18:22:60 *V/V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 244 nm.

Injection 25 µL.

Run time Twice the retention time of the (*E*)-isomer of norgestimate.

Identification of impurities Use the chromatogram supplied with *norgestimate for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity A.

Relative retention With reference to the (*E*)-isomer of norgestimate (retention time = about 14 min): impurity A = about 0.7; (*Z*)-isomer of norgestimate = about 0.9.

System suitability Reference solution (b):
— *resolution*: minimum 1.5 between the peaks due to the (*E*)- and (*Z*)-isomers of norgestimate.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of the (*Z*)-isomer of norgestimate by 1.33;
— *impurity A*: not more than twice the sum of the areas of the peaks due to the (*E*)- and (*Z*)-isomers of norgestimate in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *unspecified impurities*: for each impurity, not more than the sum of the areas of the peaks due to the (*E*)- and (*Z*)-isomers of norgestimate in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 3 times the sum of the areas of the peaks due to the (*E*)- and (*Z*)-isomers of norgestimate in the chromatogram obtained with reference solution (a) (0.3 per cent);
— *disregard limit*: 0.5 times the sum of the areas of the peaks due to the (*E*)- and (*Z*)-isomers of norgestimate in the chromatogram obtained with reference solution (a) (0.05 per cent).

Ratio of (*E*)- to (*Z*)-isomers

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution.

Calculate the (*E*)- to (*Z*)-isomer ratio by dividing the area of the peak due to the (*E*)-isomer by 1.33 times the area of the peak due to the (*Z*)-isomer. The ratio is 1.27 to 1.78.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 0.500 g by drying in an oven at 105 °C for 3 h.

ASSAY

Dissolve 0.300 g in 40 mL of *tetrahydrofuran R*. Add 10 mL of a 100 g/L solution of *silver nitrate R* and titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Rinse the electrode with *acetone R* after each titration.

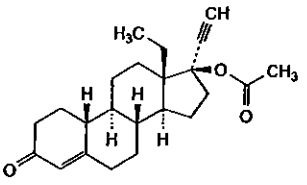
If necessary, after several titrations re-equilibrate the electrode in *water R* for 15 min to obtain sharper titration curves.

1 mL of *0.1 M sodium hydroxide* is equivalent to 36.95 mg of $C_{23}H_{31}NO_3$.

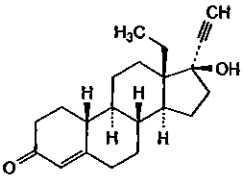
IMPURITIES

Specified impurities *A*.

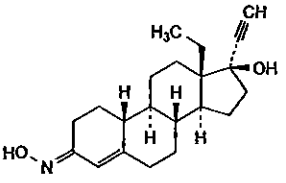
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D.*



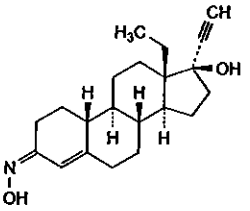
A. 13β-ethyl-3-oxo-18,19-dinor-17α-pregn-4-en-20-yn-17-yl acetate (levonorgestrel acetate),



B. 13β-ethyl-17β-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one (levonorgestrel),



C. (3*E*)-13β-ethyl-3-(hydroxyimino)-18,19-dinor-17α-pregn-4-en-20-yn-17-ol ((*E*)-norelgestromin),

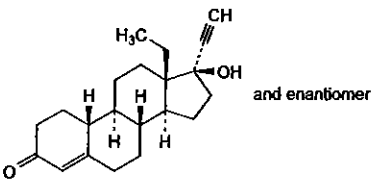


D. (3*Z*)-13β-ethyl-3-(hydroxyimino)-18,19-dinor-17α-pregn-4-en-20-yn-17-ol ((*Z*)-norelgestromin).

Ph Eur

Norgestrel

(Ph. Eur. monograph 0940)



$C_{21}H_{28}O_2$ 312.5 *6533-00-2*

Action and use
Progestogen.

Preparation
Norgestrel Tablets

Ph Eur

DEFINITION

Norgestrel contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of *rac*-13-ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water, sparingly soluble in methylene chloride, slightly soluble in alcohol.

IDENTIFICATION

A. Dissolve 0.5 g in *methylene chloride R* and dilute to 10.0 mL with the same solvent. The angle of optical rotation (*2.2.7*) is + 0.05° to −0.05°.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *norgestrel CRS*.

TESTS

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution Dissolve 0.2 g of the substance to be examined in *methylene chloride R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dilute 1 mL of the test solution to 10 mL with *methylene chloride R*. Dilute 1 mL of this solution to 20 mL with *methylene chloride R*.

Reference solution (b) Dilute 4 mL of reference solution (a) to 10 mL with *methylene chloride R*.

Reference solution (c) Dissolve 5 mg of *norgestrel CRS* and 5 mg of *ethinylestradiol CRS* in *methylene chloride R* and dilute to 50 mL with the same solvent.

Apply to the plate 10 µL of each solution. Develop over a path of 15 cm using a mixture of 20 volumes of *ethyl acetate R* and 80 volumes of *methylene chloride R*. Allow the plate to dry in air, spray with a 100 g/L solution of *phosphomolybdic acid R* in *alcohol R*, heat at 100-105 °C for 15 min and examine immediately. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the principal spot in the chromatogram obtained with reference solution (a) (0.5 per cent) and at most two such spots are more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 45 mL of *tetrahydrofuran R*. Add 10 mL of a 100 g/L solution of *silver nitrate R*. After 1 min, titrate with *0.1 M sodium hydroxide* determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 mL of *0.1 M sodium hydroxide* is equivalent to 31.25 mg of $C_{21}H_{28}O_2$.

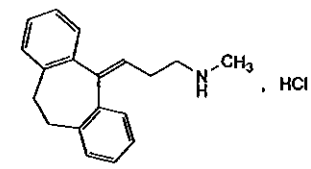
STORAGE

Store protected from light.

Ph Eur

Nortriptyline Hydrochloride

(Ph. Eur. monograph 0941)



$C_{19}H_{22}ClN$ 299.8 *894-71-3*

Action and use

Monoamine reuptake inhibitor; tricyclic antidepressant.

Preparations

Nortriptyline Capsules

Nortriptyline Tablets

Ph Eur

DEFINITION

3-(10,11-Dihydro-5*H*-dibenzo[*a*,*d*][7]annulen-5-ylidene)-*N*-methylpropan-1-amine hydrochloride.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Sparingly soluble in water, soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *nortriptyline hydrochloride CRS*.

B. Dissolve 20 mg in 2 mL of *water R*, acidify with 1 drop of *dilute sulfuric acid R* and add 1 mL of a 6.25 g/L solution of *silver sulfate R*. Shake and allow to stand. A curdled, white precipitate is formed. Centrifuge and wash the precipitate with 3 quantities, each of 1 mL, of a 0.5 per cent *V/V* solution of *sulfuric acid R*. Carry out this operation rapidly in subdued light, disregarding the fact that the supernatant solution may not become perfectly clear. Suspend the precipitate in *water R* and add *ammonia R*. Shake vigorously for at least 1 min. The precipitate dissolves easily with the possible exception of a few large particles which dissolve slowly.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution B_7 (*2.2.2, Method II*).

Dissolve 0.5 g in *water R* with gentle heating and dilute to 25 mL with the same solvent.

Acidity or alkalinity

Dissolve 0.2 g with gentle heating in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.
Add 0.1 mL of *methyl red solution R* and 0.2 mL of *0.01 M sodium hydroxide*. The solution is yellow. Add 0.4 mL of *0.01 M hydrochloric acid*. The solution is red.

Related substances

Liquid chromatography (*2.2.29*). *Protect the solutions from light.*

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *dibenzosuberone CRS* (impurity A) in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 50.0 mL with the mobile phase.

Reference solution (c) Dissolve 20 mg of *norcyclobenzaprine CRS* (impurity B) in the mobile phase and dilute to 100 mL with the mobile phase. Dilute 1 mL of the solution to 10 mL with the mobile phase.

Reference solution (d) Dissolve 10 mg of *nortriptyline for system suitability CRS* (containing impurity D) in the mobile phase, add 1 mL of reference solution (c) and dilute to 10 mL with the mobile phase.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: spherical *end-capped extra-dense bonded octylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 45 °C.

Mobile phase Mix 70 volumes of *methanol R1* and 30 volumes of a solution prepared as follows: mix 3.25 mL of *tetrabutylammonium hydroxide solution (400 g/L) R*, 0.68 g of *potassium dihydrogen phosphate R* and about 900 mL of *water for chromatography R*, then adjust to pH 7.5 with *dilute phosphoric acid R* and dilute to 1000 mL with *water for chromatography R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µL.

Run time 3 times the retention time of nortriptyline.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity A; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity B; use the chromatogram supplied with *nortriptyline for system suitability CRS* and the chromatogram obtained with reference solution (d) to identify the peak due to impurity D.

Relative retention With reference to nortriptyline (retention time = about 13 min): impurity A = about 0.5; impurity D = about 0.8; impurity B = about 0.9.

System suitability Reference solution (d):
- *resolution*: minimum 1.4 between the peaks due to impurities D and B; minimum 2.0 between the peaks due to impurity B and nortriptyline.

Limits:
- *correction factor*: for the calculation of content, multiply the peak area of impurity D by 1.7;
- *impurity D*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);
- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.05 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 30 mL of *ethanol (96 per cent) R* and add 1.0 mL of *0.1 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 29.98 mg of $C_{19}H_{22}ClN$.

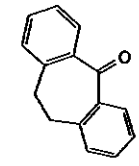
STORAGE

Protected from light.

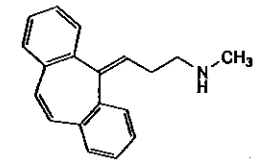
IMPURITIES

Specified impurities *A, D.*

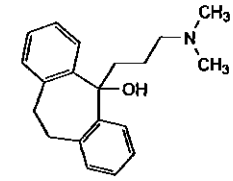
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, E, F, G, H, I, J.*



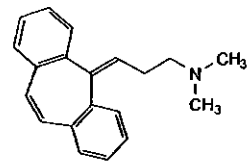
A. 10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulen-5-one (dibenzosuberone),



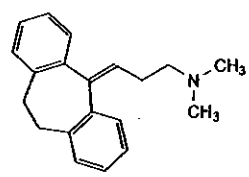
B. 3-(5*H*-dibenzo[*a*,*d*][7]annulen-5-ylidene)-*N*-methylpropan-1-amine (norcyclobenzaprine),



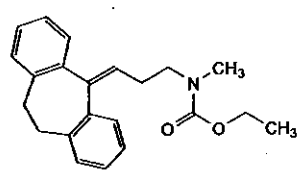
D. 5-[3-(dimethylamino)propyl]-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulen-5-ol,



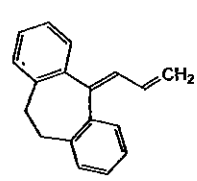
E. 3-(5*H*-dibenzo[*a*,*d*][7]annulen-5-ylidene)-*N*,*N*-dimethylpropan-1-amine (cyclobenzaprine),



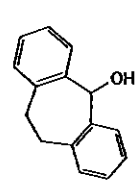
F. 3-(10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulen-5-ylidene)-*N*,*N*-dimethylpropan-1-amine (amitriptyline),



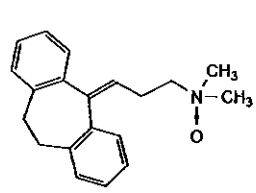
G. ethyl *N*-[3-(10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulen-5-ylidene)propyl]-*N*-methylcarbamate,



H. 5-prop-2-en-1-ylidene-10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulene,



I. 10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulen-5-ol (dibenzosuberol),



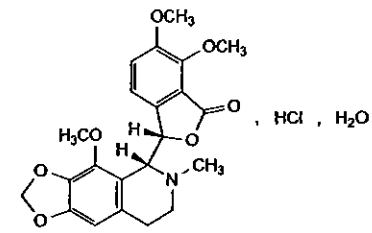
J. [3-(10,11-dihydro-5*H*-dibenzo[*a*,*d*][7]annulen-5-ylidene)propyl]dimethylamine *N*-oxide (amitriptyline-*N*-oxide).

Ph Eur

Noscapine Hydrochloride Hydrate

Noscapine Hydrochloride

(Ph. Eur. monograph 0515)



$C_{22}H_{24}ClNO_7,H_2O$ 467.9

Action and use

Opioid receptor agonist; cough suppressant.

Ph Eur

DEFINITION

(3*S*)-6,7-Dimethoxy-3-[(5*R*)-4-methoxy-6-methyl-5,6,7,8-tetrahydro-1,3-dioxolo[4,5-*g*]isoquinolin-5-yl]-2-benzofuran-1(3*H*)-one hydrochloride hydrate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals, hygroscopic.

Solubility

Freely soluble in water and in ethanol (96 per cent). Aqueous solutions are slightly acid; the base may be precipitated when the solutions are allowed to stand.

mp

About 200 °C, with decomposition.

IDENTIFICATION

First identification: C, E.

Second identification: A, B, D, E.

A. Specific optical rotation (see Tests).

B. Melting point (*2.2.14*) of the precipitate obtained in identification test E: 174 °C to 177 °C.

C. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Examine the precipitate obtained in identification test E.

Comparison *noscapine CRS.*

D. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 25 mg of the substance to be examined in *ethanol (96 per cent) R* and dilute to 100 mL with the same solvent.

Reference solution Dissolve 22 mg of *noscapine CRS* in *acetone R* and dilute to 100 mL with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *concentrated ammonia R, ethanol (96 per cent) R, acetone R, toluene R* (1:3:20:20 *V/V/V/V*).

Application 10 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *dilute potassium iodobismuthate solution R.*

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

E. Dissolve about 40 mg in a mixture of 2 mL of *water R* and 3 mL of *ethanol (96 per cent) R* and add 1 mL of *dilute ammonia R2*. Heat until dissolution is complete. Allow to cool, scratching the wall of the tube with a glass rod. Filter. The filtrate gives reaction (a) of chlorides (*2.3.1*). Wash the precipitate with *water R*, dry at 100-105 °C and reserve for identification tests B and C.

TESTS

Appearance of solution

The solution is not more intensely coloured than reference solution Y_6 or BY_6 (*2.2.2, Method II*).

Dissolve 0.5 g in *water R*, add 0.3 mL of *0.1 M hydrochloric acid* and dilute to 25 mL with *water R*.

pH (*2.2.3*)

Minimum 3.0.

Dissolve 0.2 g in 10 mL of *carbon dioxide-free water R*.

Specific optical rotation (*2.2.7*)

+ 38.5 to + 44.0 (dried substance).

Dissolve 0.500 g in *0.01 M hydrochloric acid* and dilute to 25.0 mL with the same acid.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 40.0 mg of the substance to be examined in *methanol R*, with the aid of ultrasound, and dilute to 20.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *methanol R*. Dilute 1.0 mL of this solution to 10.0 mL with *methanol R*.

Reference solution (b) Dissolve 5.0 mg of *papaverine hydrochloride CRS* (impurity A) in the mobile phase and dilute to 50.0 mL with the mobile phase (solution A). Dilute 1.0 mL of solution A to 10.0 mL with the mobile phase.

Reference solution (c) Dilute 6.0 mL of solution A to 10.0 mL with the test solution.

Column:
- *size: l* = 0.125 m, Ø = 4.6 mm;
- *stationary phase: cyanosilyl silica gel for chromatography R* (5 µm).

Mobile phase *methanol R, phosphate buffer solution pH 6.0 R1* (35:65 *V/V*).

Flow rate 1 mL/min.

Detection Spectrophotometer at 240 nm.

Injection 10 µL.

Run time 2.5 times the retention time of noscapine.

Relative retention With reference to noscapine (retention time = about 10 min): impurity A = about 1.3.

System suitability Reference solution (c):
- *resolution*: minimum 2 between the peaks due to noscapine and impurity A.

Limits:
- *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *any other impurity*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *sum of impurities other than A*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

2.5 per cent to 6.5 per cent, determined on 0.200 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

In order to avoid over heating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.

Dissolve 0.400 g in a mixture of 3 mL of *anhydrous formic acid R* and 30 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

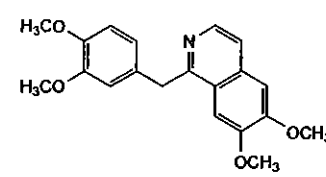
1 mL of *0.1 M perchloric acid* is equivalent to 44.99 mg of $C_{22}H_{24}ClNO_7$.

STORAGE

In an airtight container, protected from light.

IMPURITIES

Specified impurities *A.*

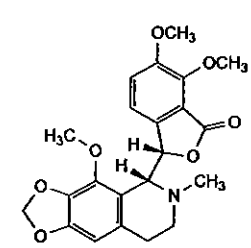


A. 1-[(3,4-dimethoxyphenyl)methyl]-6,7-dimethoxyisoquinoline (papaverine).

Ph Eur

Noscapine

(Ph. Eur. monograph 0516)



$C_{22}H_{23}NO_7$ 413.4 *128-62-1*

Action and use

Opioid receptor agonist; cough suppressant.

Ph Eur

DEFINITION

(3*S*)-6,7-Dimethoxy-3-[(5*R*)-4-methoxy-6-methyl-5,6,7,8-tetrahydro-1,3-dioxolo[4,5-*g*]isoquinolin-5-yl]isobenzofuran-1(3*H*)-one.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Practically insoluble in water, soluble in acetone, slightly soluble in ethanol (96 per cent). It dissolves in strong acids; on dilution of the acid solutions with water, the base may be precipitated.

IDENTIFICATION

First identification: C, E.

Second identification: A, B, D, E.

A. Specific optical rotation (see Tests).

B. Melting point (*2.2.14*): 174 °C to 177 °C.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *noscapine CRS.*

D. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 25 mg of the substance to be examined in *acetone R* and dilute to 100 mL with the same solvent.

Reference solution Dissolve 25 mg of *noscapine CRS* in *acetone R* and dilute to 100 mL with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *concentrated ammonia R, ethanol (96 per cent) R, acetone R, toluene R* (1:3:20:20 *V/V/V/V*).

Application 10 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *dilute potassium iodobismuthate solution R.*

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

E. To 20 mg add 10 mL of *water R* and shake. It does not dissolve.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Dissolve 0.2 g in *acetone R* and dilute to 10 mL with the same solvent. Examine immediately after dissolution.

Specific optical rotation (*2.2.7*)

+ 42 to + 48 (dried substance).

Dissolve 0.500 g in *0.1 M hydrochloric acid* and dilute to 25.0 mL with the same acid.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in 8 mL of *methanol R*, with the aid of ultrasound, and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 20.0 mL with *methanol R*. Dilute 1.0 mL of this solution to 10.0 mL with *methanol R*.

Reference solution (b) Dissolve 5 mg of *papaverine hydrochloride R* in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 10.0 mL with the mobile phase.

Reference solution (c) Dissolve 1.5 mg of *papaverine hydrochloride R* in 10 mL of the test solution and dilute to 25 mL with the mobile phase.

Column:

— *size: l* = 0.125 m, Ø = 4.6 mm;

— *stationary phase: cyanosilyl silica gel for chromatography R* (5 µm).

Mobile phase *methanol R1, phosphate buffer solution pH 6.0 R1* (350:650 *V/V*).

Flow rate 1 mL/min.

Detection Spectrophotometer at 240 nm.

Injection 10 µL.

Run time 2.5 times the retention time of noscapine.

Relative retention With reference to noscapine (retention time = about 10 min): impurity A = about 1.3.

System suitability Reference solution (c):

— *resolution*: minimum 2.0 between the peaks due to noscapine and impurity A.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *any other impurity*: for each impurity, not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *sum of impurities other than A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 0.500 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

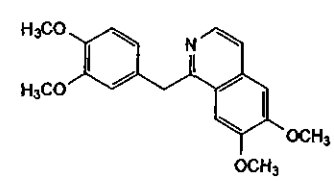
Dissolve 0.350 g in 40 mL of *anhydrous acetic acid R*, warming gently. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 41.34 mg of $C_{22}H_{23}NO_7$.

STORAGE

Protected from light.

IMPURITIES

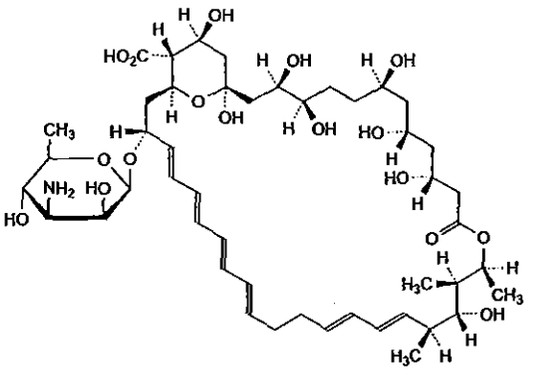


A. 1-(3,4-dimethoxybenzyl)-6,7-dimethoxyisoquinoline (papaverine).

Ph Eur

Nystatin

(Ph. Eur. monograph 0517)



$C_{47}H_{75}NO_{17}$ 926

Action and use
Antifungal.

Preparations
Nystatin Ointment
Nystatin Oral Suspension
Nystatin Vaginal Tablets
Nystatin Tablets

Ph Eur

DEFINITION

Antifungal substance obtained by fermentation using certain strains of *Streptomyces noursei* as the production micro-organism. It contains mainly tetraenes, the principal component being (1*S*,3*R*,4*R*,7*R*,9*R*,11*R*,15*S*,16*R*,17*R*,18*S*,19*E*,21*E*,25*E*,27*E*,29*E*,31*E*,33*R*,35*S*,36*R*,37*S*)-33-[(3-amino-3,6-dideoxy-β-D-mannopyranosyl)oxy]-1,3,4,7,9,11,17,37-octahydroxy-15,16,18-trimethyl-13-oxo-14,39-dioxabicyclo[33.3.1]nonatriaconta-19,21,25,27,29,31-hexaene-36-carboxylic acid (nystatin A1).

Content
Minimum 4400 IU/mg (dried substance) and minimum 5000 IU/mg (dried substance) if intended for oral administration.

CHARACTERS

Appearance
Yellow or slightly brownish powder, hygroscopic.

Solubility
Practically insoluble in water, freely soluble in dimethylformamide and in dimethyl sulfoxide, slightly soluble in methanol, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

First identification: B, E.

Second identification: A, C, D.

A. Examine the solution prepared in the test for absorbance between 220 nm and 350 nm (*2.2.25*). The solution shows 4 absorption maxima at 230 nm, 291 nm, 305 nm and 319 nm, and a shoulder at 280 nm. The ratios of the absorbances at the absorption maxima at 291 nm and 319 nm to the absorbance at the absorption maximum at 305 nm are 0.61 to 0.73 and 0.83 to 0.96, respectively. The ratio of the absorbance measured at the absorption maximum at 230 nm to that measured at the shoulder at 280 nm is 0.83 to 1.25.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *nystatin CRS.*

C. To about 2 mg add 0.1 mL of *hydrochloric acid R*. A brown colour develops.

D. To about 2 mg add 0.1 mL of *sulfuric acid R*. A brown colour develops that becomes violet on standing.

E. Examine the chromatograms obtained in the test for composition.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Absorbance (*2.2.25*)
Dissolve 0.10 g in a mixture of 5.0 mL of *glacial acetic acid R* and 50 mL of *methanol R* and dilute to 100.0 mL with *methanol R*. Dilute 1.0 mL of the solution to 100.0 mL with *methanol R*. Determined at the maximum at 305 nm within 30 min of preparation of the solution, the absorbance is not less than 0.60.

Composition
Liquid chromatography (*2.2.29*): use the normalisation procedure. *Carry out the test protected from light.*

Test solution Dissolve 20 mg of the substance to be examined in *dimethyl sulfoxide R* and dilute to 50 mL with the same solvent.

Reference solution (a) Dissolve 20 mg of *nystatin CRS* in *dimethyl sulfoxide R* and dilute to 50 mL with the same solvent.

Reference solution (b) Dissolve 20 mg of the substance to be examined in 25 mL of *methanol R* and dilute to 50 mL with *water R*. To 10.0 mL of the solution add 2.0 mL of *dilute hydrochloric acid R*. Allow to stand at room temperature for 1 h.

Reference solution (c) Dilute 1.0 mL of reference solution (a) to 100.0 mL with *dimethyl sulfoxide R*. Dilute 1.0 mL of this solution to 10.0 mL with *dimethyl sulfoxide R*.

Column:
— *size: l* = 0.15 m, Ø = 4.6 mm,
— *stationary phase: base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm),
— *temperature*: 30 °C.

Mobile phase:
— *mobile phase A: acetonitrile R*, 3.85 g/L solution of *ammonium acetate R* (29:71 *V/V*),
— *mobile phase B*: 3.85 g/L solution of *ammonium acetate R*, *acetonitrile R* (40:60 *V/V*),

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 25	100	0
25 - 35	100 → 0	0 → 100
35 - 45	0	100
45 - 50	0 → 100	100 → 0

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 305 nm.

Injection 20 µL

Retention time Nystatin A1 = about 14 min.

System suitability Reference solution (b):
— *resolution:* minimum 3.5 between the 2 principal peaks (retention time = about 13 min and 19 min).

Composition:
— *nystatin A1*: minimum 85.0 per cent,
— *any other compound*: maximum 4.0 per cent,
— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c); disregard any peak with a retention time of less than 2 min.

Loss on drying (*2.2.32*)
Maximum 5.0 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C at a pressure not exceeding 0.1 kPa for 3 h.

Sulfated ash (*2.4.14*)
Maximum 3.5 per cent, determined on 1.0 g.

ASSAY
Carry out the microbiological assay of antibiotics (*2.7.2*).
Protect the solutions from light throughout the assay.
Dissolve the substance to be examined and *nystatin CRS* separately in *dimethylformamide R* and dilute with a mixture of 5 volumes of *dimethylformamide R* and 95 volumes of buffer solution pH 6.0.

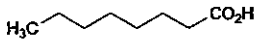
STORAGE
In an airtight container, protected from light.

LABELLING
The label states where applicable, that the substance is only for cutaneous use.

Ph Eur

Octanoic Acid

(Caprylic Acid, Ph. Eur. monograph 1401)



$C_8H_{16}O_2$ 144.2 *124-07-2*

Action and use
Excipient.

Ph Eur

DEFINITION
Octanoic acid.

Content
99.0 per cent to 100.5 per cent (anhydrous substance).

CHARACTERS
Appearance
Clear, colourless or slightly yellowish, oily liquid.

Solubility
Very slightly soluble in water, very soluble in acetone and in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION
A. Relative density (see Tests).

B. Examine the chromatograms obtained in the test for related substances.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS
Appearance
The substance to be examined is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5 (*2.2.2, Method II*).

Relative density (*2.2.5*)
0.909 to 0.912.

Related substances
Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution Dissolve 0.10 g of the substance to be examined in *ethyl acetate R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dissolve 0.10 g of *caprylic acid CRS* in *ethyl acetate R* and dilute to 10.0 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *ethyl acetate R*. Dilute 5.0 mL of this solution to 50.0 mL with *ethyl acetate R*.

Column:
— *material*: fused silica;
— *size*: *l* = 30 m, Ø = 0.25 mm;
— *stationary phase*: *macrogol 20 000 2-nitroterephthalate R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.5 mL/min.

Split ratio 1:100.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 1	100
	1 - 25	100 → 220
	25 - 35	220
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 1 µL.

System suitability Reference solution (b):
— *signal-to-noise ratio*: minimum 5 for the principal peak.

Limits:
— *any impurity*: for each impurity, maximum 0.3 per cent;
— *total*: maximum 0.5 per cent;
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

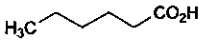
Water (*2.5.12*)
Maximum 0.7 per cent, determined on 1.000 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

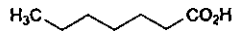
ASSAY
Dissolve 0.125 g in 25 mL of *ethanol (96 per cent) R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M sodium hydroxide* is equivalent to 14.42 mg of $C_8H_{16}O_2$.

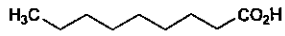
IMPURITIES



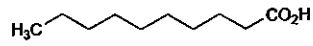
A. hexanoic acid,



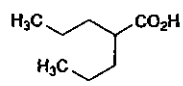
B. heptanoic acid,



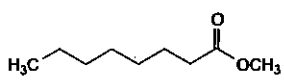
C. nonanoic acid,



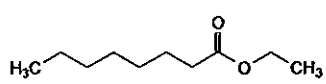
D. decanoic acid,



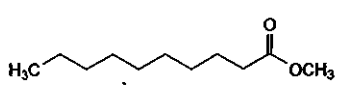
E. 2-propylpentanoic acid (valproic acid),



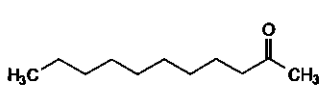
F. methyl octanoate,



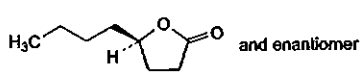
G. ethyl octanoate,



H. methyl decanoate,



I. undecan-2-one,



J. 5-butyltetrahydrofuran-2-one (γ-hydroxyoctanoic acid lactone).

Ph Eur

Octoxinol 10

(Ph. Eur. monograph 1553)

Action and use
Excipient.

Ph Eur

DEFINITION
α-[4-(1,1,3,3-Tetramethylbutyl)phenyl]-ω-hydroxydeca(oxyethylene).

Mixture consisting mainly of mono-octylphenyl ethers of macrogols corresponding to the formula $C_8H_{17}C_6H_4$-$[OCH_2\text{-}CH_2]_n$-OH where the average value of n is 10. It may contain free macrogols.

CHARACTERS

Appearance
Clear, colourless or light yellow, viscous liquid.

Solubility
Miscible with water, with ethanol (96 per cent) and with vegetable oils.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *octoxinol 10 CRS.*
Preparation Film between *sodium chloride R* plates.
B. Cloud point (see Tests).

TESTS

Acidity or alkalinity
Boil 1.0 g with 20 mL of *carbon dioxide-free water R* for 1 min, with constant stirring. Cool and filter. To 10 mL of the filtrate, add 0.05 mL of *bromothymol blue solution R1*. Not more than 0.5 mL of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Hydroxyl value (*2.5.3, Method A*)
85 to 101.

Cloud point
63 °C to 70 °C.

Dissolve 1.0 g in 99 g of *water R*. Transfer about 30 mL of this solution to a test-tube, heat on a water-bath and stir continuously until the solution becomes cloudy. Remove the test-tube from the water-bath (ensuring that the temperature does not increase more than 2 °C), and continue to stir. The cloud point is the temperature at which the solution becomes sufficiently clear that the entire thermometer bulb is plainly seen.

Ethylene oxide and dioxan (*2.4.25*)
Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Water (*2.5.12*)
Maximum 0.5 per cent, determined on 2.00 g.

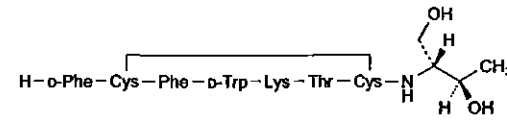
Total ash (*2.4.16*)
Maximum 0.4 per cent, determined on 1.0 g.

STORAGE
In an airtight container.

Ph Eur

Octreotide

(Ph. Eur. monograph 2414)



$C_{49}H_{66}N_{10}O_{10}S_2$ 1019 *83150-76-9*

Action and use
Somatostatin analogue; treatment of neuroendocrine tumours and acromegaly.

Ph Eur

DEFINITION
D-Phenylalanyl-L-cysteinyl-L-phenylalanyl-D-tryptophyl-L-lysyl-L-threonyl-L-cysteinyl-L-threoninol cyclic (2→7)-disulfide.

Synthetic octapeptide analogue of the natural hormone somatostatin. It is available as an acetate.

Content
95.0 per cent to 103.0 per cent (anhydrous and acetic acid-free substance).

CHARACTERS

Appearance
White or almost white powder, hygroscopic.

Solubility
Freely soluble in water, in acetic acid and in methanol.

IDENTIFICATION

Carry out either tests A, B or tests A, C.

A. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with the reference solution.

B. Nuclear magnetic resonance spectrometry (*2.2.64*).

Preparation 2 mg/mL solution in a mixture of 10 volumes of *deuterated acetic acid R* and 90 volumes of *deuterium oxide R* containing 30 µg/mL of *deuterated sodium trimethylsilylpropionate R*.

Comparison 2 mg/mL solution of *octreotide for NMR identification CRS* in a mixture of 10 volumes of *deuterated acetic acid R* and 90 volumes of *deuterium oxide R* containing 30 µg/mL of *deuterated sodium trimethylsilylpropionate R*.

Operating conditions:
- *field strength*: minimum 300 MHz;
- *temperature*: 25 °C.

Results Examine the ^{1}H NMR spectrum from 0 to 8 ppm. The ^{1}H NMR spectrum obtained is qualitatively similar to the ^{1}H NMR spectrum obtained with *octreotide for NMR identification CRS*.

C. Amino acid analysis (*2.2.56*). Method 1 for hydrolysis and method 1 for analysis are suitable.

Express the content of each amino acid in moles. Calculate the relative proportions of the amino acids taking 1/4 of the sum of the number of moles of phenylalanine, threonine and lysine as equal to 1. The values fall within the following limits: threonine: 0.7 to 1.1; threoninol: 0.7 to 1.2; lysine: 0.9 to 1.3; half-cystine: 1.0 to 2.2; phenylalanine: 1.8 to 2.2. Not more than traces of other amino acids are present.

TESTS

Specific optical rotation (*2.2.7*)
−18.5 to −14.5 (anhydrous and acetic acid-free substance).

Dissolve the substance to be examined in a 1 per cent *V/V* solution of *glacial acetic acid R* to obtain a concentration of 2.0 mg/mL.

Related substances
Liquid chromatography (*2.2.29*): use the normalisation procedure.

Solvent mixture Mix 10 volumes of *acetonitrile R* and 90 volumes of *water R*, then adjust to pH 3.5 with *acetic acid R*.

Test solution Dissolve 10.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution Dissolve the contents of a vial of *octreotide CRS* in the solvent mixture to obtain a concentration of 1.0 mg/ml.

Resolution solution Dissolve the contents of a vial of *octreotide impurity mixture CRS* (containing impurities F and G) in 1.0 mL of the reference solution.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography compatible with 100 per cent aqueous mobile phases R* (5 µm);
- *temperature*: 40 °C.

Mobile phase:
- *mobile phase A*: dissolve 4.5 g of *tetramethylammonium hydroxide R* in 800 mL of *water for chromatography R* and adjust to pH 2.0 with *phosphoric acid R*; dilute to 900 mL with *water for chromatography R* and add 100 mL of *acetonitrile R1*;
- *mobile phase B*: dissolve 4.5 g of *tetramethylammonium hydroxide R* in 300 mL of *water for chromatography R* and adjust to pH 2.0 with *phosphoric acid R*; dilute to 400 mL with *water for chromatography R* and add 600 mL of *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	90 → 80	10 → 20
15 - 40	80 → 55	20 → 45
40 - 45	55 → 30	45 → 70

Flow rate 1.8 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram supplied with *octreotide impurity mixture CRS* and the chromatogram obtained with the resolution solution to identify the peaks due to impurities F and G.

Relative retention With reference to octreotide (retention time = about 20 min): impurity A = about 0.76; impurity B = about 0.89; impurity C = about 0.94; impurity E = about 1.13; impurity F = about 1.30; impurity G = about 1.33; impurity H = about 1.66; impurity I = about 1.88.

System suitability Resolution solution:
- *resolution*: minimum 2.0 between the peaks due to impurities F and G.

Limits:
- *unspecified impurities*: for each impurity, maximum 0.5 per cent;
- *total*: maximum 2.0 per cent;
- *reporting threshold*: 0.1 per cent.

Acetic acid (*2.5.34*)
5.0 per cent to 12.8 per cent.

Test solution Dissolve 10.0 mg of the substance to be examined in a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A and dilute to 10.0 mL with the same mixture of mobile phases.

Water (*2.5.32*)
Maximum 10.0 per cent, determined on 20.0 mg.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution.

Calculate the percentage content of octreotide ($C_{49}H_{66}N_{10}O_{10}S_2$) taking into account the assigned content of $C_{49}H_{66}N_{10}O_{10}S_2$ in *octreotide CRS*.

STORAGE

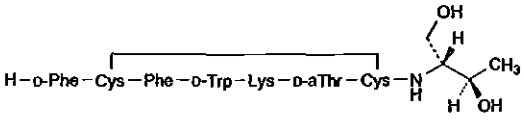
In an airtight container, protected from light, at a temperature of 2 °C to 8 °C.

LABELLING

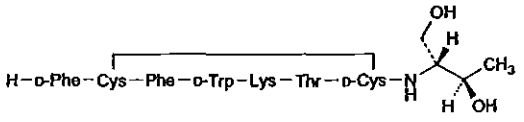
The label states the octreotide content ($C_{49}H_{66}N_{10}O_{10}S_2$).

IMPURITIES

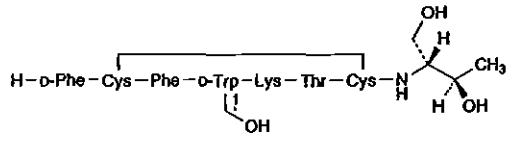
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, E, F, G, H, I.*



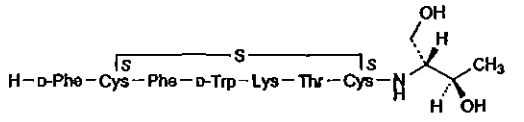
A. [6-D-allothreonine]octreotide,



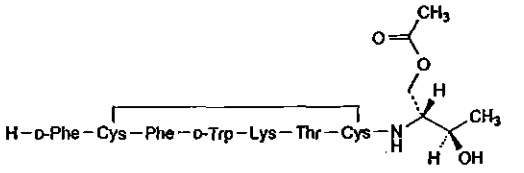
B. [7-D-cysteine]octreotide,



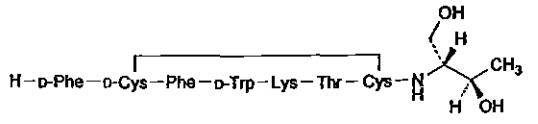
C. $N^{1.4}$-(hydroxymethyl)octreotide,



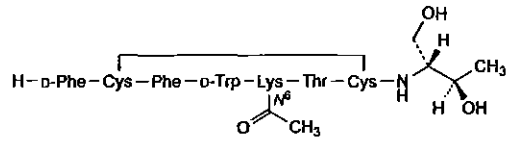
E. D-phenylalanyl-*S*-sulfanyl-L-cysteinyl-L-phenylalanyl-D-tryptophyl-L-lysyl-L-threonyl-L-cysteinyl-L-threoninol cyclic (2 → 7)-trisulfide,



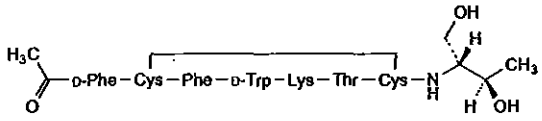
F. $O^{1.8}$-acetyloctreotide,



G. [2-D-cysteine]octreotide,



H. $N^{6.5}$-acetyloctreotide,

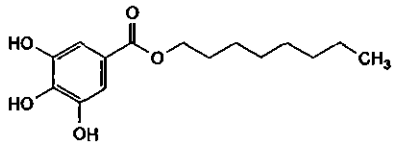


I. $N^{2.1}$-acetyloctreotide.

Ph Eur

Octyl Gallate

(Ph. Eur. monograph 2057)



$C_{15}H_{22}O_5$ 282.3 *1034-01-1*

Action and use

Used in treatment of alcohol dependence.

Ph Eur

DEFINITION

Octyl 3,4,5-trihydroxybenzoate.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

A. Melting point (*2.2.14*).

Determine the melting point of the substance to be examined. Mix equal parts of the substance to be examined and *octyl gallate CRS* and determine the melting point of the mixture. The difference between the melting points (which are about 101 °C) is not greater than 2 °C.

B. Examine the chromatograms obtained in the test for impurity A.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Impurity A

Thin-layer chromatography (*2.2.27*).

Test solution (a) Dissolve 0.20 g of the substance to be examined in *acetone R* and dilute to 10 mL with the same solvent.

Test solution (b) Dilute 1.0 mL of test solution (a) to 20 mL with *acetone R*.

Reference solution (a) Dissolve 10 mg of *octyl gallate CRS* in *acetone R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 20 mg of *gallic acid R* in *acetone R* and dilute to 20 mL with the same solvent.

Reference solution (c) Dilute 1.0 mL of reference solution (b) to 10 mL with *acetone R*.

Reference solution (d) Dilute 1.0 mL of reference solution (b) to 5 mL with test solution (a).

Plate *TLC silica gel plate R*.

Mobile phase *anhydrous formic acid R, ethyl formate R, toluene R* (10:40:50 *V/V/V*).

Application 5 µL of test solutions (a) and (b) and reference solutions (a), (c) and (d).

Development Over 2/3 of the plate.

Drying In air for 10 min.

Detection Spray with a mixture of 1 volume of *ferric chloride solution R1* and 9 volumes of *ethanol (96 per cent) R*.

System suitability Reference solution (d):
— the chromatogram shows 2 clearly separated principal spots.

Limit Test solution (a):
— *impurity A*: any spot due to impurity A is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.5 per cent).

Chlorides (*2.4.4*)
Maximum 100 ppm.

To 1.65 g add 50 mL of *water R*. Shake for 5 min. Filter. 15 mL of the filtrate complies with the test.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 70 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in *methanol R* and dilute to 250.0 mL with the same solvent. Dilute 5.0 mL of the solution to 200.0 mL with *methanol R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 275 nm.

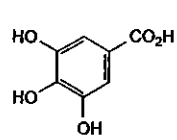
Calculate the content of $C_{15}H_{22}O_5$ taking the specific absorbance to be 387.

STORAGE

In a non-metallic container, protected from light.

IMPURITIES

Specified impurities *A*.

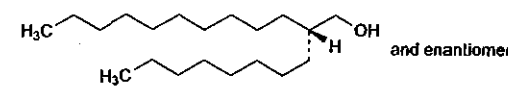


A. 3,4,5-trihydroxybenzoic acid (gallic acid).

Ph Eur

Octyldodecanol

(Ph. Eur. monograph 1136)



5333-42-6

Action and use
Excipient.

Ph Eur

DEFINITION

Condensation product of saturated liquid fatty alcohols.

Content
Minimum 90 per cent of (2*RS*)-2-octyldodecan-1-ol ($C_{20}H_{42}O$; M_r 298.6), the remainder consisting mainly of related alcohols.

CHARACTERS

Appearance
Clear, colourless or yellowish, oily liquid.

Solubility
Practically insoluble in water, miscible with ethanol (96 per cent).

Relative density
About 0.840.

Refractive index
About 1.455.

IDENTIFICATION

A. Hydroxyl value (see Tests).

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.20 g of the substance to be examined in *toluene R* and dilute to 20 mL with the same solvent.

Reference solution Dissolve 0.20 g of *octyldodecanol CRS* in *toluene R* and dilute to 20 mL with the same solvent.

Plate Suitable silica gel plate.

Mobile phase *ethyl acetate R, toluene R* (5:95 *V/V*).

Application 2 µL.

Development Over a path of 12 cm.

Drying In air.

Detection Spray with about 7 mL of a mixture of 1 volume of a 25 g/L solution of *vanillin R* in *ethanol (96 per cent) R* and 4 volumes of *sulfuric acid R* and heat at 130 °C for 5-10 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Acidity or alkalinity
Mix 5.0 g thoroughly for 1 min with a mixture of 0.1 mL of *bromothymol blue solution R1*, 2 mL of *heptane R* and 10 mL of *water R*. If the aqueous layer is blue, not more than 0.15 mL of *0.01 M hydrochloric acid* is required to change the colour of the indicator to yellow. If the aqueous layer is yellow, add 0.45 mL of *0.01 M sodium hydroxide* and shake vigorously. After standing to ensure complete separation, the aqueous layer is blue.

Optical rotation (*2.2.7*)
−0.10° to + 0.10°.

Dissolve 2.50 g in *ethanol (96 per cent) R* and dilute to 25 mL with the same solvent.

Hydroxyl value (*2.5.3, Method A*)
175 to 190.

Iodine value (*2.5.4, Method A*)
Maximum 8.0.

Peroxide value (*2.5.5, Method A*)
Maximum 5.0.

Saponification value (*2.5.6*)
Maximum 5.0.

Water (*2.5.12*)
Maximum 0.5 per cent, determined on 2.00 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Gas chromatography (*2.2.28*).

Internal standard solution Dissolve 0.4 g of *tetradecane R* in *hexane R* and dilute to 100.0 mL with the same solvent.

Test solution Dissolve 0.100 g of the substance to be examined in the internal standard solution and dilute to 10.0 mL with the same solution.

Reference solution Dissolve 0.100 g of *octyldodecanol CRS* in the internal standard solution and dilute to 10.0 mL with the same solution.

Column:
— *material*: fused silica;
— *size*: *l* = 60 m, Ø = 0.25 mm;
— *stationary phase*: *phenyl(5)methyl(95)polysiloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R.*

Flow rate 0.68 mL/min.

Split ratio 1:50.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 2	180
	2 - 22	180 → 280
	22 - 52	280
Injection port		290
Detector		300

Detection Flame ionisation.

Injection 1 µL.

Calculate the content of $C_{20}H_{42}O$ in the substance to be examined.

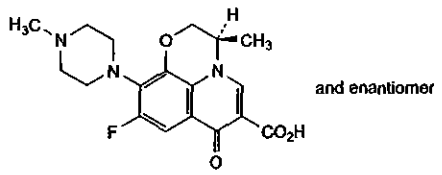
STORAGE

Protected from light.

Ph Eur

Ofloxacin

(Ph. Eur. monograph 1455)



$C_{18}H_{20}FN_3O_4$ 361.4 *82419-36-1*

Action and use
Fluoroquinolone antibacterial.

Ph Eur

DEFINITION

(3*RS*)-9-Fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
Pale yellow or bright yellow, crystalline powder.

Solubility
Slightly soluble in water, soluble in glacial acetic acid, soluble or slightly soluble in methylene chloride, slightly soluble in methanol.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *ofloxacin CRS.*

B. Optical rotation (see Tests).

TESTS

Optical rotation (*2.2.7*)
−0.10° to + 0.10°.

Dissolve 0.300 g in a mixture of 10 volumes of *methanol R* and 40 volumes of *methylene chloride R* and dilute to 10.0 mL with the same mixture of solvents.

Absorbance (*2.2.25*)
Maximum 0.25 at 440 nm.

Dissolve 0.5 g in a 10.3 g/L solution of *hydrochloric acid R* and dilute to 100.0 mL with the same solution.

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R, water R* (10:60 *V/V*).

Buffer solution Dissolve 3.08 g of *ammonium acetate R* and 5.38 g of *sodium perchlorate R* in about 900 mL of *water for chromatography R*. Adjust to pH 2.2 with *phosphoric acid R* and dilute to 1000 mL with *water for chromatography R.*

Test solution Dissolve 20.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 5.0 mg of *levofloxacin impurity F CRS* (ofloxacin impurity A; *S*-enantiomer) in 42 mL of *acetonitrile R* and dilute to 250.0 mL with *water R*. Dilute 1.0 mL of this solution to 25.0 mL with the solvent mixture.

Reference solution (c) Dissolve 5 mg of *ofloxacin impurity D CRS* and 5 mg of *ofloxacin impurity E CRS* in the solvent mixture and dilute to 25 mL with the solvent mixture. Mix 2 mL of the solution and 1 mL of the test solution and dilute to 50 mL with the solvent mixture. Dilute 1 mL of this solution to 10 mL with the solvent mixture.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm);
- *temperature*: 38 °C.

Mobile phase:
- *mobile phase A*: *acetonitrile for chromatography R*, buffer solution (16:84 *V/V*);
- *mobile phase B*: *methanol R1*, *acetonitrile for chromatography R*, buffer solution (20:30:50 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	100	0
5 - 10	100 → 82	0 → 18
10 - 15	82 → 40	18 → 60
15 - 30	40	60

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 294 nm and, for impurity A, at 240 nm.

Injection 10 µL.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity A; use the chromatogram obtained with reference solution (c) to identify the peaks due to impurities D and E.

Relative retention With reference to ofloxacin (retention time = about 10 min): impurity D = about 0.7; impurity E = about 0.93; impurity A = about 2.8.

System suitability:
- *resolution*: minimum 2.0 between the peaks due to impurity E and ofloxacin in the chromatogram obtained with reference solution (c);
- *signal-to-noise ratio*: minimum 90 for the principal peak in the chromatogram obtained with reference solution (a).

Calculation of percentage contents:
- *correction factor*: multiply the peak area of impurity D by 4.5;
- for impurity A, use the concentration of impurity A in reference solution (b) and the peak areas recorded at 240 nm;
- for impurities other than A, use the concentration of ofloxacin in reference solution (a) and the peak areas recorded at 294 nm.

Limits:
- *impurity A*: maximum 0.2 per cent;
- *impurity D*: maximum 0.10 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.4 per cent;
- *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)
Maximum 0.2 per cent, determined on 1.000 g by drying at 105 °C for 4 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

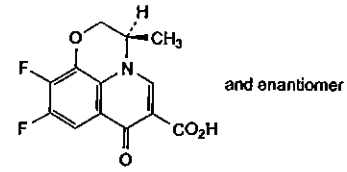
ASSAY
Dissolve 0.300 g in 100 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 36.14 mg of $C_{18}H_{20}FN_3O_4$.

STORAGE
In an airtight container, protected from light.

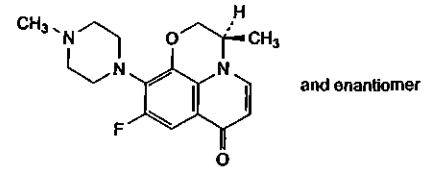
IMPURITIES
Specified impurities *A, D.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, E, F.*



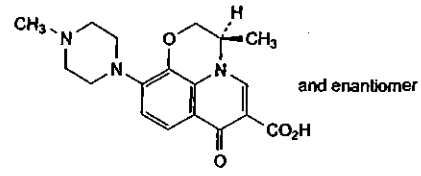
and enantiomer

A. (*3RS*)-9,10-difluoro-3-methyl-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid,



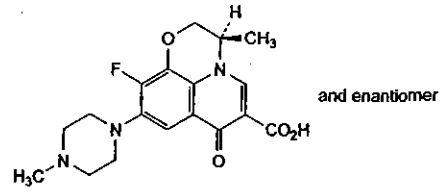
and enantiomer

B. (3*RS*)-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazin-7-one,



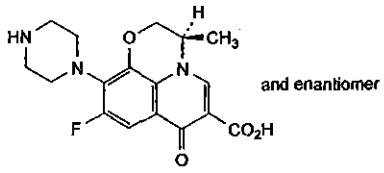
and enantiomer

C. (3*RS*)-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid,

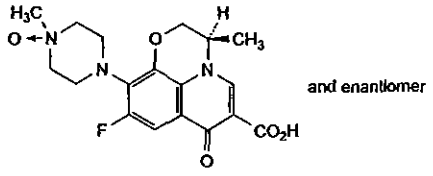


and enantiomer

D. (3*RS*)-10-fluoro-3-methyl-9-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid,



E. (3*RS*)-9-fluoro-3-methyl-7-oxo-10-(piperazin-1-yl)-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid,

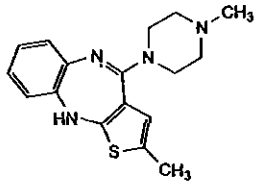


F. 4-[(3*RS*)-6-carboxy-9-fluoro-3-methyl-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazin-10-yl]-1-methylpiperazine 1-oxide.

Ph Eur

Olanzapine

(Ph. Eur. monograph 2258)



$C_{17}H_{20}N_4S$ 312.4 *132539-06-1*

Action and use
Dopamine D_2 receptor antagonist; serotonin $5HT_2$ receptor antagonist; neuroleptic.

Preparation
Olanzapine Orodispersible Tablets

Ph Eur

DEFINITION
2-Methyl-4-(4-methylpiperazin-1-yl)-10*H*-thieno[2,3-*b*][1,5]benzodiazepine.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS
Appearance
Yellow, crystalline powder.

Solubility
Practically insoluble in water, freely soluble in methylene chloride, slightly soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *olanzapine CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *ethyl acetate R*, evaporate to dryness and record new spectra using the residues.

TESTS
Related substances
Liquid chromatography (*2.2.29*). *Prepare the test and reference solutions immediately before use or keep them refrigerated and inject within 20 h of preparation.*

Solution A Dissolve 13 g of *sodium dodecyl sulfate R* in about 1450 mL of *water R*, add 5 mL of *phosphoric acid R* and adjust to pH 2.5 by slowly adding *strong sodium hydroxide solution R*. If a precipitate is formed, this precipitate has to be re-dissolved prior to final pH adjustment. Dilute to 1500 mL with *water R*.

Solvent mixture Mix 4 volumes of *acetonitrile R1* with 6 volumes of a 37 mg/L solution of *sodium edetate R* in solution A.

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 4 mg of *olanzapine for system suitability CRS* (containing impurities B, C and D) in 10.0 mL of the solvent mixture.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 35 °C.

Mobile phase:
— *mobile phase A*: *acetonitrile R1*, solution A (48:52 *V/V*);
— *mobile phase B*: solution A, *acetonitrile R1* (30:70 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	100	0
10 - 20	100 → 0	0 → 100
20 - 25	0	100

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram supplied with *olanzapine for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B, C and D.

Relative retention With reference to olanzapine (retention time = about 13 min): impurity B = about 0.3; impurity D = about 0.9; impurity C = about 1.2.

System suitability Reference solution (b):
— *resolution*: minimum 1.5 between the peaks due to impurity D and olanzapine.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity B by 0.4;
— *impurities B, C, D*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)
Maximum 1.0 per cent, determined on 0.250 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 2.0 mL of the solution to 10.0 mL with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *olanzapine CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 2.0 mL of the solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 10 mg of the substance to be examined and 1 mg of *olanzapine impurity A CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase.

Column:
— *size*: l = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 1 volume of *acetonitrile R* with 1 volume of a 6.9 g/L solution of *sodium dihydrogen phosphate monohydrate R* adjusted to pH 2.5 with *phosphoric acid R* and containing 12 g/L of *sodium dodecyl sulfate R*.

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 260 nm.

Injection 20 µL.

Run time 1.2 times the retention time of olanzapine.

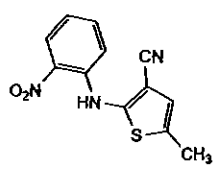
Relative retention With reference to olanzapine (retention time = about 7 min): impurity A = about 0.8.

System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the peaks due to impurity A and olanzapine.

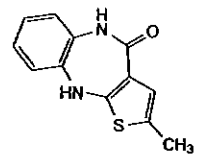
Calculate the percentage content of $C_{17}H_{20}N_4S$ using the chromatogram obtained with reference solution (a) and the declared content of *olanzapine CRS*.

IMPURITIES
Specified impurities *B, C, D.*

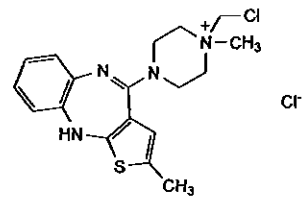
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A.*



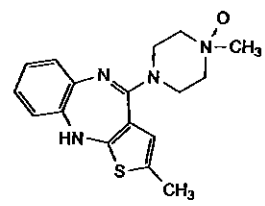
A. 5-methyl-2-[(2-nitrophenyl)amino]thiophene-3-carbonitrile,



B. 2-methyl-5,10-dihydro-4*H*-thieno[2,3-*b*][1,5]benzodiazepin-4-one,



C. 1-(chloromethyl)-1-methyl-4-(2-methyl-10*H*-thieno[2,3-*b*][1,5]benzodiazepin-4-yl)piperazin-1-ium chloride,

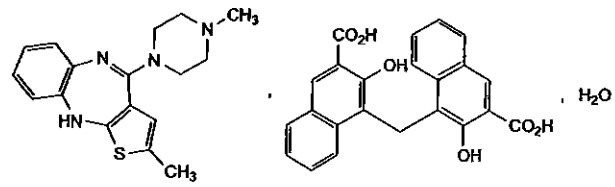


D. 1-methyl-4-(2-methyl-10*H*-thieno[2,3-*b*][1,5]benzodiazepin-4-yl)piperazin-1-oxide.

Ph Eur

Olanzapine Embonate Monohydrate

(Ph. Eur. monograph 3047)



$C_{40}H_{36}N_4O_6S,H_2O$ 719 *221373-18-8*

Action and use
Dopamine D2 receptor antagonist; serotonin 5HT2 receptorantagonist; antipsychotic.

Ph Eur

DEFINITION
2-Methyl-4-(4-methylpiperazin-1-yl)-10*H*-thieno[2,3-*b*][1,5]benzodiazepine 4,4′-methylenebis(3-hydroxynaphthalene-2-carboxylate) monohydrate.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

PRODUCTION
It is produced by methods of manufacture designed to guarantee the proper hydrate form and it complies, if tested, with a suitable test that demonstrates its monohydrate nature (e.g. X-ray powder diffraction (*2.9.33*)).

CHARACTERS

Appearance

Yellow, slightly hygroscopic powder.

Solubility

Practically insoluble in water, freely soluble in dimethyl sulfoxide, very slightly soluble in anhydrous ethanol, practically insoluble in heptane.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *olanzapine embonate monohydrate CRS.*

B. Water (see Tests).

TESTS

Related substances

Liquid chromatography (*2.2.29*). *Prepare the test and reference solutions immediately before use.*

Solution A A 1.15 g/L solution of *phosphoric acid R*, adjusted to pH 7.0 using a 330 g/L solution of *potassium hydroxide R.*

Test solution Dissolve 25.0 mg of the substance to be examined in 5.0 mL of *dimethyl sulfoxide R* and dilute to 25.0 mL with mobile phase A.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

Reference solution (b) Dissolve 5 mg of *olanzapine for system suitability CRS* (containing impurities B and D) in 1 mL of *dimethyl sulfoxide R* and dilute to 5 mL with mobile phase A.

Reference solution (c) Dissolve 5 mg of *olanzapine embonate for peak identification CRS* (containing impurities E and F) in 1 mL of *dimethyl sulfoxide R* and dilute to 5 mL with mobile phase A.

Column:
- *size: l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase: end-capped extra-dense bonded octadecylsilyl silica gel for chromatography R* (3.5 µm);
- *temperature*: 30 °C.

Mobile phase:
- *mobile phase A*: *methanol R*, solution A (40:60 *V/V*);
- *mobile phase B*: solution A, *methanol R* (30:70 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	100	0
5 - 15	100 → 0	0 → 100
15 - 19	0	100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 259 nm.

Autosampler Set at 5 °C.

Injection 10 µL.

Identification of impurities Use the chromatogram supplied with *olanzapine for system suitability CRS* and the chromatogram obained with reference solution (b) to identify the peaks due to impurities B and D; use the chromatogram supplied with *olanzapine for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to embonic acid and impurities E and F.

Relative retention With reference to olanzapine (retention time = about 18 min): embonic acid = about 0.2; impurity E = about 0.3; impurity F = about 0.4; impurity D = about 0.6; impurity B = about 0.7.

System suitability Reference solution (b):
- *resolution*: minimum 2.0 between the peaks due to impurities D and B.

Calculation of percentage contents:
- *correction factor*: multiply the peak area of impurity B by 0.7;
- for each impurity, use the concentration of olanzapine embonate monohydrate in reference solution (a).

Limits:
- *impurities B, E, F*: for each impurity, maximum 0.3 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.4 per cent;
- *reporting threshold*: 0.05 per cent; disregard the peak due to embonic acid.

Water (*2.5.32*)

2.4 per cent to 4.0 per cent, determined on 50.0 mg using the evaporation technique at 200 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*).

Solution A A 1.15 g/L solution of *phosphoric acid R*, adjusted to pH 7.0 using a 330 g/L solution of *potassium hydroxide R.*

Test solution Dissolve 25.0 mg of the substance to be examined in 10.0 mL of *dimethyl sulfoxide R* and dilute to 100.0 mL with the mobile phase.

Reference solution Dissolve 20.0 mg of *olanzapine CRS* in 20.0 mL of *dimethyl sulfoxide R* and dilute to 200.0 mL with the mobile phase.

Column:
- *size: l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase: end-capped extra-dense bonded octadecylsilyl silica gel for chromatography R* (3.5 µm);
- *temperature*: 40 °C.

Mobile phase Solution A, *methanol R* (40:60 *V/V*).

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 259 nm.

Injection 10 µL.

Run time 1.3 times the retention time of olanzapine (retention time = about 8 min).

Calculate the percentage content of $C_{40}H_{36}N_4O_6S$ taking into account the assigned content of $C_{17}H_{20}N_4S$ in *olanzapine CRS* and a conversion factor of 2.243.

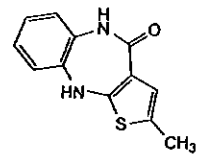
STORAGE

In an airtight container.

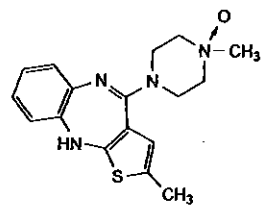
IMPURITIES

Specified impurities *B, E, F.*

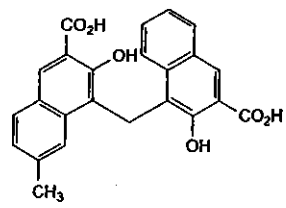
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *D.*



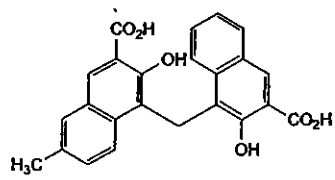
B. 2-methyl-5,10-dihydro-4*H*-thieno[2,3-*b*][1,5]benzodiazepin-4-one,



D. 1-methyl-4-(2-methyl-10*H*-thieno[2,3-*b*][1,5]benzodiazepine-4-yl)piperazine 1-oxide,



E. 4-[(3-carboxy-2-hydroxynaphthalen-1-yl)methyl]-3-hydroxy-6-methylnaphthalene-2-carboxylic acid,



F. 4-[(3-carboxy-2-hydroxynaphthalen-1-yl)methyl]-3-hydroxy-7-methylnaphthalene-2-carboxylic acid.

Ph Eur

Oleic Acid

(Ph. Eur. monograph 0799)

112-80-1

Action and use
Excipient.

Ph Eur

DEFINITION
(*Z*)-Octadec-9-enoic acid ($C_{18}H_{34}O_2$; M_r 282.5), together with varying amounts of saturated and other unsaturated fatty acids. A suitable antioxidant may be added.

Content
65.0 per cent to 88.0 per cent of $C_{18}H_{34}O_2$.

CHARACTERS
Appearance
Clear, yellowish or brownish, oily liquid.

Solubility
Practically insoluble in water, miscible with alcohol and with methylene chloride.

Relative density
About 0.892.

IDENTIFICATION
A. Acid value (see Tests).

B. Iodine value (see Tests).

C. Composition of fatty acids (see Tests).

Margaric acid Maximum 0.2 per cent for oleic acid of vegetable origin and maximum 4.0 per cent for oleic acid of animal origin.

TESTS
Appearance
The substance to be examined is not more intensely coloured than reference solution Y_1 or BY_1 (*2.2.2, Method I*).

Acid value (*2.5.1*)
195 to 204, determined on 0.5 g.

Iodine value (*2.5.4*)
89 to 105.

Peroxide value (*2.5.5*)
Maximum 10.0.

Composition of fatty acids
Gas chromatography (*2.4.22, Method C*).

Test solution Prepare as described in the method but omitting the initial hydrolysis.

Composition of the fatty acid fraction of the substance:
— *myristic acid*: maximum 5.0 per cent,
— *palmitic acid*: maximum 16.0 per cent,
— *palmitoleic acid*: maximum 8.0 per cent,
— *stearic acid*: maximum 6.0 per cent,
— *oleic acid*: 65.0 per cent to 88.0 per cent,
— *linoleic acid*: maximum 18.0 per cent,
— *linolenic acid*: maximum 4.0 per cent,
— *fatty acids of chain length greater than C_{18}*: maximum 4.0 per cent.

Total ash (*2.4.16*)
Maximum 0.1 per cent, determined on 2.00 g.

STORAGE
In an airtight, well-filled container, protected from light.

LABELLING
The label states the origin of oleic acid (animal or vegetable).

Ph Eur

Oleoyl Macrogolglycerides

(Ph. Eur. monograph 1249)

Action and use
Excipient.

Ph Eur

DEFINITION
Mixtures of monoesters, diesters and triesters of glycerol and monoesters and diesters of macrogols.

They are obtained by partial alcoholysis of an unsaturated oil mainly containing triglycerides of oleic ((9*Z*)-octadec-9-enoic) acid, using macrogol with a mean relative molecular mass between 300 and 400, or by esterification of glycerol and macrogol with unsaturated fatty acids, or by mixing glycerol esters and condensates of ethylene oxide with the fatty acids of this unsaturated oil.

CHARACTERS

Appearance
Amber oily liquid, which may give rise to a deposit after prolonged periods at 20 °C.

Solubility
Practically insoluble but dispersible in water, freely soluble in methylene chloride.

Relative density
About 0.95 at 20 °C.

Refractive index
About 1.47 at 20 °C.

Viscosity
About 35 mPa·s at 40 °C.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 1.0 g of the substance to be examined in *methylene chloride R* and dilute to 20 mL with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *hexane R, ether R* (30:70 *V/V*).

Application 10 µL.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with a 0.1 g/L solution of *rhodamine B R* in *ethanol (96 per cent) R* and examine in ultraviolet light at 365 nm.

Results The chromatogram shows a spot due to triglycerides with an R_F value of about 0.9 (R_s 1) and spots due to 1,3-diglycerides (R_s 0.7), to 1,2-diglycerides (R_s 0.6), to monoglycerides (R_s 0.1) and to esters of macrogol (R_s 0).

B. Hydroxyl value (see Tests).

C. Saponification value (see Tests).

D. Composition of fatty acids (see Tests).

TESTS

Acid value (*2.5.1*)
Maximum 2.0, determined on 2.0 g.

Hydroxyl value (*2.5.3, Method A*)
45 to 65, determined on 1.0 g.

Iodine value (*2.5.4, Method A*)
75 to 95.

Peroxide value (*2.5.5, Method A*)
Maximum 12.0, determined on 2.0 g.

Saponification value (*2.5.6*)
150 to 170, determined on 2.0 g.

Alkaline impurities
Introduce 5.0 g into a test tube and carefully add a mixture, neutralised if necessary with *0.01 M hydrochloric acid* or with *0.01 M sodium hydroxide*, of 0.05 mL of a 0.4 g/L solution of *bromophenol blue R* in *ethanol (96 per cent) R*, 0.3 mL of *water R* and 10 mL of *ethanol (96 per cent) R*. Shake and allow to stand. Not more than 1.0 mL of *0.01 M hydrochloric acid* is required to change the colour of the upper layer to yellow.

Free glycerol
Maximum 3.0 per cent.

Dissolve 1.20 g in 25.0 mL of *methylene chloride R*. Heat if necessary. After cooling, add 100 mL of *water R*. Shake and add 25.0 mL of *periodic acetic acid solution R*. Shake and allow to stand for 30 min. Add 40 mL of a 75 g/L solution of *potassium iodide R*. Allow to stand for 1 min. Add 1 mL of *starch solution R*. Titrate the iodine with *0.1 M sodium thiosulfate*. Carry out a blank titration.

1 mL of *0.1 M sodium thiosulfate* is equivalent to 2.3 mg of glycerol.

Composition of fatty acids (*2.4.22, Method A*)
Composition of the fatty-acid fraction of the substance:
— *palmitic acid*: 4.0 per cent to 9.0 per cent;
— *stearic acid*: maximum 6.0 per cent;
— *oleic acid*: 58.0 per cent to 80.0 per cent;
— *linoleic acid*: 15.0 per cent to 35.0 per cent;
— *linolenic acid*: maximum 2.0 per cent;
— *arachidic acid*: maximum 2.0 per cent;
— *eicosenoic acid*: maximum 2.0 per cent.

Ethylene oxide and dioxan (*2.4.25*)
Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Water (*2.5.12*)
Maximum 1.0 per cent, determined on 1.00 g. Use a mixture of 30 volumes of *anhydrous methanol R* and 70 volumes of *methylene chloride R* as solvent.

Total ash (*2.4.16*)
Maximum 0.1 per cent.

STORAGE

Protected from light.

LABELLING

The label states the type of macrogol used (mean relative molecular mass) or the number of units of ethylene oxide per molecule (nominal value).

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for oleoyl macrogolglycerides used as self-emulsifying agents and solubilisers.

Hydroxyl value
(see Tests).

Saponification value
(see Tests).

Composition of fatty acids
(see Tests).

Oleyl Alcohol

(Ph. Eur. monograph 2073)

Action and use
Nonionic surfactant.

Ph Eur

DEFINITION
Mixture of unsaturated and saturated long-chain fatty alcohols consisting mainly of octadec-9-enol (oleyl alcohol and elaidyl alcohol; $C_{18}H_{36}O$; M_r 268.5). It may be of vegetable or animal origin.

CHARACTERS
Appearance
Colourless or light yellow liquid.

IDENTIFICATION
A. Hydroxyl value (see Tests).
B. Composition of fatty alcohols (see Tests).

TESTS
Appearance
The substance to be examined is clear (*2.2.1*) and not more intensely coloured than reference solution B_6 (*2.2.2, Method II*).

Refractive index (*2.2.6*)
1.458 to 1.461, determined at 25 °C.

Cloud point
Maximum 10 °C.

Introduce about 60 g into a cylindrical flat-bottomed container, 30-33.5 mm in internal diameter and 115-125 mm high. Heat to 30 °C, cool, and immerse the container in iced water with the surfaces of the water and the sample at the same level. Insert a thermometer and, using it as a stirring rod, begin stirring rapidly and steadily when the temperature falls below 20 °C. Keep the thermometer immersed throughout the test, and remove and examine the container at regular intervals. The cloud point is the temperature at which the immersed portion of the thermometer, positioned vertically in the centre of the container, is no longer visible when viewed horizontally through the container and sample.

Acid value (*2.5.1*)
Maximum 1.0, determined on 5.0 g.

Hydroxyl value (*2.5.3, Method A*)
205 to 215.

Saponification value (*2.5.6*)
Maximum 2.0.

Composition of fatty alcohols
Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution Mix 25 mg of the substance to be examined with 1.0 mL of *methylene chloride R*.

Reference solution (a) Dissolve 25 mg of each of *arachidyl alcohol R, linolenyl alcohol R, linoleyl alcohol R, oleyl alcohol R, palmityl alcohol R* and *stearyl alcohol R* in *methylene chloride R* and dilute to 5 mL with the same solvent. Dilute 1 mL of this solution to 5 mL with *methylene chloride R*.

Reference solution (b) Dissolve 10 mg of *linoleyl alcohol R* and 1 g of *oleyl alcohol R* in *methylene chloride R* and dilute to 40 mL with the same solvent.

Column:
— *material:* fused silica;
— *size:* *l* = 30 m, Ø = 0.32 mm;
— *stationary phase: methylpolysiloxane R* (film thickness 1 µm).

Carrier gas *helium for chromatography R.*
Flow rate 1 mL/min.
Split ratio 1:11.
Temperature:

	Time (min)	Temperature (°C)
Column	0 - 1	170
	1 - 9	170 → 210
	9 - 65	210
Injection port		270
Detector		280

Detection Flame ionisation.
Injection 1 µL.

Identify the peaks using the chromatogram obtained with reference solution (a).

Relative retention With reference to oleyl alcohol (retention time = about 30 min): palmityl alcohol = about 0.6; linolenyl alcohol = about 0.8; linoleyl alcohol = about 0.9; stearyl alcohol = about 1.1; arachidyl alcohol = about 1.9 (elaidyl alcohol co-elutes with oleyl alcohol).

System suitability Reference solution (b):
— *peak-to-valley ratio:* minimum 1.2, where H_p = height above the baseline of the peak due to linoleyl alcohol and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to oleyl alcohol.

Limits:
— *palmityl alcohol:* maximum 8.0 per cent;
— *stearyl alcohol:* maximum 5.0 per cent;
— *oleyl alcohol* (sum of oleyl and elaidyl alcohols): minimum 80.0 per cent;
— *linoleyl alcohol:* maximum 3.0 per cent;
— *linolenyl alcohol:* maximum 0.5 per cent;
— *arachidyl alcohol:* maximum 0.3 per cent.

Ph Eur

Virgin Olive Oil

Olive Oil

(Ph. Eur. monograph 0518)

Preparation
Olive Oil Ear Drops

Ph Eur

DEFINITION
Fatty oil obtained by cold expression or other suitable mechanical means from the ripe drupes of *Olea europaea* L.

CHARACTERS
Appearance
Clear, transparent, yellow or greenish-yellow liquid.

Solubility
Practically insoluble in ethanol (96 per cent), miscible with light petroleum (bp: 50-70 °C).

When cooled, it begins to become cloudy at 10 °C and becomes a butter-like mass at about 0 °C.

Relative density
About 0.913.

IDENTIFICATION

First identification: B.

Second identification: A.

A. Identification of fatty oils by thin-layer chromatography (*2.3.2*).

Results The chromatogram obtained is similar to the corresponding chromatogram shown in Figure 2.3.2.-1. For certain types of olive oil, the difference in the size of spots E and F is less pronounced than in the corresponding chromatogram shown in Figure 2.3.2.-1.

B. Composition of fatty acids (see Tests).

TESTS

Absorbance (*2.2.25*)

Maximum 0.20 at 270 nm. The ratio of the absorbance at 232 nm to that at 270 nm is greater than 8.

To 1.00 g add *cyclohexane R* and dilute to 100.0 mL with the same solvent.

Acid value (*2.5.1*)

Maximum 2.0, determined on 5.0 g.

Peroxide value (*2.5.5, Method A*)

Maximum 20.0.

Unsaponifiable matter

Maximum 1.5 per cent.

Place 5.0 g (*m* g) in a 150 mL flask fitted with a reflux condenser. Add 50 mL of *2 M alcoholic potassium hydroxide R* and heat on a water-bath for 1 h, shaking frequently. Add 50 mL of *water R* through the top of the condenser, shake, allow to cool and transfer the contents of the flask to a separating funnel. Rinse the flask with several portions totalling 50 mL of *light petroleum R1* and add the rinsings to the separating funnel. Shake vigorously for 1 min. Allow to separate and transfer the aqueous layer to a 2nd separating funnel. If an emulsion forms, add small quantities of *ethanol (96 per cent) R* or a concentrated solution of *potassium hydroxide R*. Shake the aqueous layer with 2 quantities, each of 50 mL, of *light petroleum R1*. Combine the light petroleum layers in a 3rd separating funnel and wash with 3 quantities, each of 50 mL, of *ethanol (50 per cent V/V) R*. Transfer the light petroleum layer to a tared 250 mL flask. Rinse the separating funnel with small quantities of *light petroleum R1* and add to the tared flask. Evaporate the light petroleum on a water-bath and dry the residue at 100-105 °C for 15 min, keeping the flask horizontal. Allow to cool in a desiccator and weigh (*a* g). Repeat the drying for successive periods of 15 min until the loss of mass between 2 successive weighings does not exceed 0.1 per cent. Dissolve the residue in 20 mL of *ethanol (96 per cent) R*, previously neutralised to 0.1 mL of *bromophenol blue solution R*. If necessary, titrate with *0.1 M hydrochloric acid* (*b* mL).

Calculate the percentage content of unsaponifiable matter using the following expression:

$$\frac{100(a - 0.032b)}{m}$$

If 0.032*b* is greater than 5 per cent of *a*, the test is not valid and must be repeated.

Composition of fatty acids (*2.4.22, Method A*)

Use the mixture of calibrating substances in Table 2.4.22.-3.

Composition of the fatty-acid fraction of the oil:

— *saturated fatty acids of chain length less than C_{16}*: maximum 0.1 per cent;
— *palmitic acid*: 7.5 per cent to 20.0 per cent;
— *palmitoleic acid*: maximum 3.5 per cent;
— *stearic acid*: 0.5 per cent to 5.0 per cent;
— *oleic acid and isomer*: 56.0 per cent to 85.0 per cent;
— *linoleic acid*: 3.5 per cent to 20.0 per cent;
— *linolenic acid*: maximum 1.2 per cent;
— *arachidic acid*: maximum 0.7 per cent;
— *eicosenoic acid*: maximum 0.4 per cent;
— *behenic acid*: maximum 0.2 per cent;
— *lignoceric acid*: maximum 0.2 per cent.

Sterols (*2.4.23, Method B*)

Composition of the sterol fraction of the oil:

— *cholesterol*: maximum 0.5 per cent;
— *campesterol*: maximum 4.0 per cent;
— *Δ7-stigmasterol*: maximum 0.5 per cent;
— *sum of contents of Δ5,23-stigmastadienol, clerosterol, β-sitosterol, sitostanol, Δ5-avenasterol and Δ5,24-stigmastadienol*: minimum 93.0 per cent.

The content of stigmasterol is not greater than that of campesterol.

Sesame oil

In a ground-glass-stoppered cylinder shake 10 mL for about 1 min with a mixture of 0.5 mL of a 0.35 per cent *V/V* solution of furfural R in *acetic anhydride R* and 4.5 mL of *acetic anhydride R*. Filter through a filter paper impregnated with *acetic anhydride R*. To the filtrate add 0.2 mL of *sulfuric acid R*. No bluish-green colour develops.

Water (*2.5.32*)

Maximum 0.1 per cent, determined on 1.00 g.

STORAGE

In a well-filled container, protected from light, at a temperature not exceeding 25 °C.

Ph Eur

Refined Olive Oil

(Ph. Eur. monograph 1456)

Ph Eur

DEFINITION

Fatty oil obtained by refining of crude olive oil, obtained by cold expression or other suitable mechanical means from the ripe drupes of *Olea europaea* L. A suitable antioxidant may be added.

CHARACTERS

Appearance

Clear, colourless or greenish-yellow transparent liquid.

Solubility

Practically insoluble in ethanol (96 per cent), miscible with light petroleum (bp: 50-70 °C).

When cooled, it begins to become cloudy at 10 °C and becomes a butter-like mass at about 0 °C.

Relative density

About 0.913.

IDENTIFICATION

First identification: A, C.

Second identification: A, B.

A. Acid value (see Tests).

B. Identification of fatty oils by thin-layer chromatography (*2.3.2*).

Results The chromatogram obtained is similar to the corresponding chromatogram shown in Figure 2.3.2.-1.

For certain types of olive oil, the difference in the size of spots E and F is less pronounced than in the corresponding chromatogram shown in Figure 2.3.2.-1.

C. Composition of fatty acids (see Tests).

TESTS

Specific absorbance (*2.2.25*)
Maximum 1.20, determined at the absorption maximum at 270 nm.

To 1.00 g add *cyclohexane R* and dilute to 100.0 mL with the same solvent.

Acid value (*2.5.1*)
Maximum 0.3, determined on 10.0 g.

Peroxide value (*2.5.5, Method A*)
Maximum 10.0, or maximum 5.0 if intended for use in the manufacture of parenteral preparations.

Unsaponifiable matter
Maximum 1.5 per cent.

Place 5.0 g (*m* g) in a 150 mL flask fitted with a reflux condenser. Add 50 mL of *2 M alcoholic potassium hydroxide R* and heat on a water-bath for 1 h, shaking frequently. Add 50 mL of *water R* through the top of the condenser, shake, allow to cool and transfer the contents of the flask to a separating funnel. Rinse the flask with several portions totalling 50 mL of *light petroleum R1* and add the rinsings to the separating funnel. Shake vigorously for 1 min. Allow to separate and transfer the aqueous layer to a 2nd separating funnel. If an emulsion forms, add small quantities of *ethanol (96 per cent) R* or a concentrated solution of *potassium hydroxide R*. Shake the aqueous layer with 2 quantities, each of 50 mL, of *light petroleum R1*. Combine the light petroleum layers in a 3rd separating funnel and wash with 3 quantities, each of 50 mL, of *ethanol (50 per cent V/V) R*. Transfer the light petroleum layer to a tared 250 mL flask. Rinse the separating funnel with small quantities of *light petroleum R1* and add to the flask. Evaporate the light petroleum on a water-bath and dry the residue at 100-105 °C for 15 min, keeping the flask horizontal. Allow to cool in a desiccator and weigh (*a* g). Repeat the drying for successive periods of 15 min until the loss of mass between 2 successive weighings does not exceed 0.1 per cent. Dissolve the residue in 20 mL of *ethanol (96 per cent) R*, previously neutralised to 0.1 mL of *bromophenol blue solution R*. If necessary, titrate with *0.1 M hydrochloric acid* (*b* mL).

Calculate the percentage content of unsaponifiable matter using the following expression:

$$\frac{100(a - 0.032b)}{m}$$

If 0.032*b* is greater than 5 per cent of *a*, the test is not valid and must be repeated.

Alkaline impurities (*2.4.19*)
It complies with the test.

Composition of fatty acids (*2.4.22, Method A*)
Use the mixture of calibrating substances in Table 2.4.22.-3.

Composition of the fatty-acid fraction of the oil:
— *saturated fatty acids of chain length less than C_{16}*: maximum 0.1 per cent;
— *palmitic acid*: 7.5 per cent to 20.0 per cent;
— *palmitoleic acid*: maximum 3.5 per cent;
— *stearic acid*: 0.5 per cent to 5.0 per cent;
— *oleic acid and isomer*: 56.0 per cent to 85.0 per cent;
— *linoleic acid*: 3.5 per cent to 20.0 per cent;
— *linolenic acid*: maximum 1.2 per cent;
— *arachidic acid*: maximum 0.7 per cent;
— *eicosenoic acid*: maximum 0.4 per cent;
— *behenic acid*: maximum 0.2 per cent;
— *lignoceric acid*: maximum 0.2 per cent.

Sterols (*2.4.23, Method B*)
Composition of the sterol fraction of the oil:
— *cholesterol*: maximum 0.5 per cent;
— *campesterol*: maximum 4.0 per cent;
— *Δ7-stigmastenol*: maximum 0.5 per cent;
— *sum of contents of Δ5,23-stigmastadienol, clerosterol, β-sitosterol, sitostanol, Δ5-avenasterol and Δ5,24-stigmastadienol*: minimum 93.0 per cent.

The content of stigmasterol is not greater than that of campesterol.

Sesame oil
In a ground-glass-stoppered cylinder shake 10 mL for about 1 min with a mixture of 0.5 mL of a 0.35 per cent *V/V* solution of furfural R in *acetic anhydride R* and 4.5 mL of *acetic anhydride R*. Filter through a filter paper impregnated with *acetic anhydride R*. To the filtrate add 0.2 mL of *sulfuric acid R*. No bluish-green colour develops.

Water (*2.5.32*)
Maximum 0.1 per cent, determined on 1.00 g.

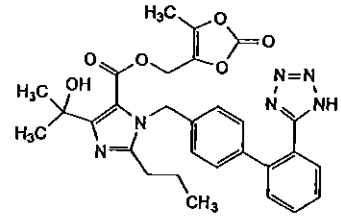
STORAGE
In a well-filled container, protected from light, at a temperature not exceeding 25 °C. If intended for use in the manufacture of parenteral preparations, store under an inert gas.

LABELLING
The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral preparations and the name of the inert gas.

Ph Eur

Olmesartan Medoxomil

(Ph. Eur. monograph 2600)



$C_{29}H_{30}N_6O_6$ 558.6 *144689-63-4*

Action and use
Angiotensin II (AT_1) receptor antagonist.

Preparation
Olmesartan Tablets

Ph Eur

DEFINITION
(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-imidazole-5-carboxylate.

Content
97.5 per cent to 102.0 per cent (anhydrous substance).

PRODUCTION

As *N*-nitrosamines are classified as probable human carcinogens, their presence in olmesartan medoxomil should be avoided or limited as much as possible. For this reason, manufacturers of olmesartan medoxomil for human use are expected to perform an assessment of the risk of *N*-nitrosamine formation and contamination during their manufacturing process; if this assessment identifies a potential risk, the manufacturing process should be modified to minimise contamination and a control strategy implemented to detect and control *N*-nitrosamine impurities in olmesartan medoxomil. The general chapter *2.5.42. N-Nitrosamines in active substances* is available to assist manufacturers.

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in heptane.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *olmesartan medoxomil CRS.*

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 25 mg of the substance to be examined in *acetonitrile R* and dilute to 25.0 mL with the same solvent.

Test solution (b) Dissolve 25.0 mg of the substance to be examined in *acetonitrile R* and dilute to 50.0 mL with the same solvent.

Reference solution (a) Dissolve 5 mg of *olmesartan medoxomil for system suitability CRS* (containing impurities A, B and C) in *acetonitrile R* and dilute to 5 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of test solution (a) to 50.0 mL with *acetonitrile R*. Dilute 1.0 mL of this solution to 10.0 mL with *acetonitrile R*.

Reference solution (c) Dissolve 25.0 mg of *olmesartan medoxomil CRS* in *acetonitrile R* and dilute to 50.0 mL with the same solvent.

Column:
- *size*: *l* = 0.10 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (3.5 µm);
- *temperature*: 40 °C.

Mobile phase:
- *mobile phase A*: mix 20 volumes of *acetonitrile R* and 80 volumes of a 2.04 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.4 with a 1.73 g/L solution of *phosphoric acid R*;
- *mobile phase B*: mix 20 volumes of a 2.04 g/L solution of *potassium dihydrogen phosphate R*, previously adjusted to pH 3.4 with a 1.73 g/L solution of *phosphoric acid R*, and 80 volumes of *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	75	25
10 - 35	75 → 0	25 → 100
35 - 45	0	100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 250 nm.

Injection 10 µL of test solution (a) and reference solutions (a) and (b).

Identification of impurities Use the chromatogram supplied with *olmesartan medoxomil for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B and C.

Relative retention With reference to olmesartan medoxomil (retention time = about 10 min): impurity A = about 0.2; impurity B = about 0.7; impurity C = about 1.5.

System suitability Reference solution (a):
- *resolution*: minimum 3.5 between the peaks due to impurity B and olmesartan medoxomil.

Limits:
- *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent);
- *impurity C*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
- *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- *total*: not more than 3.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.7 per cent);
- *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Acetone

Head-space gas chromatography (*2.2.28*): use the direct calibration method.

Internal standard solution Dilute 1.0 mL of *butanol R* to 100.0 mL with *dimethyl sulfoxide R*.

Test solution Dissolve 0.250 g of the substance to be examined in *dimethyl sulfoxide R*, add 2.0 mL of the internal standard solution and dilute to 10.0 mL with *dimethyl sulfoxide R*.

Reference solution Dilute 0.50 mL of *acetone R* to 200.0 mL with *dimethyl sulfoxide R*. Dilute 15.0 mL of the solution to 100.0 mL with *dimethyl sulfoxide R*. To 25.0 mL of this solution add 10.0 mL of the internal standard solution and dilute to 50.0 mL with *dimethyl sulfoxide R*.

Column:
- *material*: fused silica;
- *size*: *l* = 30 m, Ø = 0.53 mm;
- *stationary phase*: *macrogol 20 000 R* (film thickness 1 µm).

Carrier gas *nitrogen for chromatography R* or *helium for chromatography R*.

Flow rate 4.0 mL/min.

Split ratio 1:5.

Static head-space conditions that may be used:
- *equilibration temperature*: 80 °C;
- *equilibration time*: 30 min.

Temperature:

	Time (min)	Temperature (°C)
Column	5	50
	5 - 18	50 → 180
	18 - 23	180
Injection port		200
Detection		200

Detection Flame ionisation.

Injection 1 mL.

Calculate the content of acetone, taking its relative density to be 0.79 at 20 °C.

Limit:

— *acetone*: maximum 0.6 per cent.

Water (*2.5.32*)

Maximum 0.5 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Mobile phase Mobile phase B, mobile phase A (25:75 *V/V*).

Injection Test solution (b) and reference solution (c).

Retention time Olmesartan medoxomil = about 10 min.

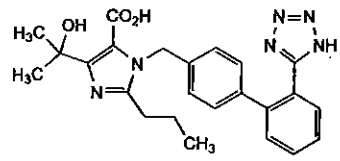
Run time 1.5 times the retention time of olmesartan medoxomil.

Calculate the percentage content of $C_{29}H_{30}N_6O_6$ taking into account the assigned content of *olmesartan medoxomil CRS*.

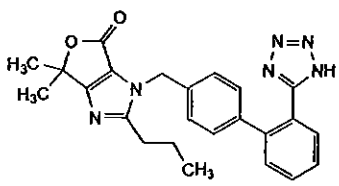
IMPURITIES

Specified impurities *A, C.*

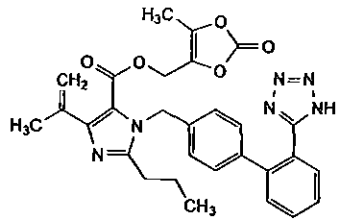
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, D.*



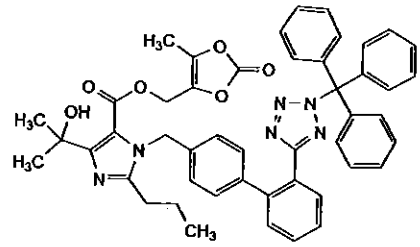
A. 4-(1-hydroxy-1-methylethyl)-2-propyl-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-imidazole-5-carboxylic acid (olmesartan),



B. 6,6-dimethyl-2-propyl-3-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-3,6-dihydro-4*H*-furo[3,4-*d*]imidazol-4-one,



C. (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl 4-(1-methylethenyl)-2-propyl-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-imidazole-5-carboxylate,

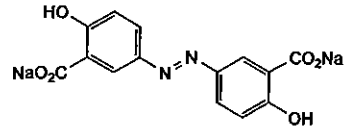


D. (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-[[2′-[(2-triphenylmethyl)-2*H*-tetrazol-5-yl]biphenyl-4-yl]methyl]-1*H*-imidazole-5-carboxylate.

Ph Eur

Olsalazine Sodium

(Ph. Eur. monograph 1457)



$C_{14}H_8N_2Na_2O_6$ 346.2 *6054-98-4*

Action and use

Aminosalicylate; treatment of ulcerative colitis.

Ph Eur

DEFINITION

Disodium 3,3′-diazenediylbis(6-hydroxybenzoate).

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

Yellow, fine, crystalline powder.

Solubility

Sparingly soluble in water, soluble in dimethyl sulfoxide, very slightly soluble in methanol.

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 40.0 mg in 5 mL of *0.1 M sodium hydroxide* and dilute to 100.0 mL with a 7.8 g/L solution of *sodium dihydrogen phosphate R* adjusted to pH 7.2 with *strong*

sodium hydroxide solution R (buffer solution). Dilute 2.0 mL of the solution to 100.0 mL with the buffer solution.

Spectral range 240 nm to 400 nm.

Absorption maxima At 255 nm and 362 nm.

Absorbance ratio A_{255}/A_{362} = 0.53 to 0.56.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *olsalazine sodium CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in a mixture of 1 volume of *dilute ammonia R2* and 4 volumes of *ethanol (96 per cent) R* and dilute to 10 mL with the same mixture of solvents.

Reference solution (a) Dissolve 10 mg of *olsalazine sodium CRS* in a mixture of 1 volume of *dilute ammonia R2* and 4 volumes of *ethanol (96 per cent) R* and dilute to 10 mL with the same mixture of solvents.

Reference solution (b) Dissolve 5 mg of *sulfasalazine CRS* in reference solution (a) and dilute to 5 mL with reference solution (a).

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *anhydrous formic acid R, acetone R, methylene chloride R* (5:50:60 *V/V/V*).

Application 10 µL.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):
— the chromatogram shows 2 separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To 0.5 g add 2 mL of *sulfuric acid R*. Progressively heat to ignition and continue heating until an almost white or at most greyish residue is obtained. Carry out the ignition at a temperature up to 800 ± 50 °C. Dissolve the residue in 10 mL of boiling *water R* and filter. 2 mL of the filtrate gives reaction (a) of sodium (*2.3.1*).

TESTS

Acetate

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.125 g of the substance to be examined in 25.0 mL of *water R* and add 1.0 mL of *dilute hydrochloric acid R*. Centrifuge and then filter the solution through a 0.45 µm filter and also through an appropriate filter for removal of chlorides.

Reference solution (a) Dissolve 0.140 g of *sodium acetate R*, 0.150 g of *sodium formate R* and 0.180 g of *dipotassium sulfate R* in 100.0 mL of *water R*. Dilute 1.0 mL of this solution to 100.0 mL with *water R*.

Reference solution (b) Use suitable amounts of *sodium acetate R* to prepare not fewer than 5 reference solutions containing 10-50 µg/mL of acetate.

Column:
— *size*: l = 0.25 m, Ø = 9 mm;
— *stationary phase*: *ion-exclusion resin for chromatography R* with a capacity of about 27 meq/column.

Suppressor column.

Mobile phase *0.0001 M hydrochloric acid.*

Flow rate 0.9 mL/min.

Detection Conductivity detector at 10 µs·cm^{-1}.

Injection 0.1 mL.

System suitability Reference solution (a):
— the chromatogram shows 3 separated peaks.

Determine the concentration of acetate in the test solution using the calibration curve generated by the average of the readings obtained with the reference solutions. Measure the peak area for acetate. Calculate the percentage content of acetate using the following expression:

$$\frac{2.6\ c}{m}$$

c = concentration of acetate in the test solution, in micrograms per millilitre, determined by linear interpolation of the standard curve for reference solution (b);
ym = mass of sample, in milligrams.

Limit:
— *acetate*: maximum 1.0 per cent.

Methanesulfonic acid

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.25 g of the substance to be examined in 20 mL of *water R*, add 1.0 mL of *dilute hydrochloric acid R* and dilute to 25.0 mL with *water R*. Centrifuge and then filter the solution through a 0.45 µm filter and also through an appropriate filter for removal of chlorides.

Reference solution (a) Dissolve 0.25 g of *methanesulfonic acid R* in 50 mL of *water R*. Add 0.58 g of *sodium acetate R* and 0.08 g of *sodium chloride R* and dilute to 100.0 mL with *water R*. Dilute 1.0 mL of this solution to 100.0 mL with *water R*.

Reference solution (b) Dissolve 0.10 g of *methanesulfonic acid R* in *water R* and dilute to 100.0 mL with *water R*. Dilute 3.0 mL of this solution to 100.0 mL with *water R*.

Precolumn:
— *size*: *l* = 0.035 m, Ø = 4 mm;
— *stationary phase*: *resin for reversed-phase ion chromatography R* (10 µm).

Column:
— *size*: *l* = 0.25 m, Ø = 4 mm;
— *stationary phase*: *resin for reversed-phase ion chromatography R* (10 µm).

Mobile phase Mix 10 volumes of *acetonitrile for chromatography R* and 990 volumes of a solution containing 1.6 g/L of *tetrabutylammonium hydroxide R* and 0.053 g/L of *anhydrous sodium carbonate R*.

Flow rate 1.0 mL/min.

Detection Conductivity detector at 50 µS·cm^{-1}.

Injection 100 µL.

System suitability Reference solution (a):
— the chromatogram shows 3 separated peaks.

Limit:
— *methanesulfonic acid*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.3 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in mobile phase A and dilute to 25.0 mL with mobile phase A.

Reference solution (a) Dilute 0.5 mL of the test solution to 100.0 mL with mobile phase A.

Reference solution (b) Dissolve 20.0 mg of *olsalazine sodium for performance test CRS* in mobile phase A and dilute to 25.0 mL with mobile phase A.

Column:
- *size*: *l* = 0.125 m, Ø = 4.0 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 30 °C.

Mobile phase:
- *mobile phase A*: dissolve 2.38 g of *tetrabutylammonium hydrogen sulfate R* and 3.6 g of *disodium hydrogen phosphate dihydrate R* in 900 mL of *water R*, adjust to pH 7.6 with *dilute sodium hydroxide solution R* and dilute to 1000.0 mL with *water R*; mix 700 mL of this buffer solution with 300 mL of *methanol R*;
- *mobile phase B*: dissolve 4.75 g of *tetrabutylammonium hydrogen sulfate R* and 3.6 g of *disodium hydrogen phosphate dihydrate R* in 900 mL of *water R*, adjust to pH 7.6 with *dilute sodium hydroxide solution R* and dilute to 1000.0 mL with *water R*; mix 350 mL of this buffer solution with 650 mL of *methanol R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	55	45
15 - 45	55 → 0	45 → 100
45 - 50	0 → 55	100 → 45
50 - 65	55	45

Flow rate 1 mL/min.

Detection Spectrophotometer at 360 nm.

Injection 20 µL.

System suitability Reference solution (b):
- the chromatogram is similar to the chromatogram obtained with *olsalazine sodium for performance test CRS*.

Limits:
- *impurities A, B, C, D, E, F, G, H, I*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent), and not more than one of the peaks has an area greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent);
- *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.025 per cent).

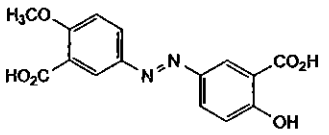
Loss on drying (*2.2.32*)
Maximum 2.0 per cent, determined on 1.000 g by drying in an oven at 150 °C.

ASSAY
Dissolve 0.100 g in 15 mL of *ethylene glycol R*. Add 40 mL of *dioxan R* and 0.2 mL of a 224 g/L solution of *potassium chloride R*. Titrate with *0.1 M hydrochloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

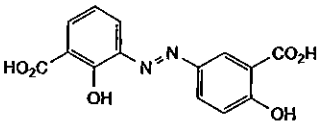
Correct the volume consumed for the content of acetate, taking the molecular mass of acetate to be 59.0.

1 mL of *0.1 M hydrochloric acid* is equivalent to 17.31 mg of $C_{14}H_8N_2Na_2O_6$.

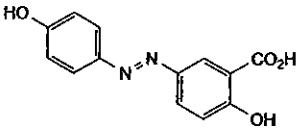
IMPURITIES
Specified impurities *A, B, C, D, E, F, G, H, I.*



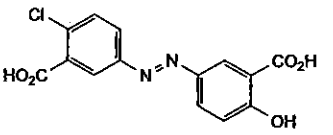
A. 6-hydroxy-6′-methoxy-3,3′-diazenediyldibenzoic acid,



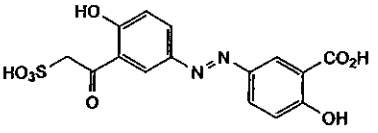
B. 2,6′-dihydroxy-3,3′-diazenediyldibenzoic acid,



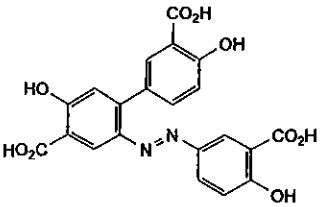
C. 2-hydroxy-5-[(4-hydroxyphenyl)diazenyl]benzoic acid,



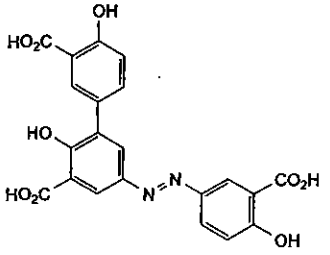
D. 6-chloro-6′-hydroxy-3,3′-diazenediyldibenzoic acid,



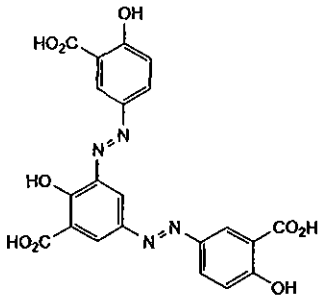
E. 2-hydroxy-5-[[4-hydroxy-3-(sulfoacetyl)phenyl]diazenyl] benzoic acid,



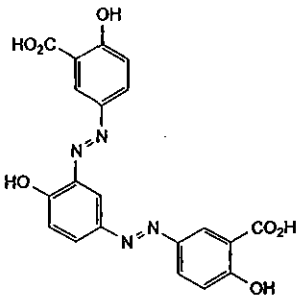
F. 2′-[(3-carboxy-4-hydroxyphenyl)diazenyl]-4,5′-dihydroxybiphenyl-3,4′-dicarboxylic acid,



G. 5-[(3-carboxy-4-hydroxyphenyl)diazenyl]-2,4′-dihydroxybiphenyl-3,3′-dicarboxylic acid,



H. 3,3′-[5-carboxy-4-hydroxy-1,3-phenylenebis(diazenediyl)] bis(6-hydroxybenzoic) acid,



I. 3,3′-[4-hydroxy-1,3-phenylenebis(diazenediyl)]bis(6-hydroxybenzoic) acid.

Ph Eur

Omega-3-Acid Ethyl Esters 60

(Ph. Eur. monograph 2063)

Action and use
Lipid-regulating drug.

Ph Eur

DEFINITION

Ethyl esters of *alpha*-linolenic acid (C18:3 n-3), moroctic acid (C18:4 n-3), eicosatetraenoic acid (C20:4 n-3), timnodonic (eicosapentaenoic) acid (C20:5 n-3; EPA), heneicosapentaenoic acid (C21:5 n-3), clupanodonic acid (C22:5 n-3) and cervonic (docosahexaenoic) acid (C22:6 n-3; DHA). Omega-3-acid ethyl esters 60 are obtained by transesterification of the body oil obtained from fish of families such as *Engraulidae, Carangidae, Clupeidae, Osmeridae, Salmonidae* and *Scombridae* or from animals of the class *Cephalopoda* and subsequent physico-chemical purification processes, including molecular distillation. The minimum content of total omega-3-acid ethyl esters and the minimum content of the omega-3-acids EPA and DHA ethyl esters are indicated in Table 2063.-1.

Table 2063.-1

Total omega-3-acid ethyl esters	EPA and DHA ethyl esters	EPA ethyl esters	DHA ethyl esters
	Minimum content (per cent)		
65	50	25	20
60	50	-	40
55	50	40	-

A suitable antioxidant may be added.

PRODUCTION

The content of dioxins and dioxin-like PCBs (polychlorinated biphenyls) is controlled using methods and limits in accordance with the requirements set in the European Union or other applicable regulations.

CHARACTERS

Appearance
Light yellow liquid.

Slight fish-like odour.

Solubility
Practically insoluble in water, very soluble in acetone, in ethanol (96 per cent), in heptane and in methanol.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay for EPA and DHA ethyl esters.

Results The peaks due to eicosapentaenoic acid ethyl ester and docosahexaenoic acid ethyl ester in the chromatogram obtained with test solution (b) are similar in retention time to the corresponding peaks in the chromatograms obtained with reference solutions (a_1) and (a_2).

B. It complies with the limits of the assay for total omega-3-acid ethyl esters.

TESTS

Absorbance *(2.2.25)*
Maximum 0.60 at 233 nm.

Dilute 0.300 g to 50.0 mL with *trimethylpentane R*. Dilute 2.0 mL of the solution to 50.0 mL with *trimethylpentane R*.

Acid value *(2.5.1)*
Maximum 2.0, determined on 10 g in 50 mL of the prescribed mixture of solvents.

Anisidine value *(2.5.36)*
Maximum 20.0.

Peroxide value *(2.5.5, Method A)*
Maximum 10.0.

Oligomers and partial glycerides
Size-exclusion chromatography *(2.2.30)*.

Test solution Dilute 50.0 mg of the substance to be examined to 10.0 mL with *tetrahydrofuran R*.

Reference solution Dissolve 50 mg of *monodocosahexaenoin R*, 30 mg of *didocosahexaenoin R* and 20 mg of *tridocosahexaenoin R* in *tetrahydrofuran R* and dilute to 100.0 mL with the same solvent.

Column 3 columns to be connected in series:
- *size*: *l* = 0.3 m, Ø = 7.8 mm;
- *stationary phase*: *styrene-divinylbenzene copolymer R* (5 µm) with the following pore sizes:
  - *column 1*: 50 nm;
  - *column 2*: 10 nm;
  - *column 3*: 5 nm;
- *connection sequence*: injector – column 1 – column 2 – column 3 – detector.

Mobile phase *tetrahydrofuran R*.

Flow rate 0.8 mL/min.

Detection Differential refractometer.

Injection 40 µL.

System suitability Reference solution:
- *elution order*: tridocosahexaenoin, didocosahexaenoin, monodocosahexaenoin;
- *resolution*: minimum 2.0 between the peaks due to didocosahexaenoin and monodocosahexaenoin;

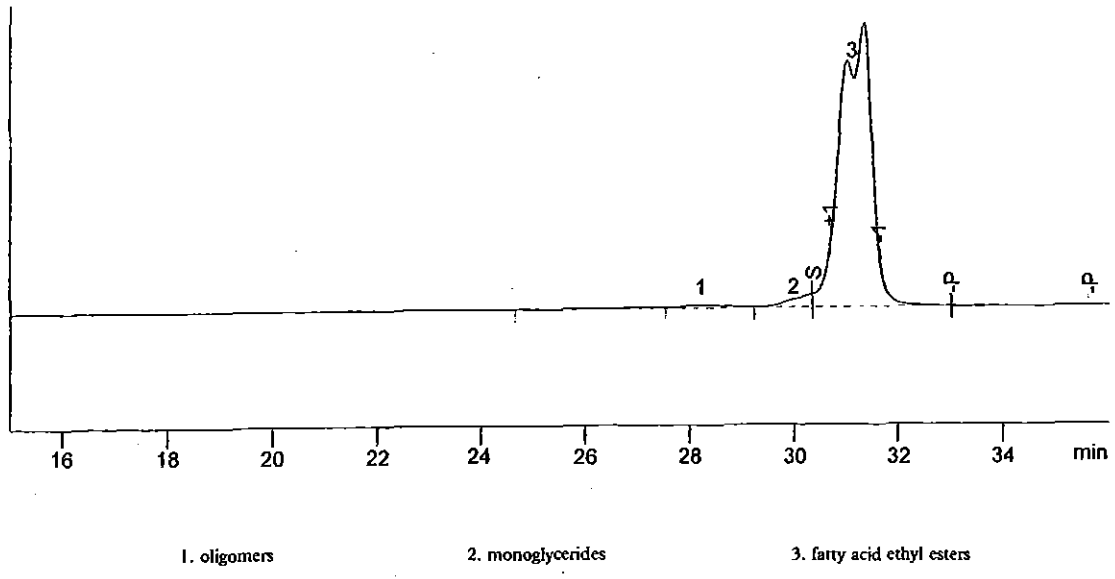
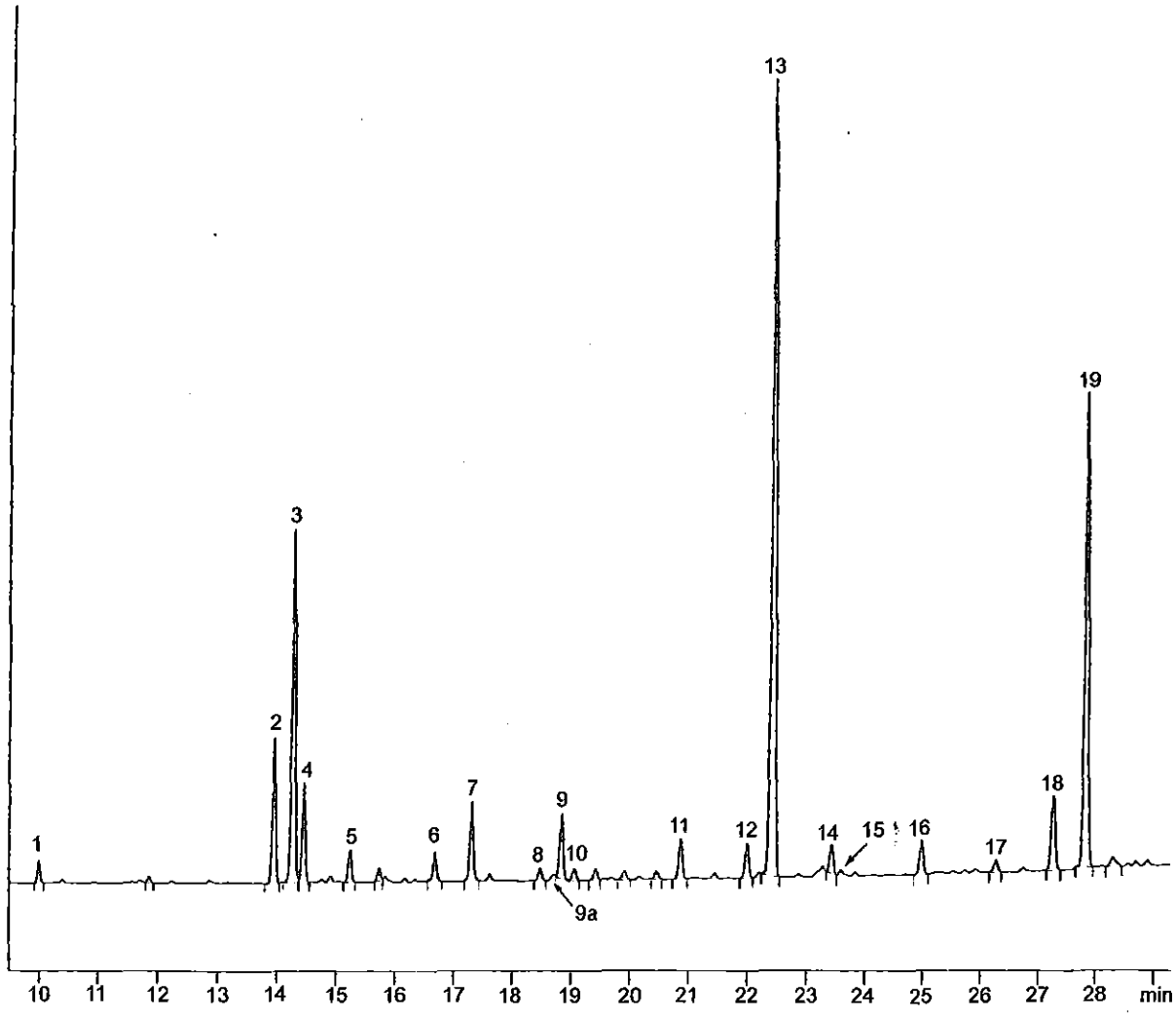


Figure 2063.-1. – *Chromatogram for the test for oligomers and partial glycerides in omega-3-acid ethyl esters 60*



1. C16:0
2. C18:0
3. C18:1 n-9
4. C18:1 n-7
5. C18:2 n-6
6. C18:3 n-3
7. C18:4 n-3
8. C20:0
9. C20:1 n-9
9a. C20:1 n-11
10. C20:1 n-7
11. C20:4 n-6
12. C20:4 n-3
13. BPA
14. C22:1 n-11
15. C22:1 n-9
16. C21:5 n-3
17. C22:5 n-6
18. C22:5 n-3
19. DHA

Figure 2063.-2. – *Chromatogram for the assays of omega-3-acid ethyl esters 60*

minimum 1.0 between the peaks due to tridocosahexaenoin and didocosahexaenoin.

Calculate the percentage content of oligomers plus partial glycerides using the following expression:

$$\frac{B}{A} \times 100$$

A = sum of the areas of all the peaks in the chromatogram;
B = sum of the areas of the peaks with a retention time less than the retention time of the peaks due to ethyl esters.

The ethyl ester peaks, which may be present in the form of an unresolved double peak, are identified as the major peaks in the chromatogram (see Figure 2063.-1).

Limit:

— *sum of oligomers and partial glycerides*: maximum 7.0 per cent.

ASSAY

EPA and DHA ethyl esters (*2.4.29*)

For identification of the peaks, see Figure 2063.-2.

Total omega-3-acid ethyl esters (*2.4.29*)

See Figure 2063.-2.

STORAGE

Under an inert gas, in an airtight container, protected from light.

LABELLING

The label states:

— the content of total omega-3-acid ethyl esters;
— the content of EPA ethyl.ester and DHA ethyl ester.

Ph Eur

Omega-3-Acid Ethyl Esters 90

(Ph. Eur. monograph 1250)

Action and use

Lipid-regulating drug.

Ph Eur

DEFINITION

Ethyl esters of *alpha*-linolenic acid (C18:3 n-3), moroctic (stearidonic) acid (C18:4 n-3), eicosatetraenoic acid (C20:4 n-3), timnodonic (eicosapentaenoic) acid (C20:5 n-3; EPA), heneicosapentaenoic acid (C21:5 n-3), clupanodonic (omega-3 docosapentaenoic) acid (C22:5 n-3) and cervonic (docosahexaenoic) acid (C22:6 n-3; DHA). Omega-3-acid ethyl esters 90 are obtained by transesterification of the body oil obtained from fish of families such as *Engraulidae, Carangidae, Clupeidae, Osmeridae, Salmonidae* and *Scombridae* or from animals of the class *Cephalopoda* and subsequent physico-chemical purification processes, including urea fractionation followed by molecular distillation.

Content

— *EPA and DHA ethyl esters*: minimum 80.0 per cent and maximum 88.2 per cent, with minimum 43.0 per cent and maximum 49.5 per cent of EPA ethyl esters and minimum 34.7 per cent and maximum 40.3 per cent of DHA ethyl esters;
— *total omega-3-acid ethyl esters (corresponding to the sum of the ethyl esters listed under Definition)*: minimum 90 per cent.

A suitable antioxidant may be added.

PRODUCTION

The content of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/PCDF), dioxin-like polychlorinated biphenyls (DLPCBs), non-dioxin-like polychlorinated biphenyls (NDLPCBs; 7PCB) and polybrominated diphenyl ethers (PBDEs) is controlled using suitable and validated methods, for example as outlined in Commission Regulation (EU) No 589/2014, Annex III, and in scientific publications of the European Food Safety Authority [EFSA, 2011].

Persistent organic pollutants	Maximum content
*Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/PCDF)**	1 pg/g (WHO-TEQ***)
*Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/PCDF) and dioxin-like polychlorinated biphenyls (DLPCBs)**	5 pg/g (WHO-TEQ)
*Non-dioxin-like polychlorinated biphenyls (NDLPCBs; 7PCB) (sum of congeners 28, 52, 101, 118, 138, 153 and 180)**	60 ng/g
*Polybrominated diphenyl ethers (PBDEs) (sum of congeners 28, 47, 49, 99, 100, 153 and 154)***	3 ng/g

*Commission Regulation (EU) No 589/2014, Annex III.
**_EFSA Journal_ 2011;9(5):2156, [274 pp.].
***WHO-TEQ = 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) toxic equivalent (TEQ) as agreed by WHO.

Calculate the upper-bound contents on the assumption that all values of the different congeners below the limit of quantification are equal to the limit of quantification.

CHARACTERS

Appearance

Light yellow liquid.

Solubility

Practically insoluble in water, very soluble in acetone, in ethanol (96 per cent), in heptane and in methanol.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay for EPA and DHA ethyl esters.

Results The peaks due to eicosapentaenoic acid ethyl ester and docosahexaenoic acid ethyl ester in the chromatogram obtained with test solution (b) are similar in retention time to the corresponding peaks in the chromatograms obtained with reference solutions (a_1) and (a_2).

B. It complies with the limits of the assay for total omega-3-acid ethyl esters.

TESTS

Absorbance (*2.2.25*)

Maximum 0.55 at 233 nm.

Dilute 0.300 g to 50.0 mL with *trimethylpentane R.* Dilute 2.0 mL of the solution to 50.0 mL with *trimethylpentane R.*

Acid value (*2.5.1*)

Maximum 2.0, determined on 10 g in 50 mL of the prescribed mixture of solvents.

Anisidine value (*2.5.36*)

Maximum 20.0.

Peroxide value (*2.5.5, Method B*)

Maximum 10.0.

Unidentified fatty acid ethyl esters (*2.4.29*)
Identify the peaks in the chromatogram obtained with test solution (b) in the assay, using Figure 1250.-2, integrating up to 1.3 times the retention time of DHA ethyl ester. Disregard any peak with an area less than 0.05 per cent of the total area.

Calculate the percentage content of the fatty acid ethyl ester corresponding to the largest single unidentified peak, using the following expression:

$$\frac{100 \times B}{A}$$

A = sum of the areas of all peaks, excluding those due to solvents, butylhydroxytoluene and the internal standard;
B = area of the largest single unidentified peak, excluding those due to solvents, butylhydroxytoluene and the internal standard.

Calculate the percentage content of total unidentified fatty acid ethyl esters, using the following expression:

$$100 - \frac{100 \times C}{A}$$

C = sum of the areas of the peaks due to ethyl esters identified in Figure 1250.-2.

Limits:
— *fatty acid ethyl ester corresponding to the largest single unidentified peak*: maximum 0.5 per cent;
— *total unidentified fatty acid ethyl esters*: maximum 2 per cent.

Cholesterol (*2.4.32*)
Maximum 3.0 mg/g.

Oligomers
Size-exclusion chromatography (*2.2.30*).

Test solution Dilute 50.0 mg of the substance to be examined to 10.0 mL with *tetrahydrofuran R*.

Reference solution Dissolve 50 mg of *monodocosahexaenoin R*, 30 mg of *didocosahexaenoin R* and 20 mg of *tridocosahexaenoin R* in *tetrahydrofuran R* and dilute to 100.0 mL with the same solvent.

Column 3 columns to be connected in series:
— *size*: *l* = 0.3 m, Ø = 7.8 mm;
— *stationary phase*: *styrene-divinylbenzene copolymer R* (5 µm) with the following pore sizes:
  — *column 1*: 50 nm;
  — *column 2*: 10 nm;
  — *column 3*: 5 nm;
— *connection sequence*: injector – column 1 – column 2 – column 3 – detector.

Mobile phase *tetrahydrofuran R*.

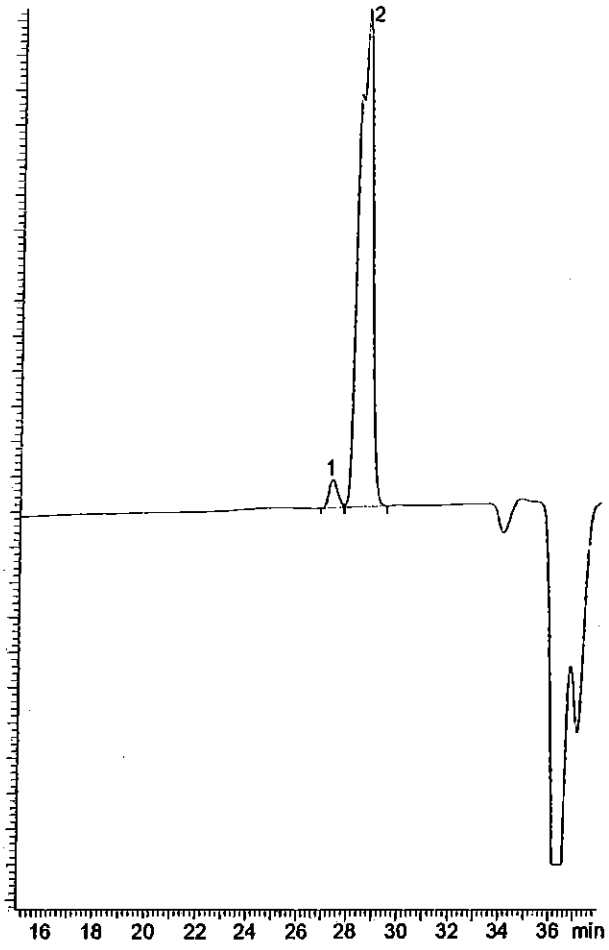
Flow rate 0.8 mL/min.

Run time 38 min.

Detection Differential refractometer.

Injection 40 µL.

System suitability Reference solution:
— *elution order*: tridocosahexaenoin, didocosahexaenoin, monodocosahexaenoin;
— *resolution*: minimum 2.0 between the peaks due to didocosahexaenoin and monodocosahexaenoin; minimum 1.0 between the peaks due to tridocosahexaenoin and didocosahexaenoin.



1. oligomers 2. ethyl esters

Figure 1250.-1. – *Chromatogram for the test for oligomers in omega-3-acid ethyl esters 90: spiked sample*

Calculate the percentage content of oligomers using the following expression:

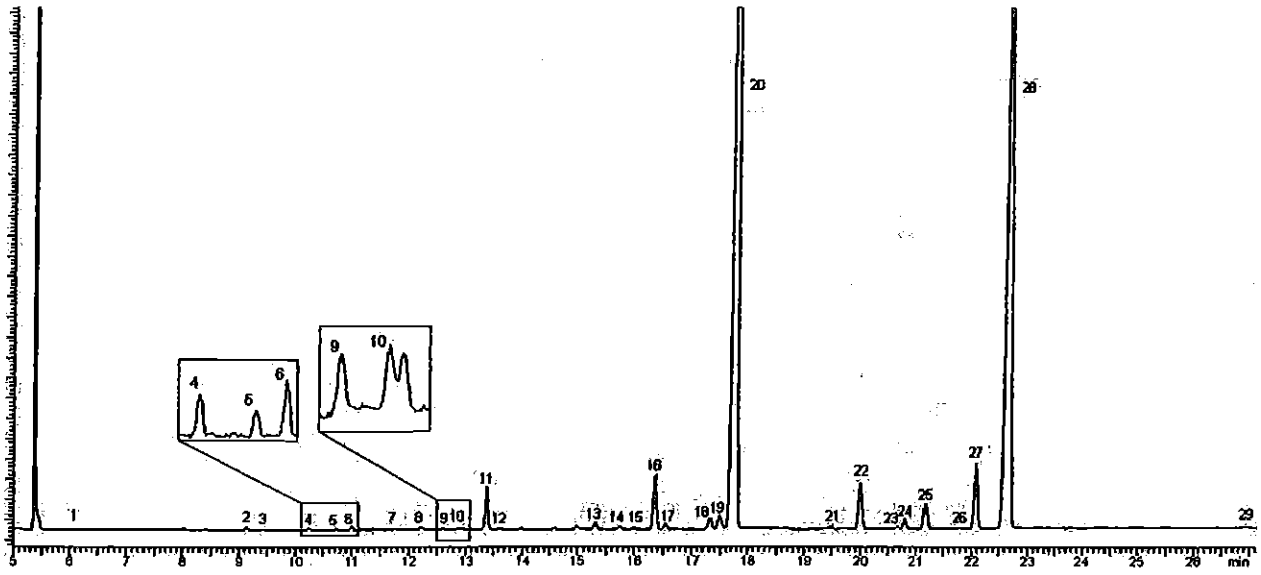
$$\frac{B}{A} \times 100$$

A = sum of the areas of all the peaks in the chromatogram;
B = sum of the areas of the peaks with a retention time less than the retention time of the peaks due to ethyl esters.

The ethyl ester peaks, which may be present in the form of an unresolved double peak, are identified as the major peaks in the chromatogram (see Figure 1250.-1).

Where the result obtained exceeds the limit due to the presence of monoglycerides, the following procedure is carried out.

Test solution Weigh 50.0 mg of the substance to be examined into a quartz tube. Add 1.5 mL of a 20 g/L solution of *sodium hydroxide R* in *methanol R*, cover with *nitrogen R*, cap tightly with a polytetrafluoroethylene-lined cap, mix and heat on a water-bath for 7 min. Allow to cool. Add 2 mL of *boron trichloride-methanol solution R*, cover with *nitrogen R*, cap tightly, mix and heat on a water-bath for 30 min. Cool to 40-50 °C, add 1 mL of *trimethylpentane R*, cap and shake vigorously for at least 30 s. Immediately add 5 mL of *saturated sodium chloride solution R*, cover with *nitrogen R*, cap and shake thoroughly for at least 15 s.



Fatty acid	r	Fatty acid	r	Fatty acid	r	Fatty acid	r
1. C14:0	0.278	8. C18:3 *n*-6	0.557	15. C20:3 *n*-6	0.720	22. C21:5 *n*-3	0.889
2. Phytanic acid	0.416	9. C18:3 *n*-4	0.574	16. C20:4 *n*-6	0.736	23. C22:4 *n*-6	0.917
3. C16:3 *n*-4	0.431	10. C18:3 *n*-3	0.585	17. Furan acid 7	0.744	24. Furan acid 10	0.922
4. C16:4 *n*-1	0.468	11. C18:4 *n*-3	0.608	18. C20:4 *n*-3	0.777	25. C22:5 *n*-6	0.939
5. C18:0	0.488	12. C18:4 *n*-1	0.618	19. Furan acid 8	0.783	26. Furan acid 11	0.963
6. C18:1 *n*-9	0.501	13. Furan acid 5	0.691	20. EPA	0.796	27. C22:5 *n*-3	0.977
7. C18:2 *n*-6	0.535	14. C19:5	0.710	21. Furan acid 9	0.867	28. DHA	1.000
						29. C24:6	1.183

r: relative retention with reference to DHA ethyl ester (retention time = about 23 min).

Figure 1250.-2. – *Chromatogram for the assay of omega-3-acid ethyl esters 90 and the test for unidentified fatty acid ethyl esters*

Transfer the upper layer to a separate tube. Shake the methanol layer once more with 1 mL of *trimethylpentane R*. Wash the combined trimethylpentane extracts with 2 quantities, each of 1 mL, of *water R*. Carefully evaporate the solvent under a current of *nitrogen R* then add 10.0 mL of *tetrahydrofuran R* to the residue. Add a small amount of *anhydrous sodium sulfate R* and filter.

Calculate the percentage content of oligomers using the following expression:

$$\frac{B'}{A} \times 100$$

A = sum of the areas of all the peaks in the chromatogram;
B' = sum of the areas of the peaks with a retention time less than the retention time of the peaks due to methyl esters.

Limit:
— *oligomers*: maximum 1.0 per cent.

ASSAY

EPA and DHA ethyl esters (*2.4.29*)
For identification of the peaks, see Figure 1250.-2.

Total omega-3-acid ethyl esters (*2.4.29*)
See Figure 1250.-2.

STORAGE
Under an inert gas, in an airtight container, protected from light.

Ph Eur

Omega-3-Marine Triglycerides

(Omega-3-Acid Triglycerides, Ph. Eur. monograph 1352)

Action and use
Lipid-regulating drug.

Ph Eur

DEFINITION
Mixture of mono-, di- and triesters of omega-3 acids with glycerol, containing mainly triesters and obtained either by esterification of concentrated and purified omega-3 acids with glycerol or by transesterification of the omega-3 acid ethyl esters with glycerol. The origin of the omega-3 acids is the body oil obtained from fish of families such as *Engraulidae, Carangidae, Clupeidae, Osmeridae, Salmonidae* and *Scombridae* or from animals of the class *Cephalopoda*. The omega-3 acids are identified as the following acids: *alpha*-linolenic acid (C18:3 n-3), moroctic acid (C18:4 n-3), eicosatetraenoic acid (C20:4 n-3), timnodonic (eicosapentaenoic) acid (C20:5 n-3; EPA), heneicosapentaenoic acid (C21:5 n-3), clupanodonic acid (C22:5 n-3) and cervonic (docosahexaenoic) acid (C22:6 n-3; DHA).

Content
— *sum of the contents of the omega-3 acids EPA and DHA, expressed as triglycerides*: minimum 45 per cent;

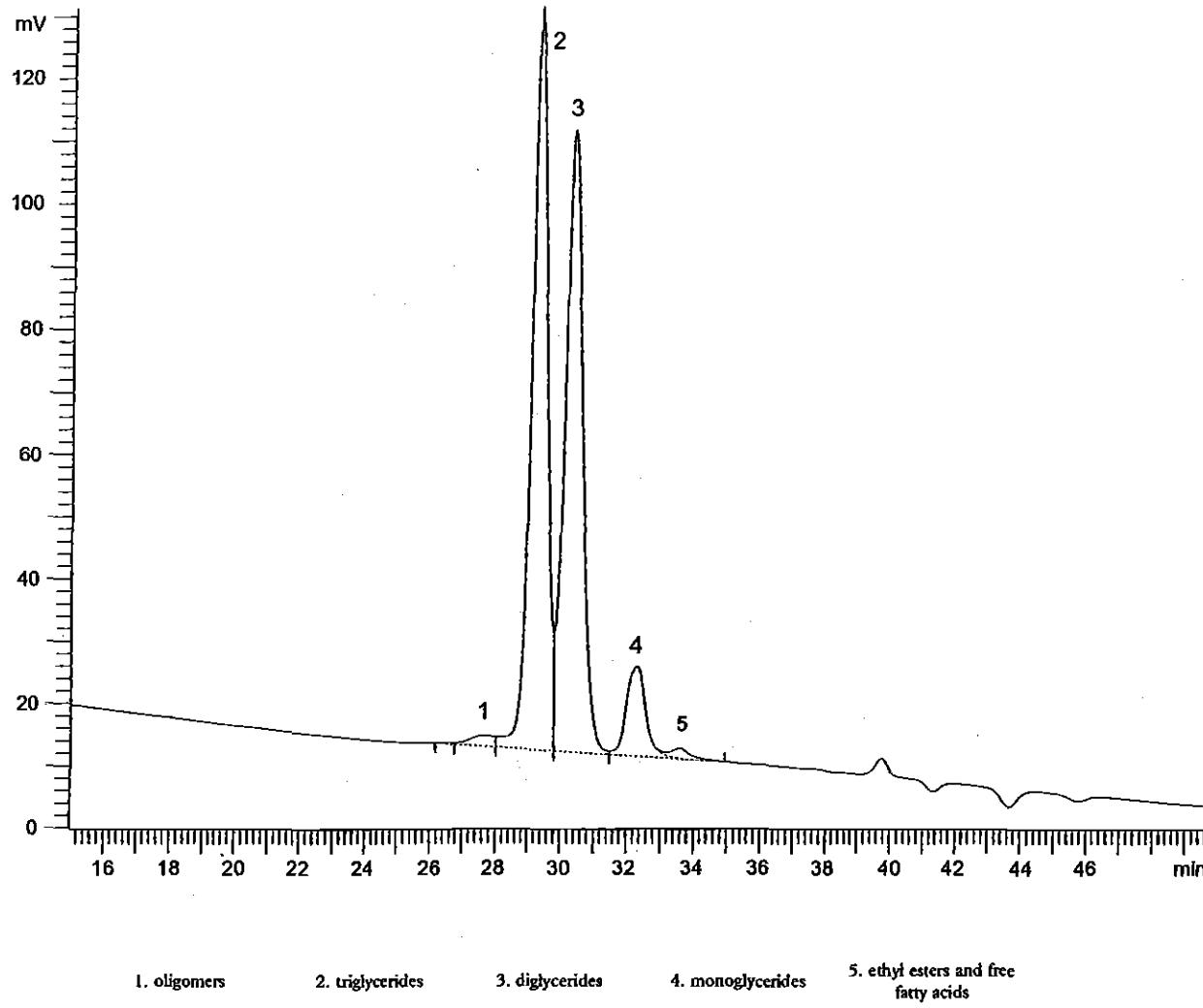


Figure 1352.-1. – *Chromatogram for the test for oligomers, triglycerides, ethyl esters and free fatty acids in omega-3 acids triglycerides*

— *total omega-3 acids, expressed as triglycerides*: minimum 60 per cent.

A suitable antioxidant may be added.

PRODUCTION

The content of dioxins and dioxin-like PCBs (polychlorinated biphenyls) is controlled using methods and limits in accordance with the requirements set in the European Union or other applicable regulations.

CHARACTERS

Appearance

Pale yellow liquid.

Solubility

Practically insoluble in water, very soluble in acetone and in heptane, slightly soluble in anhydrous ethanol.

IDENTIFICATION

Examine the chromatograms obtained in the assay for EPA and DHA.

Results The peaks due to eicosapentaenoic acid methyl ester and docosahexaenoic acid methyl ester in the chromatogram obtained with test solution (b) are similar in retention time to the corresponding peaks in the chromatograms obtained with reference solutions (a_1) and (a_2).

TESTS

Absorbance (*2.2.25*)

Maximum 0.70 at 233 nm.

Dilute 0.300 g to 50.0 mL with *trimethylpentane R*. Dilute 2.0 mL of the solution to 50.0 mL with *trimethylpentane R*.

Acid value (*2.5.1*)

Maximum 3.0, determined on 10.0 g in 50 mL of the prescribed mixture of solvents.

Anisidine value (*2.5.36*)

Maximum 20.0.

Peroxide value (*2.5.5, Method A*)

Maximum 5.0.

Oligomers, triglycerides, ethyl esters and free fatty acids

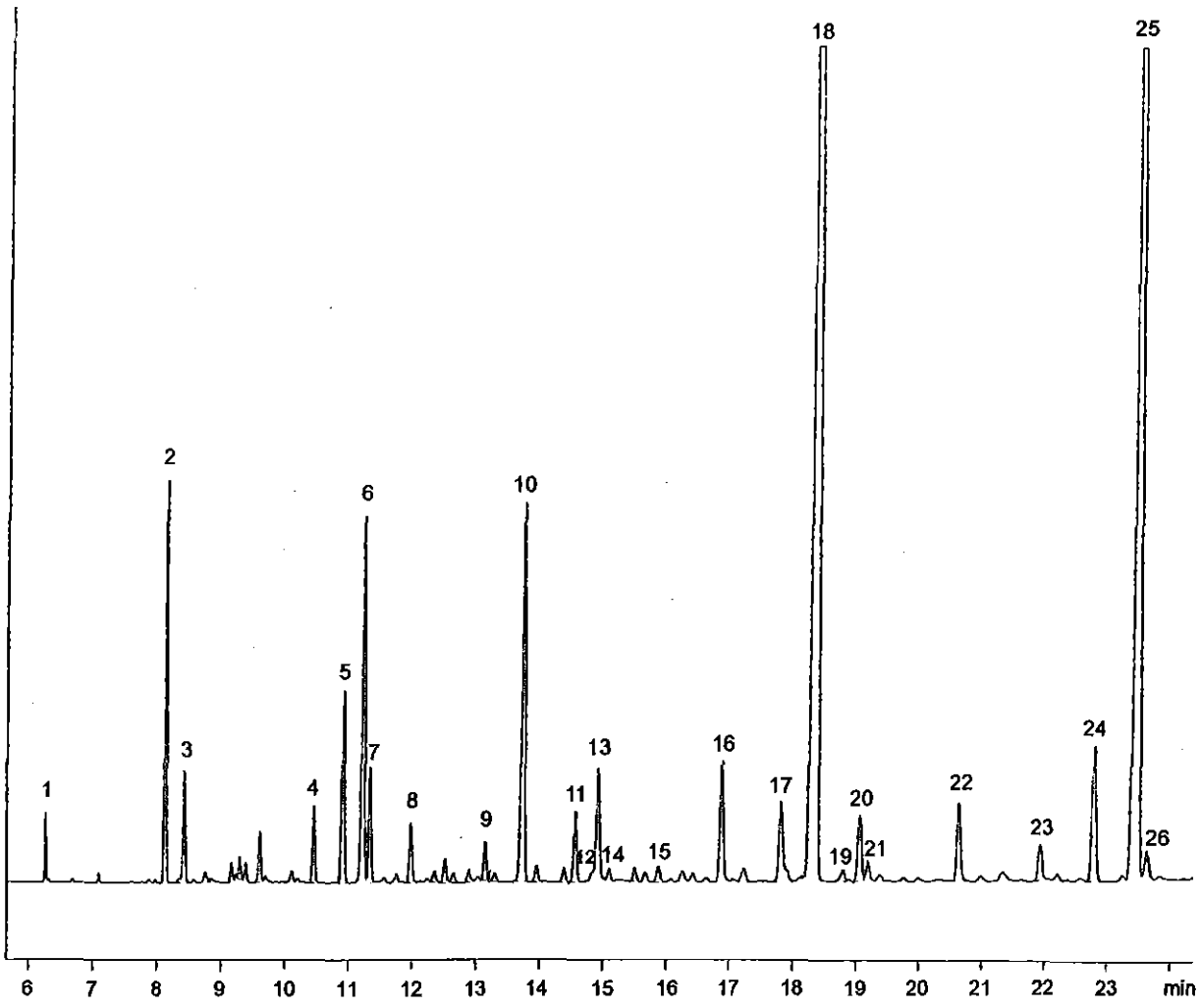
Size-exclusion chromatography (*2.2.30*).

Test solution Dilute 50.0 mg of the substance to be examined to 10.0 mL with *tetrahydrofuran R*.

Reference solution Dissolve 50 mg of *monodocosahexaenoin R*, 30 mg of *didocosahexaenoin R* and 20 mg of *tridocosahexaenoin R* in *tetrahydrofuran R* and dilute to 100.0 mL with the same solvent.

Column 3 columns to be connected in series:

— *size*: l = 0.3 m, Ø = 7.8 mm;



1. C14:0	4. C16:4 n-1	7. C18:1 n-7	10. C18:4 n-3	13. C20:1 n-9	16. C20:4 n-6	19. C22:0	22. C21:5 n-3	25. DHA
2. C16:0	5. C18:0	8. C18:2 n-6	11. C20:0	14. C20:1 n-7	17. C20:4 n-3	20. C22:1 n-11	23. C22:5 n-6	26. C24:1 n-9
3. C16:1 n-7	6. C18:1 n-9	9. C18:3 n-3	12. C20:1 n-11	15. C20:2 n-6	18. EPA	21. C22:1 n-9	24. C22:5 n-3	

Figure 1352.-2. – *Chromatogram for the assays of omega-3 acids in omega-3 acids triglycerides*

— *stationary phase*: *styrene-divinylbenzene copolymer R* (5 µm) with the following pore sizes:
  — *column 1*: 50 nm;
  — *column 2*: 10 nm;
  — *column 3*: 5 nm;
— *connection sequence*: injector – column 1 – column 2 – column 3 – detector.

Mobile phase *tetrahydrofuran R.*

Flow rate 0.8 mL/min.

Detection Differential refractometer.

Injection 40 µL.

System suitability Reference solution:
— *elution order*: tridocosahexaenoin, didocosahexaenoin, monodocosahexaenoin;
— *resolution*: minimum 2.0 between the peaks due to didocosahexaenoin and monodocosahexaenoin; minimum 1.0 between the peaks due to tridocosahexaenoin and didocosahexaenoin.

Identify the peaks using the chromatogram shown in Figure 1352.-1. Calculate the percentage content of oligomers using the following expression:

$$\frac{B}{A} \times 100$$

A = sum of the areas of all the peaks in the chromatogram;
B = area of the peak with a retention time less than the retention time of the peak due to the triglycerides.

Calculate the percentage content of triglycerides using the following expression:

$$\frac{C}{A} \times 100$$

A = sum of the areas of all the peaks in the chromatogram;
C = (sum of the) area(s) of the peak(s) due to triglycerides.

Calculate the percentage content of ethyl esters and free fatty acids using the following expression:

$$\frac{D}{A} \times 100$$

A = sum of the areas of all the peaks in the chromatogram;
D = (sum of the) area(s) of the peak(s) due to ethyl esters and free fatty acids.

Limits:
— *oligomers*: maximum 3.0 per cent;
— *triglycerides*: minimum 50.0 per cent;
— *ethyl esters and free fatty acids*: maximum 5.0 per cent.

ASSAY

EPA and DHA (*2.4.29*)
For identification of the peaks, see Figure 1352.-2.

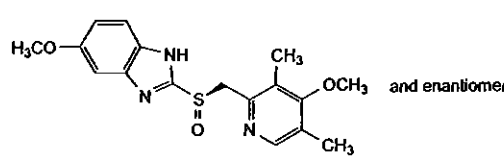
Total omega-3 acids (*2.4.29*)
See Figure 1352.-2.

STORAGE
Under an inert gas, in a well-filled, airtight container, protected from light.

Ph Eur

Omeprazole

(Ph. Eur. monograph 0942)



$C_{17}H_{19}N_3O_3S$ 345.4 *73590-58-6*

Action and use
Proton pump inhibitor; treatment of peptic ulcer disease.

Preparations
Omeprazole Gastro-resistant Tablets
Omeprazole Oral Suspension

Ph Eur

DEFINITION
5-Methoxy-2-[(*RS*)-[(4-methoxy-3,5-dimethylpyridin-2-yl) methyl]sulfinyl]-1*H*-benzimidazole.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Very slightly soluble in water, soluble in methylene chloride, sparingly soluble in ethanol (96 per cent) and in methanol. It dissolves in dilute solutions of alkali hydroxides.

It shows polymorphism (*5.9*).

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *omeprazole CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

TESTS

Solution S
Dissolve 0.50 g in *methylene chloride R* and dilute to 25 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*).

Impurities F and G
Maximum 350 ppm for the sum of the contents.

The absorbance (*2.2.25*) of solution S determined at 440 nm is not greater than 0.10.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 3 mg of the substance to be examined in the mobile phase and dilute to 25.0 mL with the mobile phase.

Reference solution (a) Dissolve 1 mg of *omeprazole CRS* and 1 mg of *omeprazole impurity D CRS* in the mobile phase and dilute to 10 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (c) Dissolve 3 mg of *omeprazole for peak identification CRS* (containing impurity E) in the mobile phase and dilute to 20 mL with the mobile phase.

Column:
— *size*: l = 0.125 m, Ø = 4.6 mm;
— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 27 volumes of *acetonitrile R* and 73 volumes of a 1.4 g/L solution of *disodium hydrogen phosphate dodecahydrate R* previously adjusted to pH 7.6 with *phosphoric acid R*.

Flow rate 1 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 40 µL.

Run time 5 times the retention time of omeprazole.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity D; use the chromatogram supplied with *omeprazole for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peak due to impurity E.

Relative retention With reference to omeprazole (retention time = about 9 min): impurity E = about 0.6; impurity D = about 0.8.

System suitability Reference solution (a):
— *resolution*: minimum 3.0 between the peaks due to impurity D and omeprazole; if necessary, adjust the pH of the aqueous part of the mobile phase or the concentration of *acetonitrile R*; an increase in the pH will improve the resolution.

Limits:
— *impurities D, E*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*
Maximum 0.2 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C at a pressure not exceeding 0.1 kPa for 4 h.

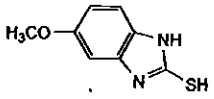
Sulfated ash *(2.4.14)*
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.250 g in a mixture of 10 mL of *water R* and 40 mL of *ethanol (96 per cent) R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

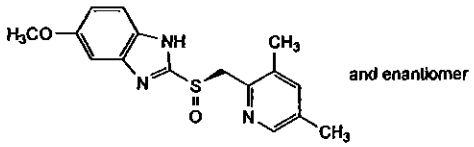
1 mL of *0.1 M sodium hydroxide* is equivalent to 34.54 mg of $C_{17}H_{19}N_3O_3S$.

STORAGE
In an airtight container, protected from light, at a temperature of 2 °C to 8 °C.

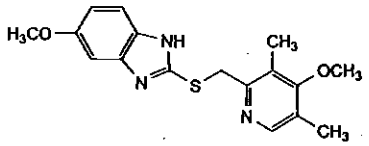
IMPURITIES
Specified impurities *D, E, F, G.*
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, H, I.*



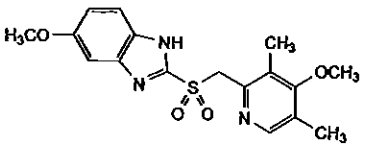
A. 5-methoxy-1*H*-benzimidazole-2-thiol,



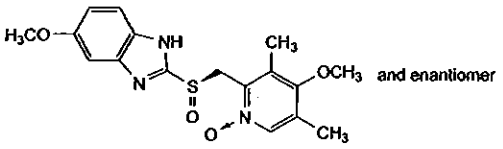
B. 2-[(*RS*)-[(3,5-dimethylpyridin-2-yl)methyl]sulfinyl]-5-methoxy-1*H*-benzimidazole,



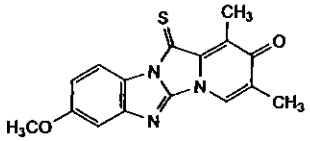
C. 5-methoxy-2-[[(4-methoxy-3,5-dimethylpyridin-2-yl) methyl]sulfanyl]-1*H*-benzimidazole (ufiprazole),



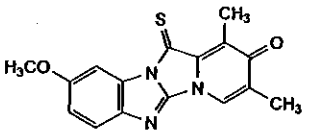
D. 5-methoxy-2-[[(4-methoxy-3,5-dimethylpyridin-2-yl) methyl]sulfonyl]-1*H*-benzimidazole (omeprazole sulfone),



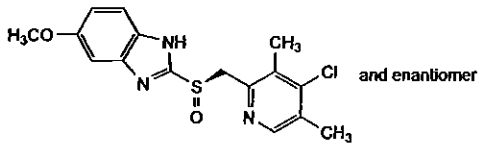
E. 4-methoxy-2-[[(*RS*)-(5-methoxy-1*H*-benzimidazol-2-yl) sulfinyl]methyl]-3,5-dimethylpyridine 1-oxide,



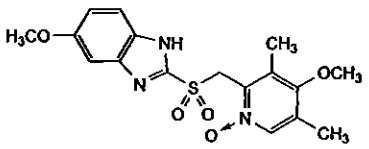
F. 8-methoxy-1,3-dimethyl-12-thioxopyrido [1′,2′:3,4]imidazo[1,2-*a*]benzimidazol-2(12*H*)-one,



G. 9-methoxy-1,3-dimethyl-12-thioxopyrido [1′,2′:3,4]imidazo[1,2-*a*]benzimidazol-2(12*H*)-one,



H. 2-[(*RS*)-[(4-chloro-3,5-dimethylpyridin-2-yl)methyl] sulfinyl]-5-methoxy-1*H*-benzimidazole,

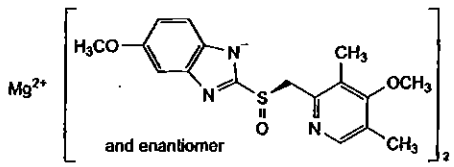


I. 4-methoxy-2-[[(5-methoxy-1*H*-benzimidazol-2-yl)sulfonyl] methyl]-3,5-dimethylpyridine 1-oxide.

Ph Eur

Omeprazole Magnesium

(Ph. Eur. monograph 2374)



$C_{34}H_{36}MgN_6O_6S_2$ 713 *95382-33-5*

Action and use
Proton pump inhibitor; treatment of peptic ulcer disease.

Ph Eur

DEFINITION
Magnesium bis[5-methoxy-2-[(*RS*)-[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]sulfinyl]-1*H*-benzimidazol-1-ide]. It contains a variable quantity of water.

Content
97.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, hygroscopic powder.

Solubility
Very slightly soluble in water, sparingly soluble in methanol, practically insoluble in heptane.

IDENTIFICATION

Carry out either tests A, B, C or tests A, B, D.

A. Optical rotation (*2.2.7*): −0.10° to + 0.10°.

Dissolve 0.250 g in *methanol R* and dilute to 25.0 mL with the same solvent.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *omeprazole magnesium CRS*.

C. Atomic absorption spectrometry (*2.2.23*) as described in the test for magnesium.

The test solution shows the absorption maximum at 285.2 nm.

D. Ignite about 0.5 g of the substance to be examined according to the procedure for the sulfated ash test (*2.4.14*). Dissolve the residue in 10 mL of *water R*. 2 mL of this solution gives the reaction of magnesium (*2.3.1*).

TESTS

Absorbance (*2.2.25*)
Maximum 0.10 at 440 nm.

Dissolve 0.500 g in *methanol R* and dilute to 25.0 mL with the same solvent. Filter the solution through a membrane filter (nominal pore size 0.45 µm).

Related substances
Liquid chromatography (*2.2.29*): use the normalisation procedure. *Prepare the solutions immediately before use.*

Test solution Dissolve 3.5 mg of the substance to be examined in the mobile phase and dilute to 25.0 mL with the mobile phase.

Reference solution (a) Dissolve 1 mg of *omeprazole CRS* and 1 mg of *omeprazole impurity D CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 3 mg of *omeprazole for peak identification CRS* (containing impurity E) in the mobile phase and dilute to 20.0 mL with the mobile phase.

Reference solution (c) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.125 m, Ø = 4.6 mm;
— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 27 volumes of *acetonitrile R* and 73 volumes of a 1.4 g/L solution of *disodium hydrogen phosphate dodecahydrate R* previously adjusted to pH 7.6 with *phosphoric acid R*.

Flow rate 1 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 40 µL.

Run time 5 times the retention time of omeprazole.

Identification of impurities:
— use the chromatogram supplied with *omeprazole for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity E;
— use the chromatogram obtained with reference solution (a) to identify the peak due to impurity D.

Relative retention With reference to omeprazole (retention time = about 9 min): impurity E = about 0.6, impurity D = about 0.8.

System suitability Reference solution (a):
— *resolution*: minimum 3.0 between the peaks due to impurity D and omeprazole; if necessary, adjust the pH of the aqueous part of the mobile phase or its proportion of acetonitrile; an increase in the pH will improve the resolution.

Limits:
— *impurities D, E*: for each impurity, maximum 0.1 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.5 per cent;
— *disregard limit*: half the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Magnesium
3.30 per cent to 3.55 per cent (anhydrous substance).

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Dissolve 0.250 g in 20.0 mL of a 103 g/L solution of *hydrochloric acid R* by slow addition of the acid and dilute to 100.0 mL with *water R*. Dilute 10.0 mL of the solution to 200.0 mL with *water R*. To 10.0 mL of this solution add 4 mL of *lanthanum chloride solution R* and dilute to 100.0 mL with *water R*.

Reference solutions Prepare the reference solutions using *magnesium standard solution (1000 ppm Mg) R*, diluting with a mixture of 1 mL of a 103 g/L solution of *hydrochloric acid R* and 1000.0 mL of *water R*.

Wavelength 285.2 nm.

Water (*2.5.12*)
7.0 per cent to 10.0 per cent, determined on 0.200 g.

ASSAY

Liquid chromatography (*2.2.29*).

Buffer pH 11.0 Mix 11 mL of a 95.0 g/L solution of *trisodium phosphate dodecahydrate R* and 22 mL of a 179.1 g/L solution of *disodium hydrogen phosphate dodecahydrate R*. Dilute to 100.0 mL with *water R*.

Test solution Dissolve 10.0 mg of the substance to be examined in about 10 mL of *methanol R*. Add 10 mL of buffer pH 11.0 and dilute to 200.0 mL with *water R*.

Reference solution Dissolve 10.0 mg of *omeprazole CRS* in about 10 mL of *methanol R*. Add 10 mL of buffer pH 11.0 and dilute to 200.0 mL with *water R*.

Column:
— *size*: *l* = 0.125 m, Ø = 4 mm;
— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 35 volumes of *acetonitrile R* and 65 volumes of a 1.4 g/L solution of *disodium hydrogen phosphate dodecahydrate R* previously adjusted to pH 7.6 with *phosphoric acid R*.

Flow rate 1 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µL.

Run time 1.5 times the retention time of omeprazole.

Retention time Omeprazole = about 4 min.

Calculate the percentage content of $C_{34}H_{36}MgN_6O_6S_2$ from the declared content of *omeprazole CRS*.

1 g of omeprazole is equivalent to 1.032 g of omeprazole magnesium.

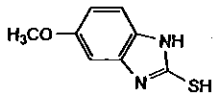
STORAGE

In an airtight container, protected from light.

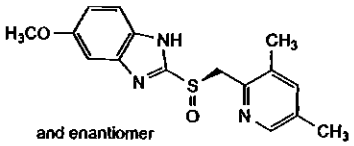
IMPURITIES

Specified impurities D, E.

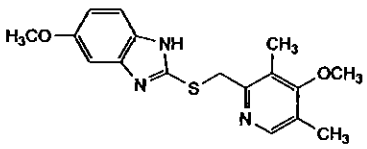
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) A, B, C.



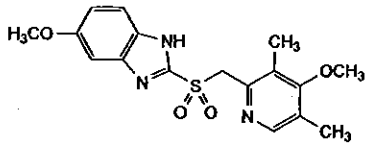
A. 5-methoxy-1*H*-benzimidazole-2-thiol,



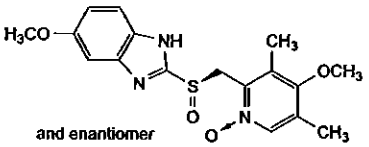
B. 2-[(*RS*)-[(3,5-dimethylpyridin-2-yl)methyl]sulfinyl]-5-methoxy-1*H*-benzimidazole,



C. 5-methoxy-2-[[(4-methoxy-3,5-dimethylpyridin-2-yl) methyl]sulfanyl]-1*H*-benzimidazole,



D. 5-methoxy-2-[[(4-methoxy-3,5-dimethylpyridin-2-yl) methyl]sulfonyl]-1*H*-benzimidazole,

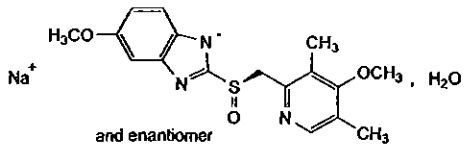


E. 4-methoxy-2-[[(*RS*)-(5-methoxy-1*H*-benzimidazol-2-yl) sulfinyl]methyl]-3,5-dimethylpyridine 1-oxide.

Ph Eur

Omeprazole Sodium

(Ph. Eur. monograph 1032)



$C_{17}H_{18}N_3NaO_3S,H_2O$ 385.4 *95510-70-6*

Action and use

Proton pump inhibitor; treatment of peptic ulcer disease.

Ph Eur

DEFINITION

Sodium 5-methoxy-2-[(*RS*)-[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]sulfinyl]-1*H*-benzimidazole monohydrate.

Content

98.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, hygroscopic powder.

Solubility

Freely soluble in water and in ethanol (96 per cent), soluble in propylene glycol, very slightly soluble in methylene chloride.

IDENTIFICATION

A. Optical rotation (*2.2.7*): −0.10° to + 0.10°, determined on solution S.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Dissolve 0.50 g of the substance to be examined in 1.50 mL of *water R*, add 3.0 mL of *methanol R* and stir; while stirring, adjust to pH 8-9 by adding, dropwise, *dilute acetic acid R* (about 0.4 mL); continue stirring until crystallisation and isolate the crystalline precipitate by filtration; wash with 5 mL of *water R*, then 2 mL of *methanol R*, and dry *in vacuo* at 40 °C for 30 min.

Comparison omeprazole CRS.

If the spectra obtained in the solid state show differences, dissolve the crystalline precipitate and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

C. Ignite 1 g and cool. Add 1 mL of *water R* to the residue and neutralise with *hydrochloric acid R*. Filter and dilute the filtrate to 4 mL with *water R*. 0.1 mL of the solution gives reaction (b) of sodium (*2.3.1*).

TESTS

Solution S

Dissolve 0.50 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_6 (*2.2.2, Method II*).

pH (*2.2.3*)

10.3 to 11.3 for solution S.

Related substances

Liquid chromatography (*2.2.29*). *Prepare solutions immediately before use.*

Test solution Dissolve 3 mg of the substance to be examined in the mobile phase and dilute to 25.0 mL with the mobile phase.

Reference solution (a) Dissolve 1 mg of *omeprazole CRS* and 1 mg of *omeprazole impurity D CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (c) Dissolve 3 mg of *omeprazole for peak identification CRS* (containing impurity E) in the mobile phase and dilute to 25.0 mL with the mobile phase.

Column:
— *size*: l = 0.125 m, Ø = 4.6 mm;
— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 27 volumes of *acetonitrile R* and 73 volumes of a 1.4 g/L solution of *disodium hydrogen phosphate dodecahydrate R*, previously adjusted to pH 7.6 with *phosphoric acid R*.

Flow rate 1 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 40 µL.

Run time 5 times the retention time of omeprazole.

Identification of impurities Use the chromatogram supplied with *omeprazole for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peak due to impurity E; use the chromatogram obtained with reference solution (a) to identify the peak due to impurity D.

Relative retention With reference to omeprazole (retention time = about 9 min): impurity E = about 0.6; impurity D = about 0.8.

System suitability Reference solution (a):
— *resolution*: minimum 3.0 between the peaks due to impurity D and omeprazole; if necessary adjust the pH of the aqueous part of the mobile phase or the concentration of *acetonitrile R*; an increase in the pH will improve the resolution.

Limits:
— *impurities D, E*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.12*)
4.5 per cent to 10.0 per cent, determined on 0.300 g.

ASSAY

Dissolve 0.300 g in 50 mL of *water R*. Titrate with *0.1 M hydrochloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M hydrochloric acid* corresponds to 36.74 mg of $C_{17}H_{18}N_3NaO_3S$.

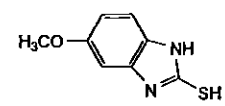
STORAGE

In an airtight container, protected from light.

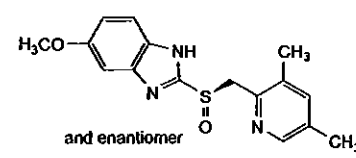
IMPURITIES

Specified impurities *D, E.*

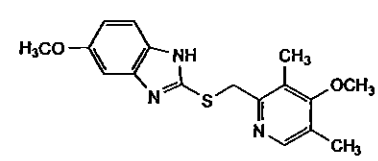
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C.*



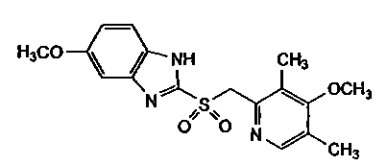
A. 5-methoxy-1*H*-benzimidazole-2-thiol,



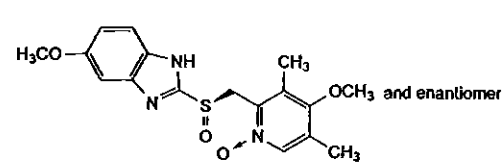
B. 2-[(*RS*)-[(3,5-dimethylpyridin-2-yl)methyl]sulfinyl]-5-methoxy-1*H*-benzimidazole,



C. 5-methoxy-2-[[(4-methoxy-3,5-dimethylpyridin-2-yl) methyl]sulfanyl]-1*H*-benzimidazole (ufiprazole),



D. 5-methoxy-2-[[(4-methoxy-3,5-dimethylpyridin-2-yl) methyl]sulfonyl]-1*H*-benzimidazole (omeprazole-sulfone),

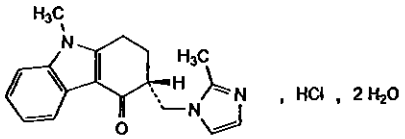


E. 4-methoxy-2-[[(*RS*)-(5-methoxy-1*H*-benzimidazol-2-yl) sulfinyl]methyl]-3,5-dimethylpyridine 1-oxide.

Ph Eur

Ondansetron Hydrochloride Dihydrate

(Ph. Eur. monograph 2016)



and enantiomer

$C_{18}H_{20}ClN_3O,2H_2O$ 365.9 *103639-04-9*

Action and use
Serotonin 5-HT_3 antagonist; treatment of nausea and vomiting.

Preparations
Ondansetron Injection
Ondansetron Tablets

Ph Eur

DEFINITION

(3*RS*)-9-Methyl-3-[(2-methyl-1*H*-imidazol-1-yl)methyl]-1,2,3,9-tetrahydro-4*H*-carbazol-4-one hydrochloride dihydrate.

Content
97.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Sparingly soluble in water, soluble in methanol, sparingly soluble in ethanol (96 per cent), slightly soluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *ondansetron hydrochloride dihydrate CRS.*

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Impurity B
Thin-layer chromatography (*2.2.27*).

Solvent mixture *concentrated ammonia R, ethanol (96 per cent) R, methanol R* (0.5:100:100 *V/V/V*).

Test solution Dissolve 0.125 g of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (a) Dissolve 12.5 mg of *ondansetron for TLC system suitability CRS* (containing impurities A and B) in the solvent mixture and dilute to 1.0 mL with the solvent mixture.

Reference solution (b) Dilute 1 mL of the test solution to 100 mL with the solvent mixture. Dilute 4.0 mL of this solution to 10.0 mL with the solvent mixture.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *concentrated ammonia R, methanol R, ethyl acetate R, methylene chloride R* (2:40:50:90 *V/V/V/V*).

Application 20 µL.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Retardation factors Impurity A = about 0.3; impurity B = about 0.4; ondansetron = about 0.6.

System suitability The chromatogram obtained with reference solution (a) shows 3 clearly separated spots.

Limit:
— *impurity B*: any spot corresponding to impurity B in the chromatogram obtained with the test solution is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.4 per cent).

Related substances
Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Test solution (b) Dissolve 90.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 10.0 mL of the solution to 100.0 mL with the mobile phase.

Reference solution (a) Dilute 2.0 mL of test solution (a) to 100.0 mL with the mobile phase. Dilute 10.0 mL of this solution to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *ondansetron impurity E CRS* and 5 mg of *ondansetron impurity A CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (c) Dissolve 5 mg of *ondansetron for LC system suitability CRS* (containing impurities C and D) in the mobile phase and dilute to 10 mL with the mobile phase.

Reference solution (d) Dissolve 5.0 mg of *ondansetron impurity D CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 100.0 mL with the mobile phase.

Reference solution (e) Dissolve 90.0 mg of *ondansetron hydrochloride dihydrate CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 10.0 mL of the solution to 100.0 mL with the mobile phase.

Reference solution (f) Dissolve 5.0 mg of *ondansetron impurity F CRS* and 5 mg of *ondansetron impurity G CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (g) To 1.0 mL of reference solution (b) add 1.0 mL of reference solution (f) and dilute to 100.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *cyanosilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 20 volumes of *acetonitrile R1* and 80 volumes of a 2.8 g/L solution of *sodium dihydrogen phosphate monohydrate R* previously adjusted to pH 5.4 with a 40 g/L solution of *sodium hydroxide R.*

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 216 nm.

Injection 20 µL of test solution (a) and reference solutions (a), (b), (c), (d), (f) and (g).

Run time 1.5 times the retention time of ondansetron.

Identification of impurities:
— use the chromatogram supplied with *ondansetron for LC system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities C and D;

— use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and E;
— use the chromatogram obtained with reference solution (f) to identify the peaks due to impurities F and G.

Relative retention With reference to ondansetron (retention time = about 18 min): impurity E = about 0.17; impurity F = about 0.20 (E and F may coelute); impurity C = about 0.35; impurity D = about 0.45; impurity A = about 0.80; impurity G = about 0.89 (A and G may coelute or be inverted).

System suitability Reference solution (c):
— *resolution*: minimum 2.5 between the peaks due to impurities C and D.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity C by 0.6;
— *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *impurity D*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.15 per cent);
— *sum of impurities A and G*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *sum of impurities E and F*: not more than the sum of the areas of the corresponding peaks in the chromatogram obtained with reference solution (g) (0.2 per cent);
— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: maximum 0.4 per cent;
— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)
9.0 per cent to 10.5 per cent, determined on 0.200 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (e).

Calculate the percentage content of $C_{18}H_{20}ClN_3O$ from the declared content of *ondansetron hydrochloride dihydrate CRS*.

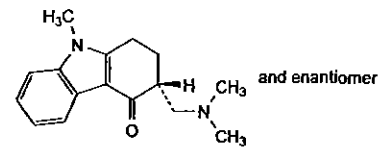
STORAGE

Protected from light.

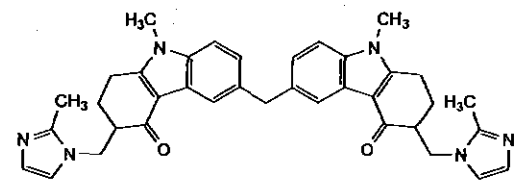
IMPURITIES

Specified impurities *A, B, C, D, E, F, G.*

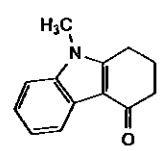
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *H.*



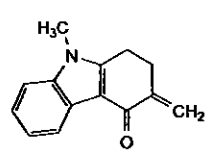
A. (3*RS*)-3-[(dimethylamino)methyl]-9-methyl-1,2,3,9-tetrahydro-4*H*-carbazol-4-one,



B. 6,6′-methylenebis[(3*RS*)-9-methyl-3-[(2-methyl-1*H*-imidazol-1-yl)methyl]-1,2,3,9-tetrahydro-4*H*-carbazol-4-one],

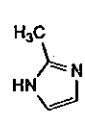


C. 9-methyl-1,2,3,9-tetrahydro-4*H*-carbazol-4-one,

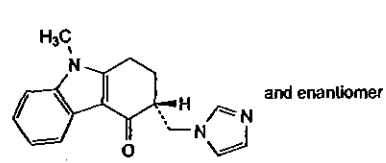


D. 9-methyl-3-methylene-1,2,3,9-tetrahydro-4*H*-carbazol-4-one,

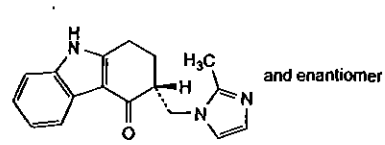
E. 1*H*-imidazole,



F. 2-methyl-1*H*-imidazole,



G. (3*RS*)-3-[(1*H*-imidazol-1-yl)methyl]-9-methyl-1,2,3,9-tetrahydro-4*H*-carbazol-4-one (*C*-desmethylondansetron),

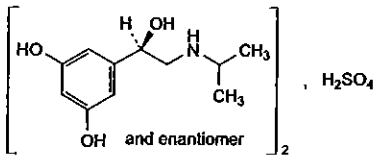


H. (3*RS*)-3-[(2-methyl-1*H*-imidazol-1-yl)methyl]-1,2,3,9-tetrahydro-4*H*-carbazol-4-one (*N*-desmethylondansetron).

Orciprenaline Sulfate

Orciprenaline Sulphate

(Ph. Eur. monograph 1033)



$C_{22}H_{36}N_2O_{10}S$ 520.6 *5874-97-5*

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

Ph Eur

DEFINITION

Bis[5-[(1*RS*)-1-hydroxy-2-[(1-methylethyl)amino] ethyl]benzene-1,3-diol] sulfate.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, slightly hygroscopic, crystalline powder.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

First identification: B, E.

Second identification: A, C, D, E.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 50.0 mg in a 0.04 per cent *V/V* solution of *hydrochloric acid R* and dilute to 50.0 mL with the same solution. Dilute 5.0 mL of this solution to 50.0 mL with a 0.04 per cent *V/V* solution of *hydrochloric acid R*.

Spectral range 240-350 nm.

Absorption maximum At 278 nm.

Specific absorbance at the absorption maximum 68.5 to 76.0 (anhydrous substance).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *orciprenaline sulfate CRS*.

If the spectra obtained show differences, dissolve separately, with heating, 50 mg of the substance to be examined and 50 mg of the reference substance, in the minimum volume of *water R*. Add 10 mL of *acetone R* and centrifuge. Dry the precipitates at 40 °C under reduced pressure for 3 h and record new spectra using the residues.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *ethanol (96 per cent) R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dissolve 10 mg of *orciprenaline sulfate CRS* in *ethanol (96 per cent) R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 10 mg of *orciprenaline sulfate CRS* and 10 mg of *salbutamol CRS* in *ethanol (96 per cent) R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *ammonia R, water R, aldehyde-free methanol R* (1.5:10:90 *V/V/V*).

Application 2 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with a 10 g/L solution of *potassium permanganate R*.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated principal spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 20 mg in 2 mL of *ethanol (96 per cent) R*. Add 2 mL of a 1 g/L solution of *dichloroquinonechlorimide R* in *ethanol (96 per cent) R* and 1 mL of *sodium carbonate solution R*. A violet colour is produced, turning to brown.

E. It gives reaction (a) of sulfates (*2.3.1*).

TESTS

Solution S

Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 20 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)

4.0 to 5.5 for solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20 mg of the substance to be examined in the mobile phase and dilute to 20 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 2 mg of *orciprenaline for system suitability CRS* (containing impurities A and B) in 2.0 mL of the mobile phase.

Column:

— *size*: *l* = 0.125 m, Ø = 4.0 mm;

— *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 45 °C.

Mobile phase Dissolve 9.1 g of *potassium dihydrogen phosphate R* and 4.6 g of *sodium octanesulfonate R* in *water R*, adjust to pH 4.0 with *dilute phosphoric acid R* and dilute to 1000 mL with *water R*. Add 140 mL of *acetonitrile R*.

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 10 µL.

Run time Twice the retention time of orciprenaline.

Identification of impurities Use the chromatogram supplied with *orciprenaline for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and B.

Relative retention With reference to orciprenaline (retention time = about 7 min): impurity A = about 0.9; impurity B = about 1.3.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to impurity A and orciprenaline.

Limits:
- — *correction factor*: for the calculation of content, multiply the peak area of impurity B by 0.3;
- — *impurities A, B*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- — *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- — *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- — *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Phenone
Maximum 0.1 per cent.

Dissolve 0.50 g in a 0.04 per cent *V/V* solution of *hydrochloric acid R* and dilute to 25.0 mL with the same solution.
The absorbance (*2.2.25*) of the solution measured at 328 nm is not greater than 0.16.

Iron (*2.4.9*)
Maximum 20 ppm.

The residue obtained in the test for sulfated ash complies with the test. Prepare the reference solution using *iron standard solution (2 ppm Fe) R*.

Water (*2.5.12*)
Maximum 2.0 per cent, determined on 1.000 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

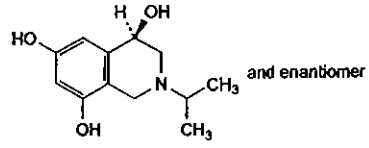
ASSAY
Dissolve 0.400 g in 5 mL of *anhydrous formic acid R* and add 30 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* using 0.1 mL of *crystal violet solution R* as indicator.

1 mL of *0.1 M perchloric acid* is equivalent to 52.06 mg of $C_{22}H_{36}N_2O_{10}S$.

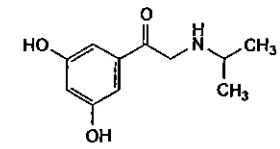
STORAGE
In an airtight container, protected from light.

IMPURITIES
Specified impurities *A, B.*

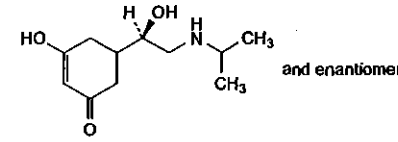
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C.*



A. (4*RS*)-2-(1-methylethyl)-1,2,3,4-tetrahydroisoquinoline-4,6,8-triol,



B. 1-(3,5-dihydroxyphenyl)-2-[(1-methylethyl) amino]ethanone,

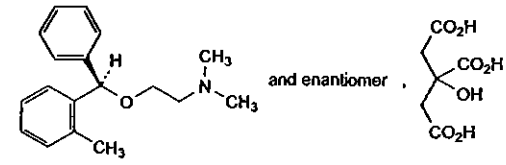


C. 3-hydroxy-5-[(1*RS*)-1-hydroxy-2-[(1-methylethyl) amino]ethyl]cyclohex-2-enone.

Ph Eur

Orphenadrine Citrate

(Ph. Eur. monograph 1759)



$C_{24}H_{31}NO_8$ 461.5 *4682-36-4*

Action and use
Anticholinergic.

Ph Eur

DEFINITION
(*RS*)-*N*,*N*-Dimethyl-2-[(2-methylphenyl) phenylmethoxy]ethanamine dihydrogen 2-hydroxypropane-1,2,3-tricarboxylate.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Sparingly soluble in water, slightly soluble in ethanol (96 per cent).

mp
About 137 °C.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *orphenadrine citrate CRS.*

TESTS
Appearance of solution
The solution is clear (*2.2.1*) and its absorbance (*2.2.25*) at 436 nm has a maximum of 0.050.

Dissolve 1.0 g in a 3.6 per cent *V/V* solution of *hydrochloric acid R* in *ethanol (96 per cent) R* and dilute to 10.0 mL with the same acid solution.

Related substances
Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution Dissolve 0.500 g of the substance to be examined in *water R* and dilute to 50 mL with the same solvent. Add 2 mL of *concentrated ammonia R* and shake with 3 quantities, each of 10 mL, of *toluene R*. To the combined upper layers add *anhydrous sodium sulfate R*, shake, filter and evaporate the filtrate by suitable means, at a temperature not exceeding 50 °C. Take up the residue with *toluene R* and dilute to 20.0 mL with the same solvent.

Reference solution (a) Dissolve 30 mg of *orphenadrine citrate CRS* and 30 mg of *orphenadrine impurity E CRS* in 20 mL of *water R*. Add 1 mL of *concentrated ammonia R* and shake with 3 quantities, each of 5 mL, of *toluene R*. To the combined upper layers add *anhydrous sodium sulfate R*, shake, filter and evaporate the filtrate by suitable means, at a temperature not exceeding 50 °C. Take up the residue with *toluene R* and dilute to 20.0 mL with the same solvent.

Reference solution (b) Dissolve the contents of a vial of *orphenadrine for peak identification CRS* (containing impurities A, B, C, D and F) in 1.0 mL of *toluene R*.

Column:
— *size*: *l* = 60 m, Ø = 0.32 mm;
— *stationary phase*: *phenyl(5)methyl(95)polysiloxane R* (film thickness 1.0 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1 mL/min.

Split ratio 1:25.

Temperature:
— *column*: 240 °C;
— *injection port and detector*: 290 °C.

Detection Flame ionisation.

Injection 2 µL.

Run time 1.3 times the retention time of orphenadrine.

Identification of impurities Use the chromatogram supplied with *orphenadrine for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C, D and F. Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity E.

Relative retention With reference to orphenadrine (retention time = about 13 min): impurity B = about 0.5; impurity A = about 0.6; impurity D = about 0.8; impurity C = about 0.9; impurity E = about 0.98; impurity F = about 1.1.

System suitability Reference solution (a):
— *resolution*: minimum of 1.5 between the peaks due to impurity E and orphenadrine.

Limits:
— *impurities A, B, C, D, E, F*: for each impurity, not more than 0.3 per cent;
— *unspecified impurities*: for each impurity, not more than 0.10 per cent;
— *total*: maximum 1.0 per cent;
— *disregard limit*: 0.05 per cent.

Loss on drying *(2.2.32)*
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulfated ash *(2.4.14)*
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.350 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

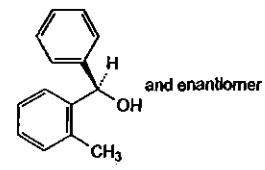
1 mL of *0.1 M perchloric acid* is equivalent to 46.15 mg of $C_{24}H_{31}NO_8$.

STORAGE

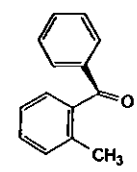
Protected from light. If the substance is sterile, store in a sterile, airtight, tamper-evident container, protected from light.

IMPURITIES

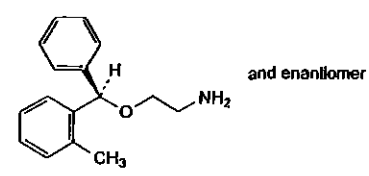
Specified impurities *A, B, C, D, E, F.*



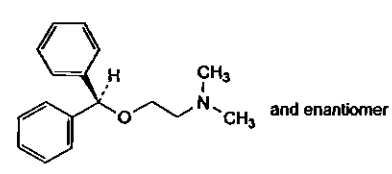
A. (*RS*)-(2-methylphenyl)phenylmethanol (2-methylbenzhydrol),



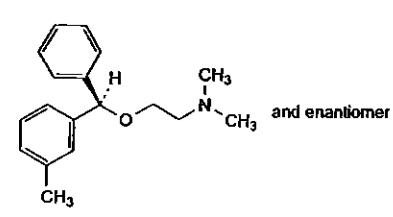
B. (2-methylphenyl)phenylmethanone (2-methylbenzophenone),



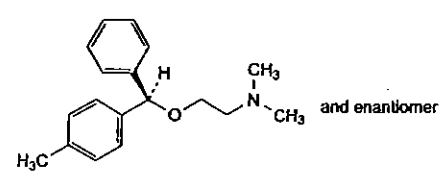
C. (*RS*)-2-[(2-methylphenyl)phenylmethoxy]ethanamine,



D. 2-(diphenylmethoxy)-*N*,*N*-dimethylethanamine (diphenhydramine),



E. (*RS*)-*N*,*N*-dimethyl-2-[(3-methylphenyl) phenylmethoxy]ethanamine (*meta*-methylbenzyl isomer),

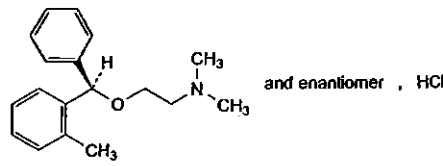


F. (*RS*)-*N*,*N*-dimethyl-2-[(4-methylphenyl) phenylmethoxy]ethanamine (*para*-methylbenzyl isomer).

Ph Eur

Orphenadrine Hydrochloride

(Ph. Eur. monograph 1760)



$C_{18}H_{24}ClNO$ 305.9 *341-69-5*

Action and use
Anticholinergic.

Preparation
Orphenadrine Hydrochloride Tablets

Ph Eur

DEFINITION

(*RS*)-*N*,*N*-Dimethyl-2-[(2-methylphenyl)phenylmethoxy]ethanamine hydrochloride.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water and in ethanol (96 per cent).

mp
About 160 °C.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *orphenadrine hydrochloride CRS.*

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and its absorbance (*2.2.25*) at 436 nm has a maximum of 0.050.

Dissolve 0.70 g in *ethanol (96 per cent) R* and dilute to 10.0 mL with the same solvent.

Related substances
Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution Dissolve 0.300 g of the substance to be examined in *water R* and dilute to 50 mL with the same solvent. Add 2 mL of *concentrated ammonia R* and shake with 3 quantities, each of 10 mL, of *toluene R*. To the combined upper layers add *anhydrous sodium sulfate R*, shake, filter and evaporate the filtrate by suitable means, at a temperature not exceeding 50 °C. Take up the residue with *toluene R* and dilute to 20.0 mL with the same solvent.

Reference solution (a) Dissolve 20 mg of *orphenadrine hydrochloride CRS* and 20 mg of *orphenadrine impurity E CRS* in 20 mL of *water R*. Add 1 mL of *concentrated ammonia R* and shake with 3 quantities, each of 5 mL, of *toluene R*. To the combined upper layers add *anhydrous sodium sulfate R*, shake, filter and evaporate the filtrate by suitable means, at a temperature not exceeding 50 °C. Take up the residue with *toluene R* and dilute to 20.0 mL with the same solvent.

Reference solution (b) Dissolve the contents of a vial of *orphenadrine for peak identification CRS* (containing impurities A, B, C, D and F) in 1.0 mL of *toluene R*.

Column:
— *size*: *l* = 60 m, Ø = 0.32 mm;
— *stationary phase*: *phenyl(5)methyl(95)polysiloxane R* (film thickness 1.0 µm).

Carrier gas *helium for chromatography R.*

Flow rate 1 mL/min.

Split ratio 1:25.

Temperature:
— *column*: 240 °C;
— *injection port and detector*: 290 °C.

Detection Flame ionisation.

Injection 2 µL.

Run time 1.3 times the retention time of orphenadrine.

Identification of impurities Use the chromatogram supplied with *orphenadrine for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C, D and F. Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity E.

Relative retention With reference to orphenadrine (retention time = about 13 min): impurity B = about 0.5; impurity A = about 0.6; impurity D = about 0.8; impurity C = about 0.9; impurity E = about 0.98; impurity F = about 1.1.

System suitability Reference solution (a):
— *resolution*: minimum 1.5 between the peaks due to impurity E and orphenadrine.

Limits:
— *impurities A, B, C, D, E, F*: for each impurity, not more than 0.3 per cent;
— *unspecified impurities*: for each impurity, not more than 0.10 per cent;
— *total*: not more than 1.0 per cent;
— *disregard limit*: 0.05 per cent.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 50 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

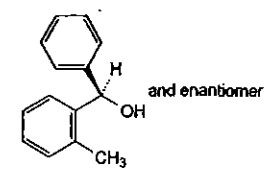
1 mL of *0.1 M perchloric acid* is equivalent to 30.59 mg of $C_{18}H_{24}ClNO$.

STORAGE

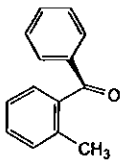
Protected from light. If the substance is sterile, store in a sterile, airtight, tamper-evident container, protected from light.

IMPURITIES

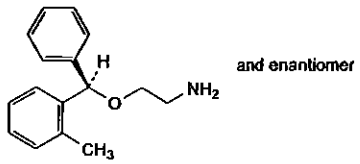
Specified impurities *A, B, C, D, E, F.*



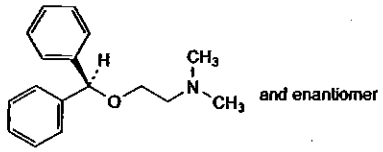
A. (*RS*)-(2-methylphenyl)phenylmethanol (2-methylbenzhydrol),



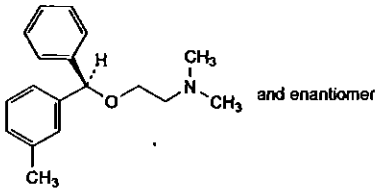
B. (2-methylphenyl)phenylmethanone (2-methylbenzophenone),



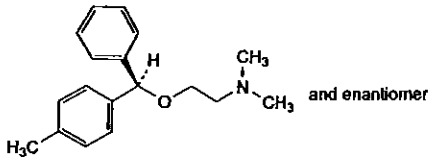
C. (*RS*)-2-[(2-methylphenyl)phenylmethoxy]ethanamine,



D. 2-(diphenylmethoxy)-*N*,*N*-dimethylethanamine (diphenhydramine),



E. (*RS*)-*N*,*N*-dimethyl-2-[(3-methylphenyl) phenylmethoxy]ethanamine (*meta*-methylbenzyl isomer),

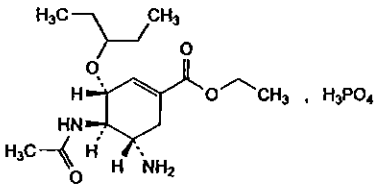


F. (*RS*)-*N*,*N*-dimethyl-2-[(4-methylphenyl) phenylmethoxy]ethanamine (*para*-methylbenzyl isomer).

Ph Eur

Oseltamivir Phosphate

(Ph. Eur. monograph 2422)



$C_{16}H_{31}N_2O_8P$ 410.4 *204255-11-8*

Action and use
Treatment of influenza.

Preparation
Paediatric Oseltamivir Oral Solution

Ph Eur

DEFINITION
Ethyl (3*R*,4*R*,5*S*)-4-acetamido-5-amino-3-(1-ethylpropoxy)cyclohex-1-ene-1-carboxylate phosphate.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Freely soluble in water and in methanol, practically insoluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION
A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *oseltamivir phosphate (impurity B-free) CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

C. Dissolve 200 mg in 10 mL of *water R*. It gives reaction (b) of phosphates (*2.3.1*).

TESTS

Specific optical rotation (*2.2.7*)
−30.7 to −32.6 (anhydrous substance), measured at 25 °C.

Dissolve 0.50 g in *water R* and dilute to 50.0 mL with the same solvent.

Impurity B
Liquid chromatography (*2.2.29*) coupled with mass spectrometry (*2.2.43*).

Test solution Dissolve 0.100 g of the substance to be examined in *water for chromatography R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dissolve 2.5 mg of *oseltamivir impurity B CRS* in 5.0 mL of *anhydrous ethanol R* and dilute to 50.0 mL with *water for chromatography R*. Dilute 2.0 mL of the solution to 100.0 mL with *water for chromatography R*.

Reference solution (b) Dissolve 50.0 mg of *oseltamivir phosphate (impurity B-free) CRS* in reference solution (a) and dilute to 5.0 mL with the same solution.

Column:
— *size*: *l* = 0.05 m, Ø = 3.0 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 40 °C.

Mobile phase Mix 10 volumes of a 1.54 g/L solution of *ammonium acetate R* in *water for chromatography R*, 30 volumes of *acetonitrile R1* and 60 volumes of *water for chromatography R*.

Flow rate 1.5 mL/min.

Post-column split ratio Use a split ratio suitable for the mass detector (e.g. 1:3).

Detection:
— mass detector: the following settings have been found to be suitable and are given as examples; if the detector has different setting parameters, adjust the detector settings so as to comply with the system suitability criterion:
  — ionisation: ESI-positive;
  — detection *m/z*: 356.2;
  — dwell: 580 ms;

— gain EMV: 1;
— fragmentator voltage: 120 V;
— gas temperature: 350 °C;
— drying gas flow: 13 L/min,
— nebuliser pressure: 345 kPa;
— capillary voltage (Vcap): 3 kV.

Injection 1 µL of the test solution and reference solution (b).

Run time 3 min.

System suitability Reference solution (b):
— *repeatability*: maximum relative standard deviation of 15 per cent determined on 6 injections.

Limit:
— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (100 ppm).

Impurity H

Gas chromatography (*2.2.28*).

Silylation reagent Mix 1.0 mL of *chlorotrimethylsilane R*, 2.0 mL of *hexamethyldisilazane R* and 10.0 mL of *anhydrous pyridine R*.

Test solution Introduce 15.0 mg of the substance to be examined into a 2 mL vial and add 1.0 mL of the silylation reagent. Close the vial, shake and heat at 60 °C for 20 min. Centrifuge and discard the precipitate.

Reference solution Introduce 15.0 mg of *oseltamivir impurity H CRS* into a 2 mL vial and add 1.0 mL of *anhydrous pyridine R*. Close the vial and shake (solution A). (Note: impurity H is hygroscopic.) Introduce 15.0 mg of the substance to be examined into another 2 mL vial and add 1.0 mL of the silylation reagent. Close the vial, shake and heat at 60 °C for 20 min. Centrifuge and discard the precipitate (solution B). Introduce 10.0 µL of solution A and 10.0 µL of solution B into a volumetric flask and dilute to 10.0 mL with *anhydrous pyridine R*.

Column:
— *material*: fused silica;
— *size*: *l* = 30 m, Ø = 0.32 mm;
— *stationary phase*: *methylpolysiloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.2 mL/min.

Split ratio 1:50.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 2	180
	2 - 11	180 → 250
	11 - 21	250
Injection port		260
Detector		260

Detection Flame ionisation.

Injection 1 µL.

Relative retention With reference to oseltamivir phosphate (retention time = about 10 min): impurity H = about 0.5.

System suitability Reference solution:
— *repeatability*: maximum relative standard deviation of 5 per cent for the peak due to impurity H after 6 injections.

Limit:
— *impurity H*: not more than 1.5 times the area of the corresponding peak in the chromatogram obtained with the reference solution (0.15 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R1, methanol R2, water for chromatography R* (135:245:620 *V/V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 5 mg of *oseltamivir impurity A CRS* and 5.0 mg of *oseltamivir impurity C CRS* in the solvent mixture and dilute to 50.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 50.0 mg of *oseltamivir phosphate (impurity B-free) CRS* in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 50 °C.

Mobile phase Mix 135 volumes of *acetonitrile R1*, 245 volumes of *methanol R2* and 620 volumes of a 6.8 g/L solution of *potassium dihydrogen phosphate R* in *water for chromatography R*, adjusted to pH 6.0 with a 1 M potassium hydroxide solution prepared from *potassium hydroxide R*.

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 207 nm.

Injection 15 µL of the test solution and reference solutions (a) and (b).

Run time Twice the retention time of oseltamivir phosphate.

Relative retention With reference to oseltamivir phosphate (retention time = about 17 min): impurity A = about 0.16; impurity C = about 0.17.

System suitability Reference solution (b):
— *resolution*: minimum 1.5 between the peaks due to impurities A and C.

Limits:
— *impurity C*: not more than 0.3 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 7 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.7 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)

Maximum 0.5 per cent, determined on 0.500 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (c).

Calculate the percentage content of $C_{16}H_{31}N_2O_8P$ from the declared content of *oseltamivir phosphate (impurity B-free) CRS.*

STORAGE

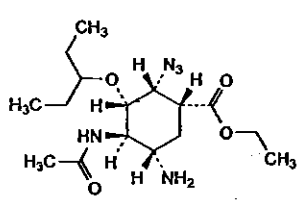
Protected from light.

IMPURITIES

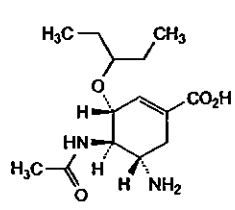
Specified impurities *B, C, H.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, D, E, F, G.*

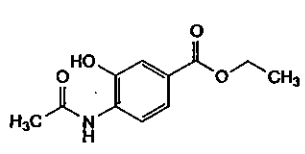
A. (3*R*,4*R*,5*S*)-5-acetamido-4-amino-3-(1-ethylpropoxy)cyclohex-1-ene-1-carboxylic acid,



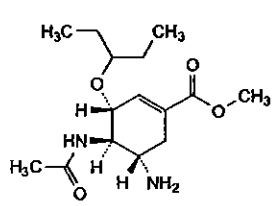
B. ethyl (1*R*,2*R*,3*S*,4*R*,5*S*)-4-acetamido-5-amino-2-azido-3(1-ethylpropoxy)cyclohexanecarboxylate,



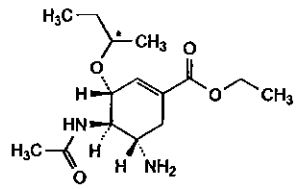
C. (3*R*,4*R*,5*S*)-4-acetamido-5-amino-3-(1-ethylpropoxy)cyclohex-1-ene-1-carboxylic acid,



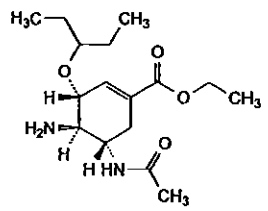
D. ethyl 4-acetamido-3-hydroxybenzoate,



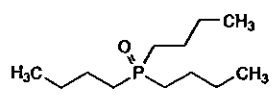
E. methyl (3*R*,4*R*,5*S*)-4-acetamido-5-amino-3-(1-ethylpropoxy)cyclohex-1-ene-1-carboxylate,



F. ethyl (3*R*,4*R*,5*S*)-4-acetamido-5-amino-3-(1-methylpropoxy)cyclohex-1-ene-1-carboxylate,



G. ethyl (3*R*,4*R*,5*S*)-5-acetamido-4-amino-3-(1-ethylpropoxy) cyclohex-1-ene-1-carboxylate,

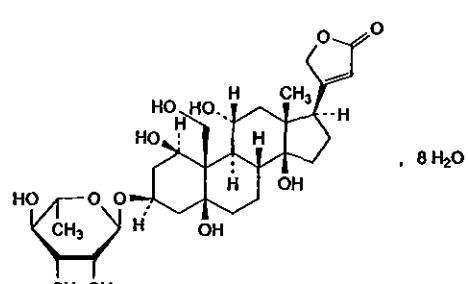


H. tributylphosphane oxide.

Ph Eur

Ouabain

(Ph. Eur. monograph 0048)



$C_{29}H_{44}O_{12},8H_2O$ 729 *11018-89-6*

Ph Eur

DEFINITION

3β-[(6-Deoxy-α-L-mannopyranosyl)oxy]-1β,5,11α,14,19-pentahydroxy-5β,14β-card-20(22)-enolide octahydrate.

Content

96.0 per cent to 104.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Sparingly soluble in water and in anhydrous ethanol, practically insoluble in ethyl acetate.

IDENTIFICATION

A. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with the test solution is similar in position, colour

and size to the spot in the chromatogram obtained with reference solution (a).

B. Dissolve 2 mg to 3 mg in 2 mL of *sulfuric acid R*; a pink colour develops which quickly changes to red. The solution shows green fluorescence in ultraviolet light.

C. Dissolve about 1 mg in 1 mL of *dinitrobenzene solution R* and add 0.2 mL of *dilute sodium hydroxide solution R*. An intense blue colour develops.

D. Dissolve 0.1 g in 5 mL of a 150 g/L solution of *sulfuric acid R* and boil for a few minutes. The solution becomes yellow and turbid. Filter and add to the filtrate 5 mL of a 120 g/L solution of *sodium hydroxide R* and 3 mL of *cupri-tartaric solution R*. Heat. A red precipitate is formed.

TESTS

Solution S
Dissolve 0.20 g in 15 mL of *water R*, heating on a water-bath. Allow to cool and dilute to 20.0 mL with *water R*.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*)
−33 to −30 (anhydrous substance), determined on solution S.

Related substances
Thin-layer chromatography (*2.2.27*).

Solvent mixture *water R, chloroform R, methanol R* (16:50:50 *V/V/V*).

Test solution Dissolve a quantity of the substance to be examined corresponding to 20 mg of the anhydrous substance in 1.0 mL of the solvent mixture.

Reference solution (a) Dissolve a quantity of *ouabain CRS* corresponding to 20 mg of the anhydrous substance in 1.0 mL of the solvent mixture.

Reference solution (b) Dissolve a quantity of *ouabain CRS* corresponding to 10 mg of the anhydrous substance in the solvent mixture and dilute to 25 mL with the solvent mixture.

Reference solution (c) Dilute 2.5 mL of reference solution (b) to 10 mL with the solvent mixture.

Plate *TLC silica gel G plate R.*

Mobile phase *water R, dimethyl sulfoxide R, methanol R, chloroform R* (4:15:15:70 *V/V/V/V*); homogenise the mixture before use.

Application 5 µL.

Development Over a path of 13 cm.

Drying Immediately at 140 °C for 30 min in a ventilated oven.

Detection Allow to cool, spray with *alcoholic solution of sulfuric acid R* and heat at 140 °C for 15 min.

System suitability:
- the principal spot in the chromatogram obtained with the test solution and the principal spot in the chromatogram obtained with reference solution (a) migrate over a distance sufficient to give unequivocal separation of the secondary spots;
- the chromatogram obtained with reference solution (c) shows a clearly visible spot.

Limit:
- *any impurity*: any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (2.0 per cent).

Alkaloids and strophanthin-K
To 5.0 mL of solution S add 0.5 mL of a 100 g/L solution of *tannic acid R*. No precipitate is formed.

Water (*2.5.12*)
18.0 per cent to 22.0 per cent, determined on 0.100 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Test solution Dissolve 40.0 mg in *ethanol (96 per cent) R* and dilute to 50.0 mL with the same solvent. Dilute 5.0 mL of the solution to 100.0 mL with *ethanol (96 per cent) R*.

Reference solution Dissolve 40.0 mg of *ouabain CRS* in *ethanol (96 per cent) R* and dilute to 50.0 mL with the same solvent. Dilute 5.0 mL of the solution to 100.0 mL with *ethanol (96 per cent) R*.

To 5.0 mL of each solution add 3.0 mL of *alkaline sodium picrate solution R*, allow to stand protected from bright light for 30 min and measure the absorbance (*2.2.25*) of both solutions at the absorption maximum at 495 nm using as the compensation liquid a mixture of 3.0 mL of *alkaline sodium picrate solution R* and 5.0 mL of *ethanol (96 per cent) R* prepared at the same time.

Calculate the percentage content of $C_{29}H_{44}O_{12}$ from the absorbances measured and the concentrations of the solutions.

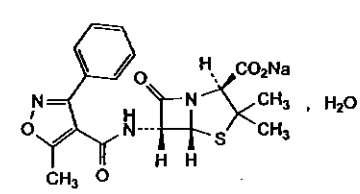
STORAGE

Protected from light.

Ph Eur

Oxacillin Sodium Monohydrate

(Ph. Eur. Monograph 2260)



$C_{19}H_{18}N_3NaO_5S,H_2O$ 441.4 *7240-38-2*

Action and use
Penicillin antibacterial.

Ph Eur

DEFINITION

Sodium (2*S*,5*R*,6*R*)-3,3-dimethyl-6-[[(5-methyl-3-phenylisoxazol-4-yl)carbonyl]amino]-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrate.

Semi-synthetic product derived from a fermentation product.

Content
95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Freely soluble in water, soluble in methanol, practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *oxacillin sodium monohydrate CRS.*

B. It gives reaction (a) of sodium (*2.3.1*).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and its absorbance (*2.2.25*) at 430 nm is not greater than 0.10.

Dissolve 2.50 g in *water R* and dilute to 25.0 mL with the same solvent.

pH (*2.2.3*)

4.5 to 7.5.

Dissolve 0.30 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Specific optical rotation (*2.2.7*)

+ 196 to + 212 (anhydrous substance).

Dissolve 0.250 g in *water R* and dilute to 25.0 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Test solution (b) Dilute 5.0 mL of test solution (a) to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *oxacillin sodium monohydrate CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 5.0 mL of the solution to 50.0 mL with the mobile phase.

Reference solution (b) Dilute 5.0 mL of test solution (b) to 50.0 mL with the mobile phase.

Reference solution (c) Dissolve 5 mg of *cloxacillin sodium CRS* (impurity E) and 5 mg of *oxacillin sodium monohydrate CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (d) In order to prepare impurities B and D *in situ*, dissolve 25 mg of the substance to be examined in 1 mL of *0.05 M sodium hydroxide*, allow to stand for 3 min, then dilute to 100 mL with the mobile phase. Inject immediately.

Reference solution (e) Dissolve 5 mg of *oxacillin for peak identification CRS* (containing impurities E, F, G, I and J) in 5 mL of the mobile phase.

Column:
- *size*: *l* = 0.25 m, Ø = 4.0 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 25 volumes of *acetonitrile R* and 75 volumes of a 2.7 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 5.0 with *dilute sodium hydroxide solution R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 225 nm.

Injection 20 µL of test solution (a) and reference solutions (b), (c), (d) and (e).

Run time 7 times the retention time of oxacillin.

Identification of impurities:
- in the chromatogram obtained with reference solution (d), the 2 principal peaks eluting before the main peak are due to impurities B and D respectively;
- use the chromatogram supplied with *oxacillin for peak identification CRS* and the chromatogram obtained with reference solution (e) to identify the peaks due to impurities E, F, G, I and J.

Relative retention With reference to oxacillin (retention time = about 5 min): impurity A = about 0.3; impurity B (isomer 1) = about 0.4; impurity B (isomer 2) = about 0.5; impurity C = about 0.65; impurity D (2 epimers) = about 0.9; impurity E = about 1.5; impurity F = about 1.9; impurity G = about 2.1; impurity I = about 3.8; impurity J = about 5.8.

System suitability:
- *resolution*: minimum 2.5 between the peaks due to oxacillin and impurity E in the chromatogram obtained with reference solution (c);
- the chromatogram obtained with reference solution (e) is similar to the chromatogram supplied with *oxacillin for peak identification CRS*.

Limits:
- *impurity B*: for the sum of the areas of the 2 isomer peaks, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent);
- *impurity E*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
- *impurities D (sum of the 2 epimers), F, G, I, J*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *any other impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent);
- *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Ethyl acetate and butyl acetate

Head-space gas chromatography (*2.2.28*).

Test solution Dissolve 0.200 g of the substance to be examined in 6.0 mL of *water R*.

Reference solution Dissolve 83 mg of *butyl acetate R* and 83 mg of *ethyl acetate R* in *water R* and dilute to 250.0 mL with the same solvent. Use 6.0 mL of this solution.

Close the vials immediately with a rubber membrane stopper coated with polytetrafluoroethylene and secured with an aluminium crimped cap. Mix to obtain a homogeneous solution.

Column:
- *material*: fused silica;
- *size*: *l* = 50 m, Ø = 0.32 mm;
- *stationary phase*: *methylpolysiloxane R* (film thickness 5 µm).

Carrier gas *helium for chromatography R.*

Flow rate 2 mL/min.

Static head-space conditions that may be used:
- *equilibration temperature*: 80 °C;
- *equilibration time*: 60 min;
- *transfer-line temperature*: 140 °C;
- *pressurisation time*: 30 s.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 6	70
	6 - 16	70 → 220
	16 - 18	220
Injection port		140
Detector		250

Detection Flame ionisation.

Retention time Ethyl acetate = about 10 min; butyl acetate = about 15.5 min.

Limits:

— *butyl acetate*: maximum 1.0 per cent;

— *ethyl acetate*: maximum 1.0 per cent.

N,N-Dimethylaniline (*2.4.26, Method B*)

Maximum 20 ppm.

2-Ethylhexanoic acid (*2.4.28*)

Maximum 0.8 per cent.

Water (*2.5.12*)

3.5 per cent to 5.0 per cent, determined on 0.300 g.

Bacterial endotoxins (*2.6.14*)

Less than 0.20 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

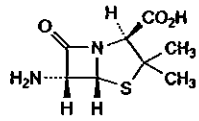
Injection Test solution (b) and reference solution (a).

Calculate the percentage content of $C_{19}H_{18}N_3NaO_5S$ taking into account the assigned content of *oxacillin sodium monohydrate CRS*.

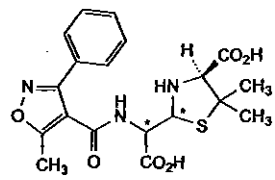
IMPURITIES

Specified impurities *B, D, E, F, G, I, J.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, C.*

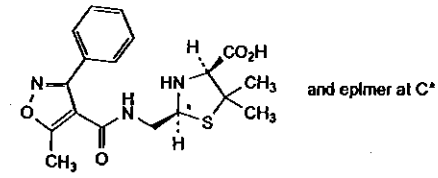


A. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),

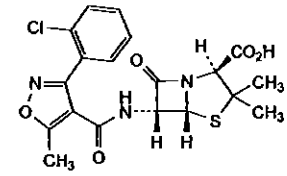


B. (4*S*)-2-[carboxy[[(5-methyl-3-phenylisoxazol-4-yl) carbonyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of oxacillin),

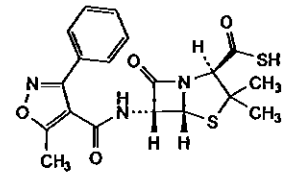
C. 5-methyl-3-phenylisoxazole-4-carboxylic acid,



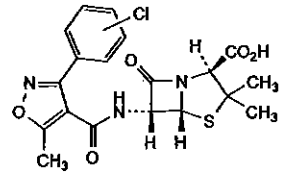
D. (2*RS*,4*S*)-5,5-dimethyl-2-[[[(5-methyl-3-phenylisoxazol-4-yl)carbonyl]amino]methyl]thiazolidine-4-carboxylic acid (penilloic acids of oxacillin),



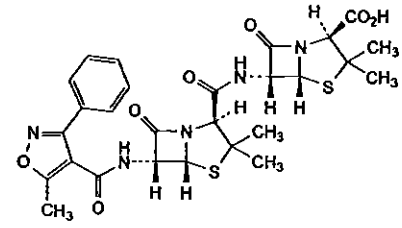
E. (2*S*,5*R*,6*R*)-6-[[[3-(2-chlorophenyl)-5-methylisoxazol-4-yl] carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylic acid (cloxacillin),



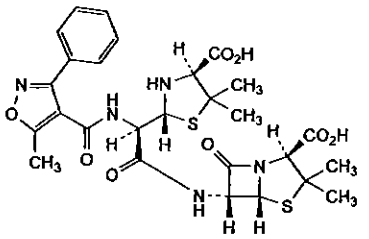
F. (2*R*,5*R*,6*R*)-3,3-dimethyl-6-[[(5-methyl-3-phenylisoxazol-4-yl)carbonyl]amino]-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carbothioic acid (thiooxacillin),



G. (2*S*,5*R*,6*R*)-6-[[[3-(chlorophenyl)-5-methylisoxazol-4-yl] carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylic acid (cloxacillin isomer),



I. (2*S*,5*R*,6*R*)-6-[[(2*S*,5*R*,6*R*)-3,3-dimethyl-6-[[(5-methyl-3-phenylisoxazol-4-yl)carbonyl]amino]-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-APA oxacillin amide),

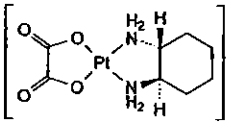


J. (2*S*,5*R*,6*R*)-6-[[(2*R*)-[(2*R*,4*S*)-4-carboxy-5,5-dimethylthiazolidin-2-yl][[(5-methyl-3-phenylisoxazol-4-yl)carbonyl]amino]acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (ozolamide of 6-APA dimer).

Ph Eur

Oxaliplatin

(Ph. Eur. monograph 2017)



$C_8H_{14}N_2O_4Pt$ 397.3 *61825-94-3*

Action and use
Platinum-containing cytotoxic.

Ph Eur

DEFINITION
(*SP*-4-2)-[(1*R*,2*R*)-Cyclohexane-1,2-diamine-κ^2N,N'][ethanedioato-κ^2O,O'(2-)]platinum.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Slightly soluble in water, very slightly soluble in methanol, practically insoluble in anhydrous ethanol.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *oxaliplatin CRS.*
B. Specific optical rotation (see Tests).

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).
Dissolve 0.10 g in *water R* and dilute to 50 mL with the same solvent.

Acidity
Dissolve 0.10 g in *carbon dioxide-free water R*, dilute to 50 mL with the same solvent and add 0.5 mL of *phenolphthalein solution R1*. The solution is colourless. Not more than 0.60 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink.

Specific optical rotation (*2.2.7*)
+ 74.5 to + 78.0 (dried substance).
Dissolve 0.250 g in *water R* and dilute to 50.0 mL with the same solvent.

Impurity D
Liquid chromatography (*2.2.29*).
Test solution Dissolve 30 mg of the substance to be examined in *methanol R* and dilute to 50.0 mL with the same solvent.
Reference solution (a) Dissolve 5 mg of *oxaliplatin impurity D CRS* in *methanol R* and dilute to 100.0 mL with the same solvent.
Reference solution (b) Dilute 15.0 mL of reference solution (a) to 50.0 mL with *methanol R*.
Reference solution (c) Dissolve 75 mg of the substance to be examined in *methanol R* and dilute to 100.0 mL with the same solvent.
Reference solution (d) Dilute 5.0 mL of reference solution (c) to 100.0 mL with *methanol R*.
Reference solution (e) To 40 mL of reference solution (c) add 1.0 mL of reference solution (b) and dilute to 50.0 mL with *methanol R*.
Reference solution (f) To 4.0 mL of reference solution (a) add 5.0 mL of reference solution (d) and dilute to 50.0 mL with *methanol R*.
Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *cellulose derivative of silica gel for chiral separation R*;
— *temperature*: 40 °C.
Mobile phase *anhydrous ethanol R, methanol R* (30:70 *V/V*).
Flow rate 0.3 mL/min.
Detection Spectrophotometer at 254 nm.
Injection 20 µL of the test solution and reference solutions (e) and (f).
Run time Twice the retention time of oxaliplatin.
Retention time Oxaliplatin = about 14 min; impurity D = about 16 min.
System suitability:
— *resolution*: minimum 1.5 between the peaks due to oxaliplatin and impurity D in the chromatogram obtained with reference solution (f);
— *signal-to-noise ratio*: minimum 10 for the peak due to impurity D in the chromatogram obtained with reference solution (e).
Limit:
— *impurity D*: not more than 3 times the difference between the heights of the peaks due to impurity D in the chromatograms obtained with reference solution (e) and the test solution (0.15 per cent).

Related substances
A. Impurity A. Liquid chromatography (*2.2.29*). *Use vigorous shaking and very brief sonication to dissolve the substance to be examined. Inject the test solution within 20 min of preparation.*
Test solution Dissolve 0.100 g of the substance to be examined in *water R* and dilute to 50.0 mL with the same solvent.
Reference solution (a) Dissolve 14.0 mg of *oxalic acid R* (impurity A) in *water R* and dilute to 250.0 mL with the same solvent.
Reference solution (b) Dilute 5.0 mL of reference solution (a) to 200.0 mL with *water R*.
Reference solution (c) Dissolve 12.5 mg of *sodium nitrate R* in *water R* and dilute to 250.0 mL with the same solvent. Dilute

a mixture of 2.0 mL of this solution and 25.0 mL of reference solution (a) to 100.0 mL with *water R*.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase Mix 20 volumes of *acetonitrile R1* and 80 volumes of a solution prepared as follows: to 10 mL of a 320 g/L solution of *tetrabutylammonium hydroxide R* add 1.36 g of *potassium dihydrogen phosphate R*, dilute to 1000 mL with *water for chromatography R* and adjust to pH 6.0 with *phosphoric acid R*.

Flow rate 2 mL/min.

Detection Spectrophotometer at 205 nm.

Injection 20 µL of the test solution and reference solutions (b) and (c).

Run time Twice the retention time of impurity A.

Retention times Nitrate = about 2.7 min; impurity A = about 4.7 min.

System suitability:
- *resolution*: minimum 9 between the peaks due to nitrate and impurity A in the chromatogram obtained with reference solution (c);
- *signal-to-noise ratio*: minimum 10 for the peak due to impurity A in the chromatogram obtained with reference solution (b).

Limit:
- *impurity A*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent).

B. Impurity B. Liquid chromatography (*2.2.29*). *Use vigorous shaking and very brief sonication to dissolve the substance to be examined. Inject the test solution within 20 min of preparation. Use suitable polypropylene containers for the preparation and injection of all solutions. Glass pipettes may be used for diluting solutions.*

Test solution Dissolve 0.100 g of the substance to be examined in *water R* and dilute to 50.0 mL with the same solvent.

Reference solution (a) Add 5.0 mg of *oxaliplatin impurity B CRS* to 25 mL of *methanol R* and dilute to 100.0 mL with *water R*. Sonicate for about 1.5 h until dissolved (solution A). Dilute 3.0 mL of solution A to 200.0 mL with *water R*.

Reference solution (b) In order to prepare impurity E *in situ*, adjust 50.0 mL of solution A to pH 6.0 with a 0.2 g/L solution of *sodium hydroxide R*, heat at 70 °C for 4 h and allow to cool.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase Mix 20 volumes of *acetonitrile R1* and 80 volumes of a solution prepared as follows: dissolve 1.36 g of *potassium dihydrogen phosphate R* and 1 g of *sodium heptanesulfonate R* in 1000 mL of *water for chromatography R* and adjust to pH 3.0 ± 0.05 with *phosphoric acid R*.

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µL.

Run time 2.5 times the retention time of impurity B.

Retention time Impurity B = about 4.3 min; impurity E = about 6.4 min.

System suitability:
- *resolution*: minimum 7 between the peaks due to impurities B and E in the chromatogram obtained with reference solution (b);
- *signal-to-noise ratio*: minimum 10 for the peak due to impurity B in the chromatogram obtained with reference solution (a).

Limit:
- *impurity B*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent).

C. Impurity C and other related substances. Liquid chromatography (*2.2.29*). *Use vigorous shaking and very brief sonication to dissolve the substance to be examined. Inject the test solution within 20 min of preparation.*

Test solution (a) Dissolve 0.100 g of the substance to be examined in *water R* and dilute to 50.0 mL with the same solvent.

Test solution (b) Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 500.0 mL with the same solvent.

Reference solution (a) Dissolve 5.0 mg of *oxaliplatin CRS* and 5.0 mg of *oxaliplatin impurity C CRS* in *water R* and dilute to 50.0 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 100.0 mL with *water R*.

Reference solution (c) Dissolve 25.0 mg of *oxaliplatin CRS* in *water R* and dilute to 250.0 mL with the same solvent.

Reference solution (d) Dissolve 5.0 mg of *dichlorodiaminocyclohexaneplatinum CRS* in reference solution (c) and dilute to 50.0 mL with reference solution (c).

Reference solution (e) Dilute 5 mL of reference solution (d) to 50.0 mL with *water R*.

Reference solution (f) To 0.100 g of the substance to be examined add 1.5 mL of reference solution (a) and dilute to 50.0 mL with *water R*.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase Mix 1 volume of *acetonitrile R1* and 99 volumes of a solution prepared as follows: dilute 0.6 mL of *dilute phosphoric acid R* in 1000 mL of *water for chromatography R* and adjust to pH 3.0 with either *sodium hydroxide solution R* or *phosphoric acid R*.

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µL of test solution (a) and reference solutions (b), (e) and (f).

Run time 3 times the retention time of oxaliplatin.

Retention time Impurity C = about 4.4 min; dichlorodiaminocyclohexaneplatinum = about 6.9 min; oxaliplatin = about 8.0 min.

System suitability:
- *resolution*: minimum 2.0 between the peaks due to dichlorodiaminocyclohexaneplatinum and oxaliplatin in the chromatogram obtained with reference solution (e);

— *signal-to-noise ratio*: minimum 50 for the peak due to impurity C and minimum 10 for the peak due to oxaliplatin in the chromatogram obtained with reference solution (b).

Limits:

— *impurity C*: not more than 0.5 times the area of the peak due to impurity C in the chromatogram obtained with reference solution (f) (0.15 per cent);

— *unspecified impurities*: for each impurity, not more than twice the area of the peak due to oxaliplatin in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *sum of unspecified impurities*: not more than 3 times the area of the peak due to oxaliplatin in the chromatogram obtained with reference solution (b) (0.15 per cent);

— *disregard limit*: the area of the peak due to oxaliplatin in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak with a retention time less than 2 min.

D. Sum of impurities other than D: maximum 0.30 per cent.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Bacterial endotoxins (*2.6.14*)
Less than 1.0 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for impurity C and other related substances with the following modifications.

Injection 20 µL of test solution (b) and reference solutions (c) and (d).

System suitability:

— *resolution*: minimum 2.0 between the peaks due to dichlorodiaminocyclohexaneplatinum and oxaliplatin in the chromatogram obtained with reference solution (d);

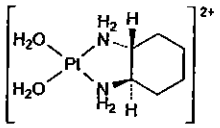
— *repeatability*: reference solution (c).

Calculate the percentage content of oxaliplatin using the chromatogram obtained with reference solution (c).

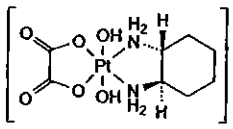
IMPURITIES
Specified impurities *A, B, C, D.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *E.*

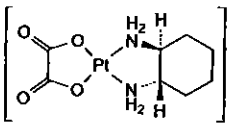
A. oxalic acid,



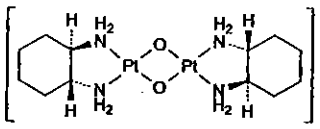
B. (*SP*-4-2)-diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-κ^2*N*,*N*′]platinum (diaquodiaminocyclohexaneplatinum; supplied as dinitrate),



C. (*OC*-6-33)-[(1*R*,2*R*)-cyclohexane-1,2-diamine-κ^2*N*,*N*′][ethanedioato-κ^2*O*,*O*′(2-)]dihydroxidoplatinum,



D. (*SP*-4-2)-[(1*S*,2*S*)-cyclohexane-1,2-diamine-κ^2*N*,*N*′][ethanedioato-κ^2*O*,*O*′(2-)]platinum (*S*,*S*-enantiomer of oxaliplatin),

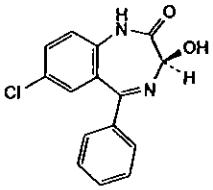


E. (*SP*-4-2)-di-µ-oxidobis[(1*R*,2*R*)-cyclohexane-1,2-diamine-1κ^2*N*,2κ^2*N*′]diplatinum (diaquodiaminocyclohexaneplatinum dimer).

Ph Eur

Oxazepam

(Ph. Eur. monograph 0778)



and enantiomer

$C_{15}H_{11}ClN_2O_2$ 286.7 *604-75-1*

Action and use
Benzodiazepine.

Preparation
Oxazepam Tablets

Ph Eur

DEFINITION
(3*RS*)-7-Chloro-3-hydroxy-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *oxazepam CRS.*

TESTS

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 40.0 mg of the substance to be examined in 25 mL of a mixture of equal volumes of *acetonitrile R* and *water R* and dilute to 50.0 mL with the same mixture of solvents.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with a mixture of equal volumes of *acetonitrile R* and *water R*. Dilute 2.0 mL of this solution to 10.0 mL with a mixture of equal volumes of *acetonitrile R* and *water R*.

Reference solution (b) Dissolve the contents of a vial of *oxazepam for peak identification CRS* (containing impurities A, B, C, D and E) in 1.0 mL of the test solution.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm) resistant to bases up to pH 11.

Mobile phase:

— *mobile phase A*: dissolve 3.48 g of *dipotassium hydrogen phosphate R* in 900 mL of *water R*, adjust to pH 10.5 with a 40 g/L solution of *sodium hydroxide R* and dilute to 1000 mL with *water R*;

— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 4	75	25
4 - 34	75 → 25	25 → 75
34 - 45	25	75
45 - 50	25 → 75	75 → 25
50 - 60	75	25

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 235 nm.

Injection 10 µL.

Identification of impurities Use the chromatogram obtained with reference solution (b) and the chromatogram supplied with *oxazepam for peak identification CRS* to identify the peaks due to impurities A, B, C, D and E.

Relative retention With reference to oxazepam (retention time = about 15 min): impurity E = about 0.7; impurity A = about 0.8; impurity B = about 1.2; impurity C = about 1.4; impurity D = about 2.0.

System suitability Reference solution (b):

— *resolution*: minimum 1.5 between the peaks due to impurities E and A.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 4.0; impurity B = 1.1;

— *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C at a pressure not exceeding 0.7 kPa.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in a mixture of 10 mL of *anhydrous acetic acid R* and 90 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

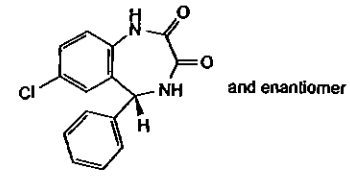
1 mL of *0.1 M perchloric acid* is equivalent to 28.67 mg of $C_{15}H_{11}ClN_2O_2$.

STORAGE

Protected from light.

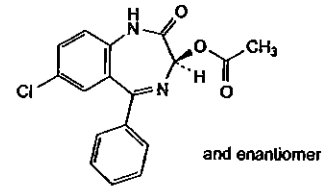
IMPURITIES

Specified impurities *A, B, C, D, E.*



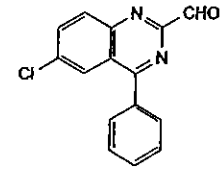
and enantiomer

A. (5*RS*)-7-chloro-5-phenyl-4,5-dihydro-1*H*-1,4-benzodiazepine-2,3-dione,

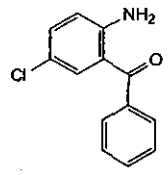


and enantiomer

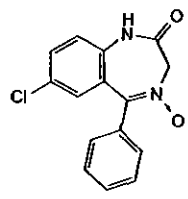
B. (3*RS*)-7-chloro-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl acetate,



C. 6-chloro-4-phenylquinazoline-2-carbaldehyde,



D. (2-amino-5-chlorophenyl)phenylmethanone,

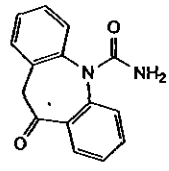


E. 7-chloro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one 4-oxide.

Ph Eur

Oxcarbazepine

(Ph. Eur. monograph 2577)



$C_{15}H_{12}N_2O_2$ 252.3 *28721-07-5*

Action and use
Antiepileptic.

Ph Eur

DEFINITION
10-Oxo-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine-5-carboxamide.

Content
97.5 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
White or faintly orange, crystalline powder.

Solubility
Practically insoluble in water and in ethanol (96 per cent), slightly soluble in methylene chloride.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *oxcarbazepine CRS*.

TESTS

Related substances
Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Solvent mixture *acetonitrile R*, solution A (50:50 *V/V*).

Phosphate buffer solution Dissolve 0.54 g of *potassium dihydrogen phosphate R* and 8.9 g of *disodium hydrogen phosphate dihydrate R* in 1.0 L of *water R*.

Solution A 1.8 g/L solution of *ascorbic acid R*.

Solution B 1.8 g/L solution of *sodium edetate R* in a mixture of equal volumes of the phosphate buffer solution and *water R*.

Test solution (a) Dissolve 50.0 mg of the substance to be examined in 25 mL of *acetonitrile R*, sonicate for 10 min, cool to room temperature and dilute to 50.0 mL with solution A.

Test solution (b) Dilute 5.0 mL of test solution (a) to 50.0 mL with the solvent mixture.

Reference solution (a) Dissolve the contents of a vial of *oxcarbazepine impurity mixture CRS* (impurities A, B, I and K) in 0.5 mL of *acetonitrile R* and dilute to 1.0 mL with solution A.

Reference solution (b) Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 50.0 mg of *oxcarbazepine CRS* in 25 mL of *acetonitrile R*, sonicate for 10 min, cool to room temperature and dilute to 50.0 mL with solution A. Dilute 5.0 mL of this solution to 50.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *phenylhexylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: *acetonitrile R*, solution B, *tetrahydrofuran R*, *water R* (5:10:10:75 *V/V/V/V*);
— *mobile phase B*: solution B, *tetrahydrofuran R*, *water R*, *acetonitrile R* (10:10:20:60 *V/V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	60	40
10 - 20	60 → 5	40 → 95
20 - 27	5	95

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 240 nm.

Injection 10 µL of test solution (a) and reference solutions (a) and (b).

Identification of impurities Use the chromatogram supplied with *oxcarbazepine impurity mixture CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, I and K.

Relative retention With reference to oxcarbazepine (retention time = about 6 min): impurity I = about 0.8; impurity A = about 1.3; impurities K and L = about 1.4; impurity B = about 1.6.

System suitability Reference solution (a):
— *peak-to-valley ratio*: minimum 4.0, where H_p = height above the baseline of the peak due to impurities K and L and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity A.

Calculation of percentage contents:
— for each impurity, use the concentration of oxcarbazepine in reference solution (b).

Limits:
— *impurities B, I*: for each impurity, maximum 0.1 per cent;
— *sum of impurities K and L*: maximum 0.1 per cent;
— *unspecified impurities*: for each impurity, maximum 0.05 per cent;
— *total*: maximum 0.5 per cent;

— *reporting threshold*: 0.03 per cent.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Mobile phase:

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 7	60	40
7 - 8	60 → 5	40 → 95
8 - 13	5	95

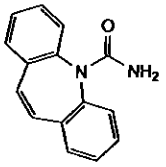
Injection Test solution (b) and reference solution (c).

Calculate the percentage content of $C_{15}H_{12}N_2O_2$ taking into account the assigned content of *oxcarbazepine CRS*.

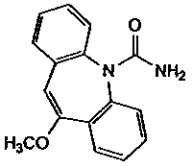
IMPURITIES

Specified impurities *B, I, K, L.*

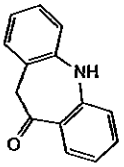
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, C, D, E, F, G, H, M.*



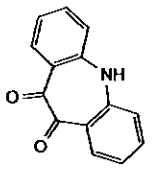
A. 5*H*-dibenzo[*b*,*f*]azepine-5-carboxamide (carbamazepine),



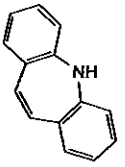
B. 10-methoxy-5*H*-dibenzo[*b*,*f*]azepine-5-carboxamide (10-methoxycarbamazepine),



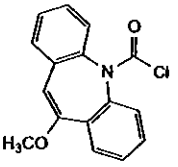
C. 5,11-dihydro-10*H*-dibenzo[*b*,*f*]azepin-10-one,



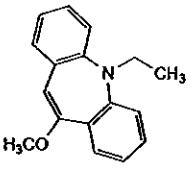
D. 5*H*-dibenzo[*b*,*f*]azepine-10,11-dione,



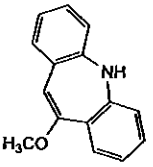
E. 5*H*-dibenzo[*b*,*f*]azepine,



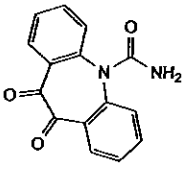
F. 10-methoxy-5*H*-dibenzo[*b*,*f*]azepine-5-carbonyl chloride,



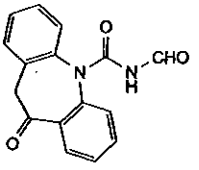
G. 5-ethyl-10-methoxy-5*H*-dibenzo[*b*,*f*]azepine,



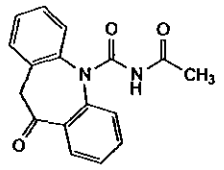
H. 10-methoxy-5*H*-dibenzo[*b*,*f*]azepine,



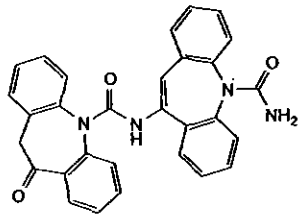
I. 10,11-dioxo-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine-5-carboxamide,



K. *N*-formyl-10-oxo-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine-5-carboxamide,



L. *N*-acetyl-10-oxo-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine-5-carboxamide,

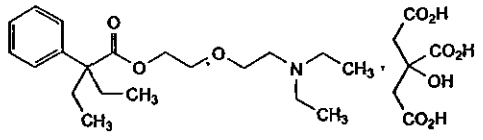


M. 10-{[(10-oxo-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepin-5-yl) carbonyl]amino}-5*H*-dibenzo[*b*,*f*]azepine-5-carboxamide.

Ph Eur

Oxeladin Hydrogen Citrate

(Ph. Eur. monograph 1761)



$C_{26}H_{41}NO_{10}$ 527.6 *52432-72-1*

Action and use
Cough suppressant.

Ph Eur

DEFINITION
2-[2-(Diethylamino)ethoxy]ethyl 2-ethyl-2-phenylbutanoate dihydrogen 2-hydroxypropane-1,2,3-tricarboxylate.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water, slightly soluble or very slightly soluble in ethyl acetate.

It shows polymorphism (*5.9*).

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *oxeladin hydrogen citrate CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *anhydrous ethanol R*, evaporate to dryness and record new spectra using the residues.

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Dissolve 2.0 g in *water R* and dilute to 10.0 mL with the same solvent.

Related substances
Gas chromatography (*2.2.28*): use the normalisation procedure. *Prepare the solutions immediately before use.*

Test solution Dissolve 0.500 g of the substance to be examined in *water R* and dilute to 50 mL with the same solvent. Add 1 mL of a 10.3 g/L solution of *hydrochloric acid R* and shake with 3 quantities, each of 10 mL, of *methylene chloride R*. Combine the lower layers. Add 5 mL of *concentrated ammonia R* to the aqueous layer and shake with 3 quantities, each of 10 mL, of *methylene chloride R*. Combine the lower layers with the lower layers obtained previously, add *anhydrous sodium sulfate R*, shake, filter and evaporate the filtrate by suitable means at a temperature not exceeding 30 °C. Take up the residue with *methylene chloride R* and dilute to 20.0 mL with the same solvent.

Reference solution (a) Dissolve 5 mg of *oxeladin impurity D CRS* in 10 mL of *water R*, add 0.5 mL of *concentrated ammonia R* and shake with 3 quantities, each of 2 mL, of *methylene chloride R*. To the combined lower layers, add 0.2 mL of the test solution and dilute to 10.0 mL with *methylene chloride R*.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *methylene chloride R*. Dilute 1.0 mL of this solution to 20.0 mL with *methylene chloride R*.

Reference solution (c) Dissolve 5 mg of *oxeladin impurity C CRS* in 10 mL of *water R*, add 0.5 mL of *concentrated ammonia R* and shake with 3 quantities, each of 2 mL, of *methylene chloride R*. Combine the lower layers and dilute to 10 mL with *methylene chloride R*.

Column:
— *material*: fused silica;
— *size*: *l* = 25 m, Ø = 0.32 mm;
— *stationary phase*: *phenyl(5)methyl(95)polysiloxane R* (film thickness 0.4 µm).

Carrier gas *helium for chromatography R.*

Flow rate 1.0 mL/min; adjust the flow rate if necessary to obtain a retention time of about 13 min for oxeladin.

Split ratio 1:15.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 4	160
	4 - 12	160 → 240
	12 - 21	240
	21 - 30	240 → 160
Injection port		280
Detector		280

Detection Flame ionisation.

Injection 1 µL.

Relative retention With reference to oxeladin (retention time = about 13 min): impurity A = about 0.2; impurity B = about 0.4; impurity C = about 0.8; impurity D = about 0.9.

System suitability Reference solution (a):
— *resolution*: minimum 10 between the peaks due to impurity D and oxeladin.

Limits:
— *impurity C*: maximum 0.2 per cent;
— *impurity D*: maximum 0.3 per cent;

— *any other impurity*: for each impurity, maximum 0.1 per cent;
— *total*: maximum 1.0 per cent;
— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 3 h.

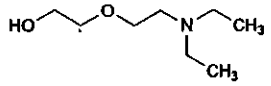
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.400 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

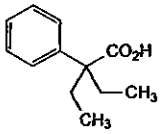
1 mL of *0.1 M perchloric acid* is equivalent to 52.76 mg of $C_{26}H_{41}NO_{10}$.

IMPURITIES
Specified impurities *C, D.*

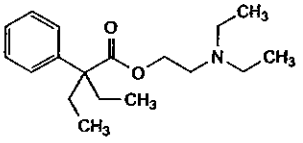
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B.*



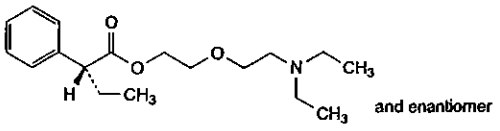
A. 2-[2-(diethylamino)ethoxy]ethanol,



B. 2-ethyl-2-phenylbutanoic acid,



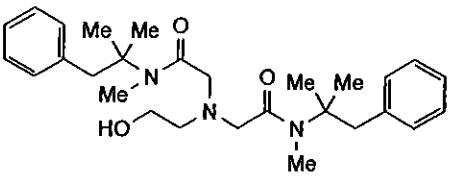
C. 2-(diethylamino)ethyl 2-ethyl-2-phenylbutanoate,



D. 2-[2-(diethylamino)ethoxy]ethyl (2*RS*)-2-phenylbutanoate.

Ph Eur

Oxetacaine



$C_{28}H_{41}N_3O_3$ 467.6 *126-27-6*

Action and use
Local anaesthetic.

DEFINITION
Oxetacaine is 2,2′-(2-hydroxyethylimino)bis[*N*-(α,α-dimethylphenethyl)-*N*-methylacetamide]. It contains not less than 99.0% and not more than 100.5% of $C_{28}H_{41}N_3O_3$, calculated with reference to the dried substance.

CHARACTERISTICS
A white or almost white powder.

Practically insoluble in water; freely soluble in methanol; soluble in ethyl acetate.

IDENTIFICATION
The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of oxetacaine *(RS 254)*.

TESTS

Melting point
100°C to 104°C, Appendix V A.

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *ethyl acetate*.

(1) 10.0% w/v of the substance being examined.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel 60 precoated plate (Merck plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of warm air and spray liberally with a solution containing 6% w/v of *ammonium thiocyanate* and 2% w/v of *cobalt(II) chloride*. Carefully remove excess solution by applying filter paper to the plate and allow the plate to dry in air for 10 minutes or until spots appear.

MOBILE PHASE

1 volume of 18M *ammonia*, 20 volumes of *absolute ethanol* and 79 volumes of *toluene*.

LIMITS

In the chromatogram obtained with solution (1):

any *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%);

not more than one *secondary spot* is more intense than the spot in the chromatogram obtained with solution (3) (0.1%).

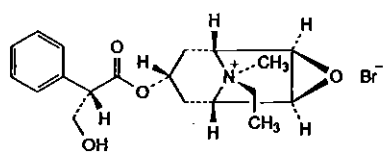
Loss on drying
When dried at 60°C at a pressure not exceeding 0.7 kPa for 4 hours, loses not more than 0.5% of its weight. Use 1 g.

Sulfated ash
Not more than 0.1%, Appendix IX A.

ASSAY
Dissolve 1 g in 50 mL of *anhydrous acetic acid* and carry out Method I for *non-aqueous titration*, Appendix VIII A, determining the end-point potentiometrically. Each mL of *0.1M perchloric acid VS* is equivalent to 46.76 mg of $C_{28}H_{41}N_3O_3$.

Oxitropium Bromide

(Ph. Eur. monograph 2170)



$C_{19}H_{26}BrNO_4$ 412.3 *30286-75-0*

Action and use
Anticholinergic; treatment of reversible airways obstruction.

Ph Eur

DEFINITION
(1*R*,2*R*,4*S*,5*S*,7*s*,9*s*)-9-Ethyl-7-[[(2*S*)-3-hydroxy-2-phenylpropanoyl]oxy]-9-methyl-3-oxa-9-azoniatricyclo [3.3.1.$0^{2,4}$]nonane bromide (ethylhyoscine).

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Very soluble in water, freely soluble in methanol, sparingly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *oxitropium bromide CRS.*

If the spectra obtained in the solid state show differences at about 1700 cm^{-1} and about 3300 cm^{-1}, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

B. It gives reaction (a) of bromides (*2.3.1*).

TESTS

Specific optical rotation (*2.2.7*)
−26 to −24 (dried substance).

Dissolve 1.0 g in *water R* and dilute to 20.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 75.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 5.0 mL of the solution to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 7.5 mg of *oxitropium bromide impurity B CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (b) Dilute 5.0 mL of reference solution (a) to 50.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 50.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution (c) Mix 5.0 mL of the test solution and 5.0 mL of reference solution (a).

Reference solution (d) Dilute 15.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution (e) Dilute 5.0 mL of the test solution to 50.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 50.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 50.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.125 m, Ø = 4.0 mm;
— *stationary phase*: *base-deactivated octylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 350 m^2/g and a pore size of 6 nm.

Mobile phase *acetonitrile for chromatography R*, 7.8 g/L solution of *sodium dihydrogen phosphate R* (10:100 *V/V*).

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 50 µL of the test solution and reference solutions (b), (c), (d) and (e).

Relative retention With reference to oxitropium (retention time = about 6 min): impurity A = about 0.8; impurity B = about 0.9; impurity C = about 1.3.

System suitability Reference solution (c):
— *resolution*: minimum 1.6 between the peaks due to impurity B and oxitropium.

Limits:
— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (e) (0.1 per cent);
— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
— *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (1.5 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (e) (0.10 per cent);
— *sum of unspecified impurities*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (e) (0.2 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (e) (0.05 per cent).

Impurity D
Liquid chromatography (*2.2.29*).

Test solution Dissolve 75.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 5.0 mL of the solution to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 6.0 mg of *oxitropium bromide impurity D CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (b) Dilute 5.0 mL of reference solution (a) to 200.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution (c) To 5.0 mL of the test solution add 5.0 mL of reference solution (a).

Column:
— *size*: *l* = 0.125 m, Ø = 4.0 mm;
— *stationary phase*: *base-deactivated octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile for chromatography R*, 7.8 g/L solution of *sodium dihydrogen phosphate R* (18.5:100 *V/V*).

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 50 µL of the test solution and reference solutions (b) and (c).

System suitability Reference solution (c):
— *resolution*: minimum 3.0 between the peaks due to impurity D and oxitropium.

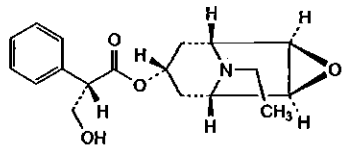
Limit:
— *impurity D*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.2 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

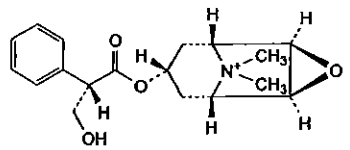
ASSAY
Dissolve 0.350 g in 100 mL of *water R* and add 5.0 mL of *dilute nitric acid R*. Titrate with *0.1 M silver nitrate*. Determine the end-point potentiometrically (*2.2.20*) using a silver indicator electrode and a silver-silver chloride reference electrode.

1 mL of *0.1 M silver nitrate* is equivalent to 41.23 mg of $C_{19}H_{26}BrNO_4$.

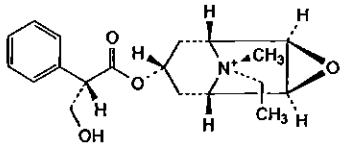
IMPURITIES
Specified impurities *A, B, C, D.*



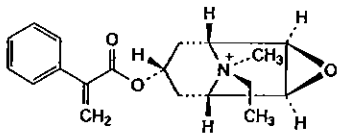
A. (1*R*,2*R*,4*S*,5*S*,7*s*)-9-ethyl-3-oxa-9-azatricyclo [3.3.1.$0^{2,4}$]non-7-yl (2*S*)-3-hydroxy-2-phenylpropanoate (*N*-ethylnorhyoscine),



B. (1*R*,2*R*,4*S*,5*S*,7*s*)-7-[[(2*S*)-3-hydroxy-2-phenylpropanoyl]oxy]-9,9-dimethyl-3-oxa-9-azoniatricyclo [3.3.1.$0^{2,4}$]nonane (methylhyoscine),



C. (1*R*,2*R*,4*S*,5*S*,7*s*,9*r*)-9-ethyl-7-[[(2*S*)-3-hydroxy-2-phenylpropanoyl]oxy]-9-methyl-3-oxa-9-azoniatricyclo [3.3.1.$0^{2,4}$]nonane (pseudo-isomer),

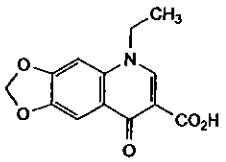


D. (1*R*,2*R*,4*S*,5*S*,7*s*,9*s*)-9-ethyl-9-methyl-7-[(2-phenylacryloyl)oxy]-3-oxa-9-azoniatricyclo[3.3.1.$0^{2,4}$] nonane (apo-*N*-ethylhyoscine).

Ph Eur

Oxolinic Acid

(Ph. Eur. monograph 1353)



$C_{13}H_{11}NO_5$ 261.2 *14698-29-4*

Action and use
Antibacterial.

Ph Eur

DEFINITION
5-Ethyl-8-oxo-5,8-dihydro-1,3-dioxolo[4,5-*g*]quinoline-7-carboxylic acid.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS
Appearance
Almost white or pale yellow, crystalline powder.

Solubility
Practically insoluble in water, very slightly soluble in methylene chloride, practically insoluble in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION
First identification: B.

Second identification: A, C.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 25.0 mg in 5 mL of *0.1 M sodium hydroxide*, heating on a water-bath. Allow to cool and dilute to 100.0 mL with *methanol R*. Dilute 2.0 mL of this solution to 100.0 mL with *0.1 M hydrochloric acid*.

Spectral range 220-350 nm.

Absorption maxima At 260 nm, 322 nm and 336 nm.

Absorbance ratio A_{260}/A_{336} = 4.9 to 5.2.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *oxolinic acid CRS*.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in 3 mL of *dilute sodium hydroxide solution R* and dilute to 20 mL with *ethanol (96 per cent) R*.

Reference solution (a) Dissolve 10 mg of *oxolinic acid CRS* in 3 mL of *dilute sodium hydroxide solution R* and dilute to 20 mL with *ethanol (96 per cent) R*.

Reference solution (b) Dissolve 5 mg of *ciprofloxacin hydrochloride CRS* in *methanol R* and dilute to 10 mL with the same solvent. Dilute 1 mL of this solution to 2 mL with reference solution (a).

Plate *TLC silica gel plate R.*

Mobile phase *acetonitrile R, concentrated ammonia R, methanol R, methylene chloride R* (10:20:40:40 *V/V/V/V*).

Application 10 µL.

Development At the bottom of a chromatographic tank, place an evaporating dish containing 50 mL of *concentrated ammonia R* and expose the plate to the ammonia vapour for 15 min in the closed tank; withdraw the plate, transfer to a second chromatographic tank and proceed with development over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):
— the chromatogram shows 2 clearly separated principal spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Solution S
Dissolve 0.6 g in 20 mL of a 40 g/L solution of *sodium hydroxide R.*

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_7 (*2.2.2, Method II*).

Related substances
Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.10 g of the substance to be examined in 3 mL of *dilute sodium hydroxide solution R* and dilute to 10 mL with *ethanol (96 per cent) R.*

Reference solution (a) Dilute 1 mL of the test solution to 50.0 mL with *ethanol (96 per cent) R.* Dilute 1.0 mL of this solution to 5.0 mL with *ethanol (96 per cent) R.*

Reference solution (b) Dissolve 2 mg of *oxolinic acid impurity B CRS* in *ethanol (96 per cent) R* and dilute to 10 mL with the same solvent. Dilute 1.0 mL of this solution to 10 mL with *ethanol (96 per cent) R.*

Reference solution (c) Dissolve 5 mg of the substance to be examined and 5 mg of *oxolinic acid impurity A CRS* in 2 mL of *dilute sodium hydroxide solution R* and dilute to 40 mL with *ethanol (96 per cent) R.*

Plate *cellulose for chromatography R* as the coating substance.

Mobile phase *ammonia R, water R, propanol R* (15:30:55 *V/V/V*).

Application 5 µL, in sufficiently small portions to obtain small spots.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (c):
— the chromatogram shows 2 clearly separated principal spots.

Limits:
— *impurity B*: any spot due to impurity B is not more intense than the corresponding spot in the chromatogram obtained with reference solution (b) (0.2 per cent);
— *impurities A, C*: any spot due to impurities A or C is not more intense than the principal spot in the chromatogram obtained with reference solution (a) (0.4 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by heating in an oven at 105 °C.

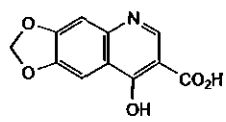
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.200 g in 150 mL of *dimethylformamide R.* Titrate with *0.1 M tetrabutylammonium hydroxide,* determining the end-point potentiometrically (*2.2.20*). Use a glass indicator electrode and a suitable reference electrode.

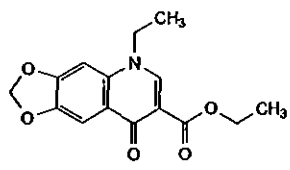
1 mL of *0.1 M tetrabutylammonium hydroxide* is equivalent to 26.12 mg of $C_{13}H_{11}NO_5$.

STORAGE
Protected from light.

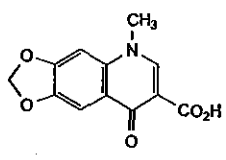
IMPURITIES



A. 8-hydroxy-1,3-dioxolo[4,5-*g*]quinoline-7-carboxylic acid,



B. ethyl 5-ethyl-8-oxo-5,8-dihydro-1,3-dioxolo[4,5-*g*] quinoline-7-carboxylate,

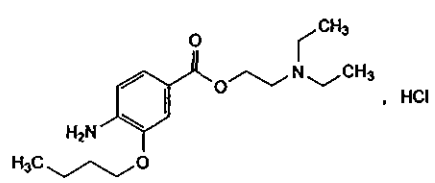


C. 5-methyl-8-oxo-5,8-dihydro-1,3-dioxolo[4,5-*g*]quinoline-7-carboxylic acid.

Ph Eur

Oxybuprocaine Hydrochloride

(Ph. Eur. monograph 1251)



$C_{17}H_{29}ClN_2O_3$ 344.9 *5987-82-6*

Action and use
Local anaesthetic.

Preparation
Oxybuprocaine Eye Drops

Ph Eur

DEFINITION

2-(Diethylamino)ethyl 4-amino-3-butoxybenzoate hydrochloride.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 158 °C to 162 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *oxybuprocaine hydrochloride CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 40 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dissolve 40 mg of *oxybuprocaine hydrochloride CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 20 mg of *procaine hydrochloride R* in reference solution (a) and dilute to 5 mL with reference solution (a).

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *anhydrous formic acid R, methanol R, water R, ethyl acetate R* (10:15:15:60 *V/V/V/V*).

Application 5 µL.

Development Over a path of 10 cm.

Drying In a current of warm air for 10 min.

Detection Spray with *dimethylaminobenzaldehyde solution R7* and examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dilute 0.2 mL of solution S (see Tests) to 2 mL with *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5 (*2.2.2, Method II*).

pH (*2.2.3*)

4.5 to 6.0 for solution S.

Related substances

Liquid chromatography (*2.2.29*).

Buffer solution pH 2.5 Add 6 mL of *perchloric acid solution R* and 12 mL of *dilute phosphoric acid R* to 950 mL of *water R*. Adjust to pH 2.5 with a 40 g/L solution of *sodium hydroxide R* and dilute to 1000.0 mL with *water R*.

Test solution Dissolve 10.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 20.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 100.0 mL with the mobile phase.

Reference solution (b) Mix 1.0 mL of the test solution with 1 mL of a 40 g/L solution of *sodium hydroxide R* and allow to stand for 20 min. Add 1 mL of *dilute phosphoric acid R* and dilute to 100.0 mL with the mobile phase. Dilute 25 mL of this solution to 100.0 mL with the mobile phase.

Column:

— *size: l* = 0.15 m, Ø = 3.9 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R1* (5 µm) with a pore size of 10 nm and a carbon loading of 19 per cent;

— *temperature:* 35 °C.

Mobile phase *acetonitrile R*, buffer solution pH 2.5 (25:75 *V/V*).

Flow rate 1 mL/min.

Detection Spectrophotometer at 309 nm.

Injection 20 µL.

Run time 4 times the retention time of oxybuprocaine.

Retention time Oxybuprocaine = about 9 min.

System suitability Reference solution (b):

— *resolution:* minimum 12 between the peaks due to oxybuprocaine and impurity B (hydrolysis product).

Limits:

— *any impurity:* for each impurity, not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);

— *total:* not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent);

— *disregard limit:* 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.0125 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

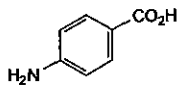
Dissolve 0.300 g in a mixture of 20 mL of *anhydrous acetic acid R* and 20 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 34.49 mg of $C_{17}H_{29}ClN_2O_3$.

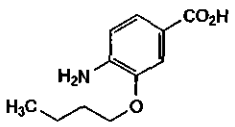
STORAGE

Protected from light.

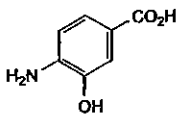
IMPURITIES



A. 4-aminobenzoic acid,



B. 4-amino-3-butoxybenzoic acid,

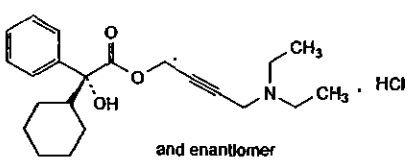


C. 4-amino-3-hydroxybenzoic acid.

Ph Eur

Oxybutynin Hydrochloride

(Ph. Eur. monograph 1354)



and enantiomer

$C_{22}H_{32}ClNO_3$ 394.0 *1508-65-2*

Action and use
Anticholinergic.

Preparations
Oxybutynin Oral Solution
Oxybutynin Tablets
Oxybutynin Prolonged-release Tablets

Ph Eur

DEFINITION
4-(Diethylamino)but-2-ynyl (*RS*)-2-cyclohexyl-2-hydroxy-2-phenylacetate hydrochloride.

Content
99.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water and in ethanol (96 per cent), soluble in acetone, practically insoluble in cyclohexane.

IDENTIFICATION
First identification: B, D.
Second identification: A, C, D.
A. Melting point (*2.2.14*): 124 °C to 129 °C.
B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *oxybutynin hydrochloride CRS.*
C. Thin-layer chromatography (*2.2.27*).
Test solution Dissolve 50 mg of the substance to be examined in *ethanol (96 per cent) R* and dilute to 10 mL with the same solvent.
Reference solution Dissolve 10 mg of *oxybutynin hydrochloride CRS* in *ethanol (96 per cent) R* and dilute to 2 mL with the same solvent.
Plate *TLC silica gel plate R.*
Mobile phase *methanol R.*
Application 5 µL.
Development Over 2/3 of the plate.
Drying In air.
Detection Expose to iodine vapour for 30 min.
Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.
D. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S
Dissolve 2.00 g in *water R* and dilute to 20.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

Optical rotation (*2.2.7*)
−0.10° to + 0.10°, determined on solution S.

Related substances
Liquid chromatography (*2.2.29*).
Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.
Reference solution (a) Dissolve 5.0 mg of *oxybutynin hydrochloride CRS* and 5.0 mg of *oxybutynin impurity A CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 50.0 mL with the mobile phase.
Reference solution (b) Dilute 1.0 mL of the test solution to 200.0 mL with the mobile phase.
Column:
— *size:* l = 0.15 m, Ø = 3.9 mm;
— *stationary phase:* octylsilyl silica gel for chromatography R2 (5 µm).
Mobile phase Mix 49 volumes of a solution containing 3.4 g/L of *potassium dihydrogen phosphate R* and 4.36 g/L of *dipotassium hydrogen phosphate R* and 51 volumes of *acetonitrile R1.*
Flow rate 1 mL/min.
Detection Spectrophotometer at 210 nm.
Injection 10 µL.
Run time Twice the retention time of oxybutynin.
Retention time Oxybutynin = about 15 min; impurity A = about 24 min.
System suitability Reference solution (a):
— *resolution:* minimum 11.0 between the peaks due to oxybutynin and impurity A.
Limits:
— *impurity A:* not more than 1.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (a) (1.5 per cent);
— *unspecified impurities:* for each impurity, not more than 0.2 times the area of the principal peak in the

chromatogram obtained with reference solution (b) (0.10 per cent);
— *sum of impurities other than A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 3.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

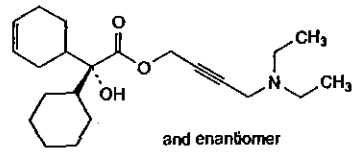
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.300 g in a mixture of 5.0 mL of *0.01 M hydrochloric acid* and 50 mL of *ethanol (96 per cent) R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

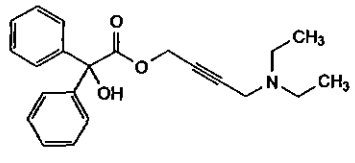
1 mL of *0.1 M sodium hydroxide* is equivalent to 39.4 mg of $C_{22}H_{32}ClNO_3$.

STORAGE
Protected from light.

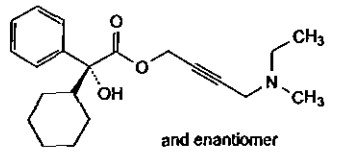
IMPURITIES
Specified impurities *A*.
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D, E*.



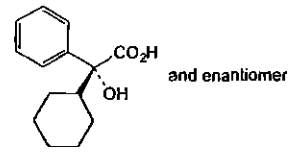
A. 4-(diethylamino)but-2-ynyl (*RS*)-2-(cyclohex-3-enyl)-2-cyclohexyl-2-hydroxyacetate,



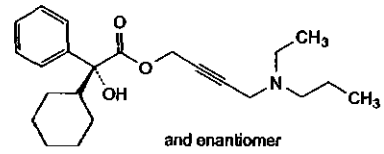
B. 4-(diethylamino)but-2-ynyl 2-hydroxy-2,2-diphenylacetate (diphenyl analogue of oxybutynin),



C. 4-(ethylmethylamino)but-2-ynyl (*RS*)-2-cyclohexyl-2-hydroxy-2-phenylacetate (methylethyl analogue of oxybutynin),



D. (*RS*)-2-cyclohexyl-2-hydroxy-2-phenylacetic acid (phenylcyclohexylglycolic acid),

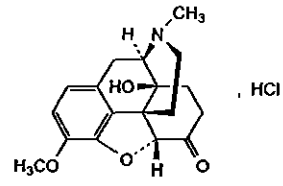


E. 4-(ethylpropylamino)but-2-ynyl (*RS*)-2-cyclohexyl-2-hydroxy-2-phenylacetate (ethylpropyl analogue of oxybutynin).

Ph Eur

Oxycodone Hydrochloride

(Ph. Eur. monograph 1793)



$C_{18}H_{22}ClNO_4$ 351.9 *124-90-3*

Action and use
Opioid receptor agonist; analgesic.

Preparations
Oxycodone Capsules
Oxycodone Injection
Oxycodone Oral Solution
Oxycodone Prolonged-release Tablets

Ph Eur

DEFINITION
4,5α-Epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one hydrochloride.

Content
98.5 per cent to 101.5 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white powder, hygroscopic.

Solubility
Freely soluble in water, sparingly soluble in anhydrous ethanol, practically insoluble in toluene.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Preparation Discs.
Dissolve 50 mg in *water R* and dilute to 5 mL with the same solvent. Render the solution alkaline with *dilute ammonia R1*. Allow the mixture to stand until a precipitate is formed. Filter, wash the precipitate with 10 mL of cold *water R*, and dry for 1 h at 105 °C. Examine the precipitate.

Comparison Repeat the operations using 50 mg of *oxycodone hydrochloride CRS*.

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 1.00 g in *carbon dioxide-free water R* and dilute to 50.0 mL with the same solvent.

Acidity or alkalinity

To 10 mL of solution S add 0.05 mL of *methyl red solution R*. Not more than 0.2 mL of *0.02 M sodium hydroxide* or *0.02 M hydrochloric acid* is required to change the colour of the indicator.

Specific optical rotation (*2.2.7*)

−140 to −148 (anhydrous substance), determined on solution S.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions protected from light.*

Test solution Dissolve 0.100 g of the substance to be examined in a 1 per cent *V/V* solution of *dilute acetic acid R* and dilute to 50.0 mL with the same solvent.

Reference solution (a) Dissolve 20.0 mg of *oxycodone impurity D CRS* in a 1 per cent *V/V* solution of *dilute acetic acid R* and dilute to 10.0 mL with the same solution.

Reference solution (b) To 1.0 mL of the test solution, add 1.0 mL of reference solution (a) and dilute to 100.0 mL with a 1 per cent *V/V* solution of *dilute acetic acid R*. Dilute 1.0 mL of the solution to 10.0 mL with a 1 per cent *V/V* solution of *dilute acetic acid R*.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase:
- *mobile phase A*: mix 830 mL of a 1.1 g/L solution of *sodium heptanesulfonate monohydrate R* previously adjusted to pH 2.0 with a mixture of equal volumes of *phosphoric acid R* and *water R*, with 70 mL of *acetonitrile R* and 100 mL of *methanol R*;
- *mobile phase B*: mix 600 mL of a 1.1 g/L solution of *sodium heptanesulfonate monohydrate R* previously adjusted to pH 2.0 with a mixture of equal volumes of *phosphoric acid R* and *water R*, with 150 mL of *acetonitrile R* and 250 mL of *methanol R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 60	100 → 50	0 → 50

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µL.

Relative retention With reference to oxycodone (retention time = about 24 min): impurity A = about 0.4; impurity B = about 0.7; impurity C = about 1.14; impurity D = about 1.18; impurity E = about 1.18; impurity F = about 2.4.

System suitability Reference solution (b):
- *resolution*: minimum 3 between the peaks due to oxycodone and impurity D.

Limits:
- *correction factor*: for the calculation of content, multiply the peak area of impurity F by 0.5;
- *sum of impurities D and E*: not more than 10 times the area of the peak due to oxycodone in the chromatogram obtained with reference solution (b) (1.0 per cent);
- *impurities A, B, C, F*: for each impurity, not more than the area of the peak due to oxycodone in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *any other impurity*: for each impurity, not more than the area of the peak due to oxycodone in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *total*: not more than 15 times the area of the peak due to oxycodone in the chromatogram obtained with reference solution (b) (1.5 per cent);
- *disregard limit*: 0.5 times the area of the peak due to oxycodone in the chromatogram obtained with reference solution (b) (0.05 per cent).

Ethanol (*2.4.24, System A*)

Maximum 1.0 per cent.

Water (*2.5.12*)

Maximum 7.0 per cent, determined on 0.250 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in a mixture of 5.0 mL of *0.01 M hydrochloric acid* and 60 mL of *ethanol (96 per cent) R*. Titrate with *0.1 M ethanolic sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Measure the volume used between the 2 inflexion points.

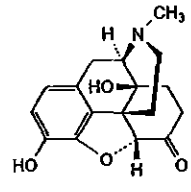
1 mL of *0.1 M ethanolic sodium hydroxide* is equivalent to 35.19 mg of $C_{18}H_{22}ClNO_4$.

STORAGE

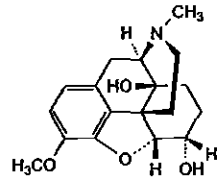
In an airtight container, protected from light.

IMPURITIES

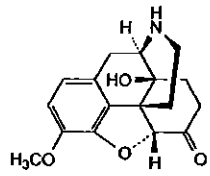
Specified impurities *A, B, C, D, E, F.*



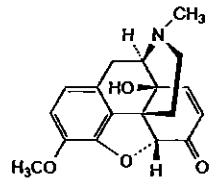
A. 4,5α-epoxy-3,14-dihydroxy-17-methylmorphinan-6-one (oxymorphone),



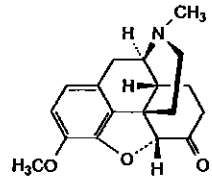
B. 4,5α-epoxy-3-methoxy-17-methylmorphinan-6α,14-diol (7,8-dihydro-14-hydroxycodeine),



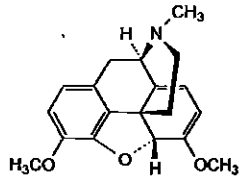
C. 4,5α-epoxy-14-hydroxy-3-methoxymorphinan-6-one (noroxycodone),



D. 7,8-didehydro-4,5α-epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one (14-hydroxycodeinone),



E. 4,5α-epoxy-3-methoxy-17-methylmorphinan-6-one (hydrocodone),



F. 6,7,8,14-tetradehydro-4,5α-epoxy-3-6-dimethoxy-17-methylmorphinan (thebain).

Ph Eur

Oxygen

(Ph. Eur. monograph 0417)

Oxygen should be kept in approved metal cylinders, the shoulders of which are painted white and the remainder black. The cylinder should carry a label stating 'Oxygen'. In addition, 'Oxygen' or the symbol 'O2' should be stencilled in paint on the shoulder of the cylinder.

When Oxygen is intended for use in a room in which magnetic resonance imaging (MRI) is being performed, the cylinder and fittings should be made from suitable non-ferromagnetic materials and labelled accordingly.

O_2	32.00	7782-44-7

Ph Eur

DEFINITION

Content

Minimum 99.5 per cent *V/V* of O_2.

This monograph applies to oxygen for medicinal use.

CHARACTERS

Appearance

Colourless gas.

Solubility

At 20 °C and at a pressure of 101 kPa, 1 volume dissolves in about 32 volumes of water.

PRODUCTION

Oxygen is produced by a purification process followed by cryodistillation of the ambient air.

Carbon dioxide

Maximum 300 ppm *V/V*, determined using an infrared analyser (*2.5.24*).

Gas to be examined Filter the substance to be examined to avoid stray light phenomena.

Reference gas (a) *Oxygen R.*

Reference gas (b) Mixture containing 300 ppm *V/V* of *carbon dioxide R1* in *nitrogen R1*.

Calibrate the apparatus and set the sensitivity using reference gases (a) and (b). Measure the content of carbon dioxide in the gas to be examined.

Carbon monoxide

Maximum 5 ppm *V/V*, determined using an infrared analyser (*2.5.25*).

Gas to be examined Filter the substance to be examined to avoid stray light phenomena.

Reference gas (a) *Oxygen R.*

Reference gas (b) Mixture containing 5 ppm *V/V* of *carbon monoxide R* in *nitrogen R1*.

Calibrate the apparatus and set the sensitivity using reference gases (a) and (b). Measure the content of carbon monoxide in the gas to be examined.

Water

Maximum 67 ppm *V/V*, determined using an electrolytic hygrometer (*2.5.28*).

Assay

Determine the concentration of oxygen using a paramagnetic analyser (*2.5.27*).

IDENTIFICATION

It complies with the limits of the assay.

TESTS

Carbon dioxide

Maximum 300 ppm *V/V*, determined using a carbon dioxide detector tube (*2.1.6*).

Carbon monoxide

Maximum 5 ppm *V/V*, determined using a carbon monoxide detector tube (*2.1.6*).

Water vapour

Maximum 67 ppm *V/V*, determined using a water vapour detector tube (*2.1.6*).

STORAGE

As a compressed gas or liquid in appropriate containers, complying with the legal regulations. Oils and grease are not to be used unless they are oxygen-compatible.

IMPURITIES

Specified impurities *A, B, C.*

A. CO_2: carbon dioxide,

B. CO: carbon monoxide,

C. H_2O: water.

Ph Eur

Oxygen (93 per cent)

(Ph. Eur. monograph 2455)

O_2 32.00

Ph Eur

DEFINITION

Content

90.0 per cent *V/V* to 96.0 per cent *V/V* of O_2, the remainder mainly consisting of argon and nitrogen.

This monograph applies to oxygen (93 per cent) for medicinal use. It does not apply to gas produced using individual concentrators for domiciliary use.

PRODUCTION

Oxygen (93 per cent) is produced in single-stage concentrators by adsorption purification of ambient air using zeolites. During production, the oxygen content is continuously monitored by means of a paramagnetic analyser (*2.5.27*). Following the design and installation of the concentrator, and after any modification or significant intervention, the gas produced complies with the following requirements.

Carbon dioxide

Maximum 300 ppm *V/V*, determined using an infrared analyser (*2.5.24*).

Gas to be examined The substance to be examined. It must be filtered to avoid stray light phenomena.

Reference gas (a) *Oxygen R.*

Reference gas (b) A mixture of 7 per cent *V/V* of *nitrogen R1* and 93 per cent *V/V* of *oxygen R*, containing 300 ppm *V/V* of *carbon dioxide R1.*

Calibrate the apparatus and set the sensitivity using reference gases (a) and (b). Measure the content of carbon dioxide in the gas to be examined.

Carbon monoxide

Maximum 5 ppm *V/V*, determined using an infrared analyser (*2.5.25*).

Gas to be examined The substance to be examined. It must be filtered to avoid stray light phenomena.

Reference gas (a) *Oxygen R.*

Reference gas (b) A mixture containing 5 ppm *V/V* of *carbon monoxide R* in *nitrogen R1.*

Calibrate the apparatus and set the sensitivity using reference gases (a) and (b). Measure the content of carbon monoxide in the gas to be examined.

Nitrogen monoxide and nitrogen dioxide

Maximum 2 ppm *V/V* in total, determined using a chemiluminescence analyser (*2.5.26*).

Gas to be examined The substance to be examined.

Reference gas (a) A mixture of 21 per cent *V/V* of *oxygen R* and 79 per cent *V/V* of *nitrogen R1*, containing less than 0.05 ppm *V/V* of nitrogen monoxide and nitrogen dioxide.

Reference gas (b) A mixture containing 2 ppm *V/V* of *nitrogen dioxide R* in *nitrogen R1.*

Calibrate the apparatus and set the sensitivity using reference gases (a) and (b). Measure the content of nitrogen monoxide and nitrogen dioxide in the gas to be examined.

Sulfur dioxide

Maximum 1 ppm *V/V*, determined using an ultraviolet fluorescence analyser (Figure 2455.-1.).

The apparatus consists of the following:

- a system generating ultraviolet radiation with a wavelength of 210 nm, made up of an ultraviolet lamp, a collimator, and a selective filter; the beam is blocked periodically by a chopper rotating at high speeds;
- a reaction chamber, through which flows the gas to be examined;
- a system that detects radiation emitted at a wavelength of 350 nm, made up of a selective filter, a photomultiplier tube and an amplifier.

Gas to be examined The substance to be examined. It must be filtered.

Reference gas (a) A mixture of 7 per cent *V/V* of *nitrogen R1* and 93 per cent *V/V* of *oxygen R.*

Reference gas (b) A mixture of 7 per cent *V/V* of *nitrogen R1* and 93 per cent *V/V* of *oxygen R*, containing 0.5 ppm *V/V* to 2 ppm *V/V* of *sulfur dioxide R1.*

Calibrate the apparatus and set the sensitivity using reference gases (a) and (b). Measure the content of sulfur dioxide in the gas to be examined.

Oil

Maximum 0.1 mg/m^3, determined using an oil detector tube (*2.1.6*).

Water

Maximum 67 ppm *V/V*, determined using an electrolytic hygrometer (*2.5.28*).

Assay

Determine the concentration of oxygen using a paramagnetic analyser (*2.5.27*).

CHARACTERS

Appearance

Colourless gas.

IDENTIFICATION

It complies with the limits of the assay.

TESTS

Carbon dioxide

Maximum 300 ppm *V/V*, determined using a carbon dioxide detector tube (*2.1.6*).

Carbon monoxide

Maximum 5 ppm *V/V*, determined using a carbon monoxide detector tube (*2.1.6*).

Nitrogen monoxide and nitrogen dioxide

Maximum 2 ppm *V/V* in total, determined using a nitrogen monoxide and nitrogen dioxide detector tube (*2.1.6*).

Sulfur dioxide

Maximum 1 ppm *V/V*, determined using a sulfur dioxide detector tube (*2.1.6*).

Oil

Maximum 0.1 mg/m^3, determined using an oil detector tube (*2.1.6*).

Water vapour

Maximum 67 ppm *V/V*, determined using a water vapour detector tube (*2.1.6*).

ASSAY

Determine the content of oxygen using a paramagnetic analyser (*2.5.27*).

STORAGE

Oxygen 93 per cent obtained from an oxygen concentrator is normally used on the site where it is produced. It is fed directly into a medicinal gas pipeline or administration system. Where authorised by the competent authority, it may be stored in suitable containers complying with the legal

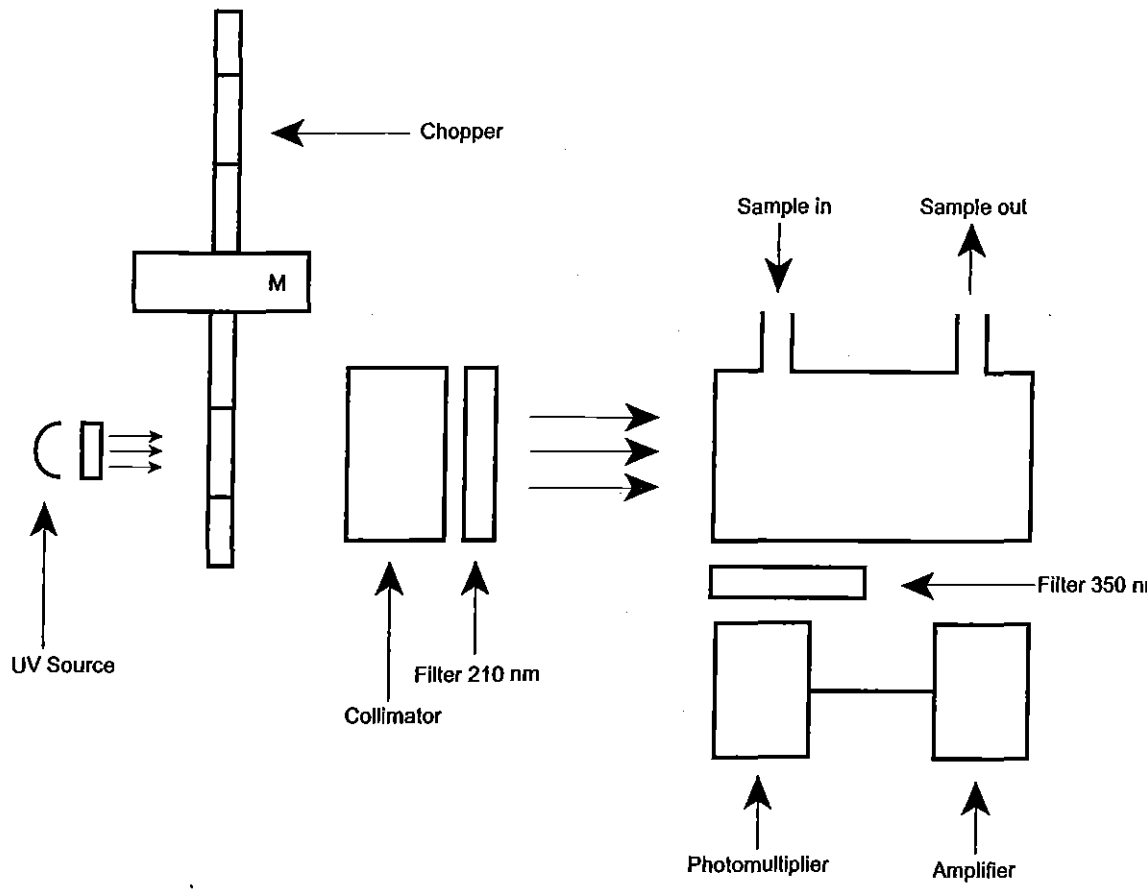


Figure 2455.-1. – *UV fluorescence analyser*

regulations. Oils and grease are not to be used unless they are oxygen-compatible.

IMPURITIES

A. CO_2: carbon dioxide,

B. CO: carbon monoxide,

C. SO_2: sulfur dioxide,

D. NO and NO_2: nitrogen monoxide and nitrogen dioxide,

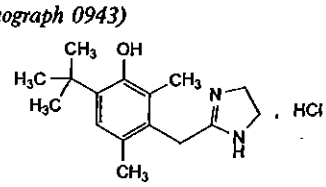
E. oil,

F. H_2O: water.

Ph Eur

Oxymetazoline Hydrochloride

(Ph. Eur. monograph 0943)



$C_{16}H_{25}ClN_2O$ 296.8 *2315-02-8*

Action and use

Alpha-adrenoceptor agonist; decongestant.

Ph Eur

DEFINITION

3-[(4,5-Dihydro-1*H*-imidazol-2-yl)methyl]-6-(1,1-dimethylethyl)-2,4-dimethylphenol hydrochloride.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water and in ethanol (96 per cent).

IDENTIFICATION

First identification: A, D.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *oxymetazoline hydrochloride CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 20 mg of the substance to be examined in a mixture of equal volumes of *ethyl acetate R* and *methanol R* and dilute to 5 mL with the same mixture of solvents.

Reference solution Dissolve 20 mg of *oxymetazoline hydrochloride CRS* in a mixture of equal volumes of *ethyl acetate R* and *methanol R* and dilute to 5 mL with the same mixture of solvents.

Plate *TLC silica gel G plate R.*

Mobile phase *diethylamine R, cyclohexane R, anhydrous ethanol R* (6:15:79 *V/V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying In a current of warm air for 5 min, then allow to cool.

Detection Spray with a freshly prepared 5.0 g/L solution of *potassium ferricyanide R* in *ferric chloride solution R2*; examine in daylight.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. Dissolve about 2 mg in 1 mL of *water R*, then add 0.2 mL of a 50 g/L solution of *sodium nitroprusside R* and 0.2 mL of *dilute sodium hydroxide solution R*. Allow to stand for 10 min. Add 2 mL of *sodium hydrogen carbonate solution R*. A violet colour develops.

D. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_7 (*2.2.2, Method II*).

Dissolve 2.5 g in *water R* and dilute to 50 mL with the same solvent.

Acidity or alkalinity

Dissolve 0.25 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent. Add 0.1 mL of *methyl red solution R* and 0.2 mL of *0.01 M hydrochloric acid*. The solution is red. Not more than 0.4 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to yellow.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 50.0 mL with the same solvent.

Reference solution (a) Dilute 5.0 mL of the test solution to 100.0 mL with *water R*. Dilute 2.0 mL of this solution to 100.0 mL with *water R*.

Reference solution (b) Dissolve 5.0 mg of *oxymetazoline impurity A CRS* and 5 mg of the substance to be examined in *water R* and dilute to 50.0 mL with the same solvent. Dilute 10.0 mL of the solution to 50.0 mL with *water R*.

Reference solution (c) Dilute 1.0 mL of reference solution (b) to 20.0 mL with *water R*.

Column:
- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: end-capped octadecylsilyl silica gel for chromatography with embedded polar groups R* (5 µm).

Mobile phase:
- *mobile phase A*: 1.36 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*;
- *mobile phase B*: *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	70	30
5 - 20	70 → 15	30 → 85
20 - 35	15	85

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µL.

Relative retention With reference to oxymetazoline (retention time = about 5.0 min): impurity A = about 0.9.

System suitability Reference solution (b):
- *resolution*: minimum 4.0 between the peaks due to impurity A and oxymetazoline.

Limits:
- *impurity A*: not more than 1.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water

(*2.5.32*): maximum 0.3 per cent, determined on 1.00 g .

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

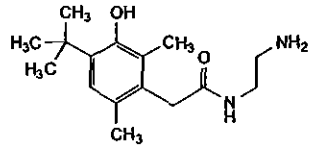
Dissolve 0.200 g in a mixture of 20 mL of *acetic anhydride R* and 20 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 29.68 mg of $C_{16}H_{25}ClN_2O$.

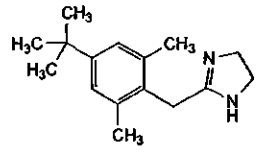
IMPURITIES

Specified impurities *A*.

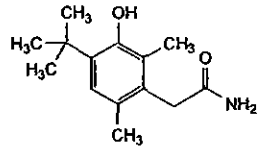
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D, E.*



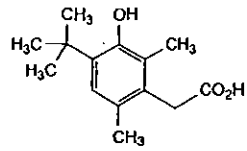
A. *N*-(2-aminoethyl)-2-[4-(1,1-dimethylethyl)-3-hydroxy-2,6-dimethylphenyl]acetamide,



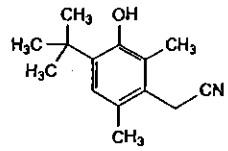
B. 2-[[4-(1,1-dimethylethyl)-2,6-dimethylphenyl]methyl]-4,5-dihydro-1*H*-imidazole (xylometazoline),



C. 2-[4-(1,1-dimethylethyl)-3-hydroxy-2,6-dimethylphenyl]acetamide,



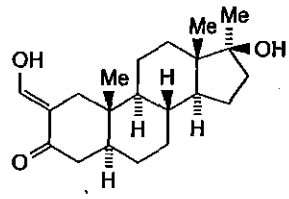
D. 2-[4-(1,1-dimethylethyl)-3-hydroxy-2,6-dimethylphenyl]acetic acid,



E. 2-[4-(1,1-dimethylethyl)-3-hydroxy-2,6-dimethylphenyl]acetonitrile.

Ph Eur

Oxymetholone



$C_{21}H_{32}O_3$ 332.5 *434-07-1*

Action and use
Anabolic steroid; androgen.

Preparation
Oxymetholone Tablets

DEFINITION
Oxymetholone is 17β-hydroxy-2-hydroxymethylene-17α-methyl-5α-androstan-3-one. It contains not less than 97.0% and not more than 103.0% of $C_{21}H_{32}O_3$, calculated with reference to the dried substance.

CHARACTERISTICS
A white to creamy white crystalline powder. It exhibits polymorphism.

Practically insoluble in *water*; soluble in *ethanol (96%)*; slightly soluble in *ether*.

IDENTIFICATION
A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.002% w/v solution in 0.01M *ethanolic sodium hydroxide* exhibits a maximum only at 315 nm.
The *absorbance* at 315 nm is about 1.1.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.003% w/v solution in 0.01M *ethanolic hydrochloric acid* exhibits a maximum only at 277 nm.
The *absorbance* at 277 nm is about 1.0.

C. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of oxymetholone *(RS 256)*. If the spectra are not concordant, dissolve the substance in the minimum of *chloroform*, evaporate to dryness, dry the residue over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa and prepare a new spectrum.

D. Complies with the test for identification of steroids, Appendix III A, using impregnating solvent II and mobile phase D.

TESTS
Melting point
175° to 180°, Appendix V A.

Specific optical rotation
In a 2% w/v solution in *1,4-dioxan*, +34 to +38, calculated with reference to the dried substance, Appendix V F.

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 98 volumes of *toluene* and 2 volumes of *absolute ethanol* as the mobile phase. Apply separately to the plate 10 µL of each of two solutions of the substance being examined in a mixture of equal volumes of *chloroform* and *ethanol (96%)* containing (1) 1.0% w/v and (2) 0.0050% w/v. After removal of the plate, allow it to dry in air and spray with *vanillin-ethanolic sulfuric acid reagent*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

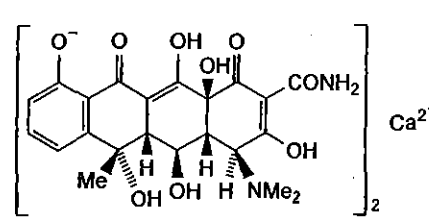
Loss on drying
When dried to constant weight at 105°, loses not more than 0.5% of its weight. Use 1 g.

Sulfated ash
Not more than 0.1%, Appendix IX A.

ASSAY
Dissolve 0.1 g in sufficient 0.01M *ethanolic sodium hydroxide* to produce 200 mL, dilute 5 mL to 250 mL with 0.01M *ethanolic sodium hydroxide* and measure the *absorbance* of the resulting solution at the maximum at 315 nm, Appendix II B. Calculate the content of $C_{21}H_{32}O_3$ taking 547 as the value of A(1%, 1 cm) at the maximum at 315 nm.

STORAGE
Oxymetholone should be kept free from contact with ferrous metals and protected from light.

Oxytetracycline Calcium



$(C_{22}H_{23}N_2O_9)_2Ca$ 958.9 *15251-48-6*

Action and use
Tetracycline antibacterial.

DEFINITION
Oxytetracycline Calcium is the calcium salt of (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxamide, a substance produced by the growth of certain strains of *Streptomyces rimosus* or obtained by any other means. It contains not less than 94.5% and not more than 102.0% of

$(C_{22}H_{23}N_2O_9)_2Ca$, calculated with reference to the anhydrous substance.

CHARACTERISTICS

A pale yellow to greenish fawn, crystalline powder.

Practically insoluble in *water*; soluble in dilute acids. It dissolves slowly in 5M *ammonia*.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in 0.01M *methanolic hydrochloric acid*.

(1) 0.05% w/v of the substance being examined.

(2) 0.05% w/v of *oxytetracycline BPCRS*.

(3) 0.05% w/v of each of *oxytetracycline BPCRS* and *demeclocycline hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel* precoated plate (Merck silica gel 60 plates are suitable). Adjust the pH of a 10% w/v solution of *disodium edetate* to 7.0 with 10M *sodium hydroxide* and spray the solution evenly onto the plate (about 10 mL for a plate 100 mm × 200 mm). Allow the plate to dry in a horizontal position for at least 1 hour. Before use, dry the plate at 110° for 1 hour.

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of air and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

6 volumes of *water*, 35 volumes of *methanol* and 59 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position, colour and size to that in the chromatogram obtained with solution (2).

B. To 2 mg add 5 mL of *sulfuric acid*; a deep red colour is produced. Add the solution to 2.5 mL of *water*; the colour changes to yellow.

C. Yields reaction B characteristic of *calcium salts*, Appendix VI.

TESTS

Acidity or alkalinity

pH of a 2.5% w/v suspension, 6.0 to 7.5, Appendix V L.

Light absorption

Absorbance of a 0.002% w/v solution in *0.1M chloride buffer pH 2.0* at the maximum at 353 nm, 0.56 to 0.61, calculated with reference to the anhydrous substance, Appendix II B.

Specific optical rotation

In a 1% w/v solution in *0.1M hydrochloric acid*, -194 to -210, calculated with reference to the anhydrous substance, Appendix V F. Allow the solution to stand protected from light for 30 minutes before measurement.

Light-absorbing impurities

A. Dissolve 0.2 g in 6 mL of 1M *hydrochloric acid* and add sufficient *methanol* to produce 100 mL. The *absorbance* at 430 nm, when measured within 1 hour of preparing the solution, is not more than 0.30, calculated with reference to the anhydrous substance, Appendix II B.

B. Dissolve 1 g in 6 mL of 1M *hydrochloric acid* and add sufficient *methanol* to produce 100 mL. The *absorbance* at 490 nm, when measured within 1 hour of preparing the solution, is not more than 0.20, calculated with reference to the anhydrous substance, Appendix II B.

Calcium

3.90 to 4.30%, calculated with reference to the anhydrous substance, when determined by the following method. Transfer about 1 g, accurately weighed, to a Kjeldahl flask, cautiously add 10 mL of *nitric acid* and mix. Allow to stand for 5 minutes, add a glass bead and heat on a water bath for 5 minutes. Remove from the water bath, cautiously add 5 mL of 9M *perchloric acid* and heat, adding further 5 mL quantities of the perchloric acid at intervals until the liquid is almost colourless. Add 0.1 mL of *nitric acid* and allow any further reaction to subside. Do not allow the volume of the liquid in the flask to be reduced below 3 mL at any stage in the oxidation. Wash the walls of the flask with 40 mL of *water*, collecting the washings in the flask, and boil for 3 to 4 minutes to expel chlorine. Cool, transfer the contents of the flask to a conical flask with the aid of *water* and dilute to about 200 mL with *water*. Adjust to pH 9 with 5M *sodium hydroxide* and then add 100 mL of *water* followed by 12 mL of 10M *sodium hydroxide* and mix. Add about 15 mg of *calconcarboxylic acid triturate* and titrate with 0.05M *disodium edetate VS* until the colour changes from violet to full blue. Each mL of 0.05M *disodium edetate VS* is equivalent to 2.004 mg of Ca.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 20 volumes of *acetonitrile* and 80 volumes of 0.01M oxalic acid (solvent A). *Prepare the solutions immediately before use.*

(1) 0.008% w/v of the substance being examined.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) 0.08% w/v of *oxytetracycline for system suitability A EPCRS*.

(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Intertsil C8 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.3 mL per minute.

(d) Use a column temperature of 50°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 0.05% v/v *trifluoroacetic acid*.

Mobile phase B 5 volumes of *tetrahydrofuran*, 15 volumes of *methanol* and 80 volumes of *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	90	10	isocratic
5-20	90→65	10→35	linear gradient
20-21	65→90	35→10	linear gradient
21-27	90	10	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to oxytetracycline (retention time about 7 minutes) are:

impurity A, about 0.9; impurity B, about 1.2; impurity C, about 1.3; impurity D, about 1.4; impurity E, about 2.2; impurity F, about 2.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 3.0, where *Hp* is the height above the baseline of the peak due to impurity A and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to oxytetracycline.

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 3.0, where *Hp* is the height above the baseline of the peak due to impurity B and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to oxytetracycline.

LIMITS

Identify any peak corresponding to impurities A, B, C, D, E and F in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (3). Multiply the areas of the peaks due to Impurity D and E by a correction factor of 0.4.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to impurity C is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of all the*secondary peaks* is not greater than 3.5 times the area of the principal peak in the chromatogram obtained with solution (2) (3.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.10%).

Water

Not more than 15.0% w/w, Appendix IX C. Use 0.15 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 20 volumes of *acetonitrile* and 80 volumes of 0.01M *oxalic acid* (solvent A). Prepare the solutions immediately before use.

(1) 0.008% w/v of the substance being examined.

(2) 0.008% w/v of *oxytetracycline BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions stated under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $(C_{22}H_{23}N_2O_9)_2Ca$ using the declared content of $C_{22}H_{24}N_2O_9$ in *oxytetracycline BPCRS*. Each mg of $C_{22}H_{24}N_2O_9$ is equivalent to 1.041 mg of $(C_{22}H_{23}N_2O_9)_2Ca$.

STORAGE

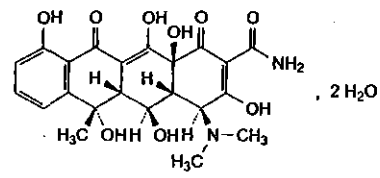
Oxytetracycline Calcium should be protected from light and stored at a temperature of 2° to 8°.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Oxytetracycline Dihydrate.

Oxytetracycline Dihydrate

(Ph. Eur. monograph 0199)



$C_{22}H_{24}N_2O_9,2H_2O$ 496.4 *6153-64-6*

Action and use

Tetracycline antibacterial.

Preparation

Oxytetracycline Tablets

Ph Eur

DEFINITION

(4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide dihydrate.

Substance produced by the growth of certain strains of *Streptomyces rimosus*.

Content

94.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Yellow, crystalline powder, slightly hygroscopic.

Solubility

Very slightly soluble in water. It dissolves in dilute acid and alkaline solutions.

IDENTIFICATION

First identification: B, C, D.

Second identification: A, C, D.

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 5 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dissolve 5 mg of *oxytetracycline CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 5 mg of *oxytetracycline CRS*, 5 mg of *tetracycline hydrochloride R* and 5 mg of *minocycline hydrochloride R* in *methanol R* and dilute to 10 mL with the same solvent.

Plate *TLC octadecylsilyl silica gel F_{254} plate R.*

Mobile phase Mix 20 volumes of *acetonitrile R*, 20 volumes of *methanol R* and 60 volumes of a 63 g/L solution of *oxalic acid R* previously adjusted to pH 2 with *concentrated ammonia R*.

Application 1 µL.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability The chromatogram obtained with reference solution (b) shows 3 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

C. To about 2 mg add 5 mL of *sulfuric acid R*. A deep red colour develops. Add the solution to 2.5 mL of *water R*. The colour becomes yellow.

D. Water (see Tests).

TESTS

pH (*2.2.3*)
4.5 to 7.5.

Suspend 0.1 g in 10 mL of *carbon dioxide-free water R*.

Light-absorbing impurities

Carry out the measurements within 1 h of preparing the solutions.

Dissolve 20.0 mg in a mixture of 1 volume of a 103 g/L solution of *hydrochloric acid R* and 99 volumes of *methanol R* and dilute to 10.0 mL with the same mixture of solvents. The absorbance (*2.2.25*) determined at 430 nm has a maximum of 0.25 (anhydrous substance).

Dissolve 0.100 g in a mixture of 1 volume of a 103 g/L solution of *hydrochloric acid R* and 99 volumes of *methanol R* and dilute to 10.0 mL with the same mixture of solvents. The absorbance (*2.2.25*) determined at 490 nm has a maximum of 0.20 (anhydrous substance).

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution (a) Dissolve 20.0 mg of the substance to be examined in a 1 g/L solution of *hydrochloric acid R* and dilute to 25.0 mL with the same solution.

Test solution (b) Dilute 5.0 mL of test solution (a) to 50.0 mL with a 1 g/L solution of *hydrochloric acid R*.

Reference solution (a) Dissolve 20.0 mg of *oxytetracycline CRS* in a 1 g/L solution of *hydrochloric acid R* and dilute to 25.0 mL with the same acid. Dilute 5.0 mL of the solution to 50.0 mL with a 1 g/L solution of *hydrochloric acid R*.

Reference solution (b) Dilute 1.0 mL of test solution (a) to 100.0 mL with a 1 g/L solution of *hydrochloric acid R*.

Reference solution (c) Dissolve 4.0 mg of *oxytetracycline for system suitability A CRS* (containing impurities A, B, C, D and E) in a 1 g/L solution of *hydrochloric acid R* and dilute to 5.0 mL with the same solution.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 50 °C.

Mobile phase:
- *mobile phase A*: 0.05 per cent *V/V* solution of *trifluoroacetic acid R*;
- *mobile phase B*: *tetrahydrofuran for chromatography R*, *methanol R1*, *acetonitrile R1* (5:15:80 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	90	10
5 - 20	90 → 65	10 → 35

Flow rate 1.3 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µL of test solution (a) and reference solutions (b) and (c).

Identification of impurities Use the chromatogram supplied with *oxytetracycline for system suitability A CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, C, D and E.

Relative retention With reference to oxytetracycline (retention time = about 6.5 min): impurity A = about 0.9; impurity B = about 1.2; impurity C = about 1.3; impurity D = about 1.4; impurity E = about 2.2.

System suitability Reference solution (c):
- *peak-to-valley ratio*: minimum 3.0, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to oxytetracycline; minimum 3.0, where H_p = height above the baseline of the peak due to impurity B and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to oxytetracycline.

Calculation of percentage contents:
- *correction factors*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity D = 0.4; impurity E = 0.4;
- for each impurity, use the concentration of oxytetracycline dihydrate in reference solution (b).

Limits:
- *impurity C*: maximum 2.0 per cent;
- *impurity B*: maximum 1.0 per cent;
- *impurity A*: maximum 0.7 per cent;
- *impurities D, E*: for each impurity, maximum 0.2 per cent;
- *any other impurity*: for each impurity, maximum 0.1 per cent;
- *total*: maximum 3.5 per cent;
- *reporting threshold*: 0.05 per cent.

Water (*2.5.12*)
6.0 per cent to 9.0 per cent, determined on 0.250 g.

Sulfated ash (*2.4.14*)
Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (a).

Calculate the percentage content of $C_{22}H_{24}N_2O_9$ taking into account the assigned content of *oxytetracycline CRS*.

STORAGE

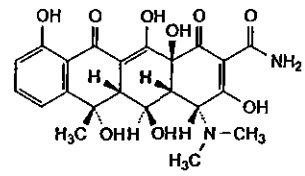
In an airtight container, protected from light.

IMPURITIES

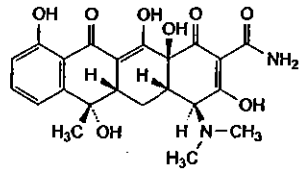
Specified impurities *A, B, C, D, E.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of

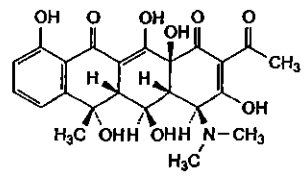
compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) F.



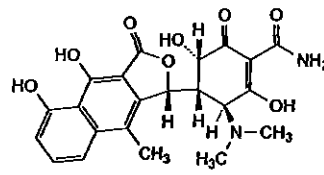
A. (4*R*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(dimethylamino)-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epioxytetracycline),



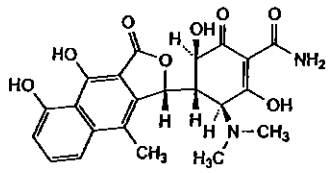
B. (4*S*,4a*S*,5a*S*,6*S*,12a*S*)-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (tetracycline),



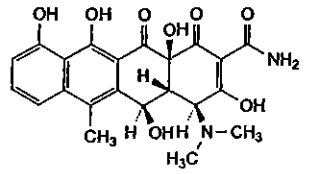
C. (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-2-acetyl-4-(dimethylamino)-3,5,6,10,12,12a-hexahydroxy-6-methyl-4a,5a,6,12a-tetrahydrotetracene-1,11(4*H*,5*H*)-dione (2-acetyl-2-decarbamoyloxytetracycline),



D. (3*S*,4*S*,5*S*)-4-[(1*R*)-4,5-dihydroxy-9-methyl-3-oxo-1,3-dihydronaphtho[2,3-*c*]furan-1-yl]-3-(dimethylamino)-2,5-dihydroxy-6-oxocyclohex-1-ene-1-carboxamide,



E. (3*S*,4*S*,5*R*)-4-[(1*R*)-4,5-dihydroxy-9-methyl-3-oxo-1,3-dihydronaphtho[2,3-*c*]furan-1-yl]-3-(dimethylamino)-2,5-dihydroxy-6-oxocyclohex-1-ene-1-carboxamide,

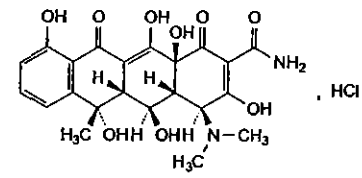


F. (4*S*,4a*R*,5*R*,12a*S*)-4-(dimethylamino)-3,5,10,11,12a-pentahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide (anhydrooxytetracycline).

Ph Eur

Oxytetracycline Hydrochloride

(Ph. Eur. monograph 0198)



$C_{22}H_{25}ClN_2O_9$ 496.9 *2058-46-0*

Action and use
Tetracycline antibacterial.

Preparations
Oxytetracycline Capsules
Oxytetracycline Cutaneous Spray

Ph Eur

DEFINITION

(4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide hydrochloride.

Substance produced by the growth of certain strains of *Streptomyces rimosus*.

Content
94.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
Yellow, crystalline powder, hygroscopic.

Solubility
Freely soluble in water, sparingly soluble in ethanol (96 per cent). Solutions in water become turbid on standing, owing to the precipitation of oxytetracycline.

IDENTIFICATION

First identification: B, C, D.

Second identification: A, C, D.

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 5 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dissolve 5 mg of *oxytetracycline hydrochloride CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 5 mg of *oxytetracycline hydrochloride CRS*, 5 mg of *tetracycline hydrochloride R* and 5 mg of *minocycline hydrochloride R* in *methanol R* and dilute to 10 mL with the same solvent.

Plate *TLC octadecylsilyl silica gel F_{254} plate R.*

Mobile phase Mix 20 volumes of *acetonitrile R*, 20 volumes of *methanol R* and 60 volumes of a 63 g/L solution of *oxalic acid R* previously adjusted to pH 2 with *concentrated ammonia R*.

Application 1 µL.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability The chromatogram obtained with reference solution (b) shows 3 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

B. Examine the chromatograms obtained in the test for related substances.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

C. To about 2 mg add 5 mL of *sulfuric acid R*. A deep red colour develops. Add the solution to 2.5 mL of *water R*. The colour becomes yellow.

D. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

pH (*2.2.3*)

2.3 to 2.9.

Dissolve 0.1 g in 10 mL of *carbon dioxide-free water R*.

Light-absorbing impurities

Carry out the measurements within 1 h of preparing the solutions.

Dissolve 20.0 mg in a mixture of 1 volume of a 103 g/L solution of *hydrochloric acid R* and 99 volumes of *methanol R* and dilute to 10.0 mL with the same mixture of solvents. The absorbance (*2.2.25*), determined at 430 nm has a maximum of 0.50 (anhydrous substance).

Dissolve 0.100 g in a mixture of 1 volume of a 103 g/L solution of *hydrochloric acid R* and 99 volumes of *methanol R* and dilute to 10.0 mL with the same mixture of solvents. The absorbance (*2.2.25*) determined at 490 nm has a maximum of 0.20 (anhydrous substance).

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution (a) Dissolve 20.0 mg of the substance to be examined in a 1 g/L solution of *hydrochloric acid R* and dilute to 25.0 mL with the same solution.

Test solution (b) Dilute 5.0 mL of test solution (a) to 50.0 mL with a 1 g/L solution of *hydrochloric acid R*.

Reference solution (a) Dissolve 20.0 mg of *oxytetracycline CRS* in a 1 g/L solution of *hydrochloric acid R* and dilute to 25.0 mL with the same acid. Dilute 5.0 mL of the solution to 50.0 mL with a 1 g/L solution of *hydrochloric acid R*.

Reference solution (b) Dilute 1.0 mL of test solution (a) to 100.0 mL in a 1 g/L solution of *hydrochloric acid R*.

Reference solution (c) Dissolve 4.0 mg of *oxytetracycline for system suitability A CRS* (containing impurities A, B, C, D, E and F) in a 1 g/L solution of *hydrochloric acid R* and dilute to 5.0 mL with the same solution.

Column:
- *size*: l = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 50 °C.

Mobile phase:
- *mobile phase A*: 0.05 per cent *V/V* solution of *trifluoroacetic acid R*;
- *mobile phase B*: *tetrahydrofuran for chromatography R*, *methanol R1*, *acetonitrile R1* (5:15:80 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	90	10
5 - 20	90 → 65	10 → 35

Flow rate 1.3 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µL of test solution (a) and reference solutions (b) and (c).

Identification of impurities Use the chromatogram supplied with *oxytetracycline for system suitability A CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, C, D, E and F.

Relative retention With reference to oxytetracycline (retention time = about 6.5 min): impurity A = about 0.9; impurity B = about 1.2; impurity C = about 1.3; impurity D = about 1.4; impurity E = about 2.2; impurity F = about 2.3.

System suitability Reference solution (c):
- *peak-to-valley ratio*: minimum 3.0, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to oxytetracycline; minimum 3.0, where H_p = height above the baseline of the peak due to impurity B and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to oxytetracycline.

Calculation of percentage contents:
- *correction factor*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity D = 0.4; impurity E = 0.4;
- for each impurity, use the concentration of oxytetracycline hydrochloride in reference solution (b).

Limits:
- *impurity C*: maximum 2.0 per cent;
- *impurity B*: maximum 1.0 per cent;
- *impurity A*: maximum 0.5 per cent;
- *impurities D, E*: for each impurity, maximum 0.2 per cent;
- *sum of impurities D, E and F*: maximum 1.0 per cent;
- *any other impurity*: maximum 0.1 per cent;
- *total*: maximum 3.5 per cent;
- *reporting threshold*: 0.05 per cent.

Water (*2.5.12*)

Maximum 2.0 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*)

Maximum 0.2 per cent, determined on 1.0 g.

Bacterial endotoxins (*2.6.14*)

Less than 0.4 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (a).

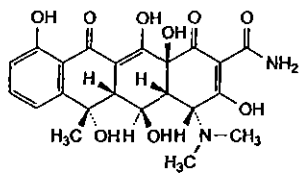
Calculate the percentage content of $C_{22}H_{25}ClN_2O_9$ taking into account the assigned content of *oxytetracycline CRS* and a conversion factor of 1.079.

STORAGE

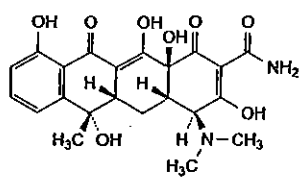
In an airtight container, protected from light. If the substance is sterile, store in a sterile, airtight, tamper-evident container.

IMPURITIES

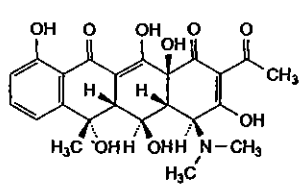
Specified impurities *A, B, C, D, E, F.*



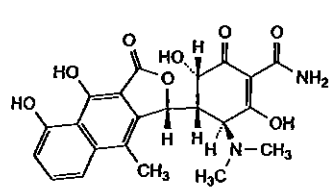
A. (4*R*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(dimethylamino)-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epioxytetracycline),



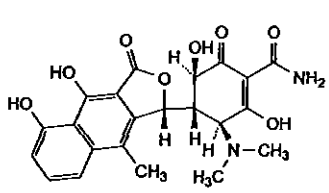
B. (4*S*,4a*S*,5a*S*,6*S*,12a*S*)-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (tetracycline),



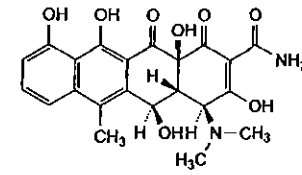
C. (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-2-acetyl-4-(dimethylamino)-3,5,6,10,12,12a-hexahydroxy-6-methyl-4a,5a,6,12a-tetrahydrotetracene-1,11(4*H*,5*H*)-dione (2-acetyl-2-decarbamoyloxytetracycline),



D. (3*S*,4*S*,5*S*)-4-[(1*R*)-4,5-dihydroxy-9-methyl-3-oxo-1,3-dihydronaphtho[2,3-*c*]furan-1-yl]-3-(dimethylamino)-2,5-dihydroxy-6-oxocyclohex-1-ene-1-carboxamide,



E. (3*S*,4*S*,5*R*)-4-[(1*R*)-4,5-dihydroxy-9-methyl-3-oxo-1,3-dihydronaphtho[2,3-*c*]furan-1-yl]-3-(dimethylamino)-2,5-dihydroxy-6-oxocyclohex-1-ene-1-carboxamide,

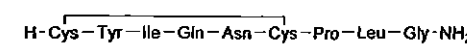


F. (4*S*,4a*R*,5*R*,12a*S*)-4-(dimethylamino)-3,5,10,11,12a-pentahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide (anhydrooxytetracycline).

Ph Eur

Oxytocin

(Ph. Eur. monograph 0780)



$C_{43}H_{66}N_{12}O_{12}S_2$ 1007 *50-56-6*

Action and use

Oxytocic.

Preparations

Ergometrine and Oxytocin Injection

Oxytocin Injection

Ph Eur

DEFINITION

L-Cysteinyl-L-tyrosyl-L-isoleucyl-L-glutamyl-L-asparaginyl-L-cysteinyl-L-prolyl-L-leucylglycinamide cyclic (1→6)-disulfide.

Synthetic cyclic nonapeptide having the structure of the hormone produced by the posterior lobe of the pituitary gland that stimulates contraction of the uterus and milk ejection in receptive mammals. It is available in the freeze-dried form as an acetate.

Content

93.0 per cent to 102.0 per cent (anhydrous and acetic acid-free substance).

By convention, for the purpose of labelling oxytocin preparations, 1 mg of oxytocin peptide ($C_{43}H_{66}N_{12}O_{12}S_2$) is equivalent to 600 IU of biological activity.

CHARACTERS

Appearance

White or almost white, hygroscopic powder.

Solubility

Very soluble in water. It dissolves in dilute solutions of acetic acid and of ethanol (96 per cent).

IDENTIFICATION

A. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with the reference solution.

B. Amino acid analysis (*2.2.56*). For hydrolysis use Method 1 and for analysis use Method 1.

Express the content of each amino acid in moles. Calculate the relative proportions of the amino acids, taking 1/6 of the sum of the number of moles of aspartic acid, glutamic acid, proline, glycine, isoleucine and leucine as equal to 1. The values fall within the following limits: aspartic acid: 0.90 to 1.10; glutamic acid: 0.90 to 1.10; proline: 0.90 to

1.10; glycine: 0.90 to 1.10; leucine: 0.90 to 1.10; isoleucine: 0.90 to 1.10; tyrosine: 0.7 to 1.05; half-cystine: 1.4 to 2.1. Not more than traces of other amino acids are present.

TESTS

pH (*2.2.3*)
3.0 to 6.0.

Dissolve 0.200 g in *carbon dioxide-free water R* and dilute to 10.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution Prepare a 0.25 mg/mL solution of the substance to be examined in a 15.6 g/L solution of *sodium dihydrogen phosphate R*.

Resolution solution Dissolve the contents of a vial of *oxytocin/desmopressin validation mixture CRS* in 1 mL of a 15.6 g/L solution of *sodium dihydrogen phosphate R*.

Column:
— *size*: l = 0.125 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: 15.6 g/L solution of *sodium dihydrogen phosphate R*;
— *mobile phase B*: *acetonitrile for chromatography R*, *water R* (50:50 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	70 → 40	30 → 60
30 - 30.1	40 → 70	60 → 30
30.1 - 45	70	30

Flow rate 1 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 50 µL.

Retention time Oxytocin = about 7.5 min; desmopressin = about 10 min.

System suitability Resolution solution:
— *resolution*: minimum 5.0 between the peaks due to desmopressin and oxytocin.

Limits:
— *any impurity*: maximum 1.5 per cent;
— *total*: maximum 5 per cent;
— *disregard limit*: 0.1 per cent.

Acetic acid (*2.5.34*)
6.0 per cent to 10.0 per cent.

Test solution Dissolve 15.0 mg of the substance to be examined in a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A and dilute to 10.0 mL with the same mixture of mobile phases.

Water (*2.5.12*)
Maximum 5.0 per cent, determined on at least 50 mg.

Bacterial endotoxins (*2.6.14*)
Less than 300 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Reference solution Dissolve the contents of a vial of *oxytocin CRS* in a 15.6 g/L solution of *sodium dihydrogen phosphate R* to obtain a concentration of 0.25 mg/mL.

Injection 25 µL.

Calculate the content of oxytocin ($C_{43}H_{66}N_{12}O_{12}S_2$) from the declared content of $C_{43}H_{66}N_{12}O_{12}S_2$ in *oxytocin CRS*.

STORAGE

In an airtight container, protected from light, at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-evident container.

LABELLING

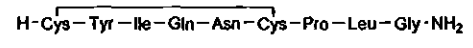
The label states the oxytocin peptide content ($C_{43}H_{66}N_{12}O_{12}S_2$).

Ph Eur

Oxytocin Concentrated Solution

Oxytocin Bulk Solution

(Ph. Eur. monograph 0779)



$C_{43}H_{66}N_{12}O_{12}S_2$ 1007

Action and use
Oxytocic.

Preparations
Ergometrine and Oxytocin Injection
Oxytocin Injection

Ph Eur

DEFINITION

L-Cysteinyl-L-tyrosyl-L-isoleucyl-L-glutamyl-L-asparaginyl-L-cysteinyl-L-prolyl-L-leucylglycinamide cyclic (1→6)-disulfide.

Solution of oxytocin, a synthetic cyclic nonapeptide having the structure of the hormone produced by the posterior lobe of the pituitary gland that stimulates contraction of the uterus and milk ejection in receptive mammals. It is available as a solution with a stated concentration of not less than 0.25 mg of oxytocin per millilitre, in a solvent that may contain an appropriate antimicrobial preservative.

Content
95.0 per cent to 105.0 per cent of the amount of the peptide stated per millilitre.

By convention, for the purpose of labelling oxytocin preparations, 1 mg of oxytocin peptide ($C_{43}H_{66}N_{12}O_{12}S_2$) is equivalent to 600 IU of biological activity.

CHARACTERS

Appearance
Clear, colourless liquid.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with the reference solution.

B. Amino acid analysis (*2.2.56*). For hydrolysis use Method 1 and for analysis use Method 1.

Express the content of each amino acid in moles. Calculate the relative proportions of the amino acids taking 1/6 of the

sum of the number of moles of aspartic acid, glutamic acid, proline, glycine, isoleucine and leucine as equal to 1.
The values fall within the following limits: aspartic acid: 0.90 to 1.10; glutamic acid: 0.90 to 1.10; proline: 0.90 to 1.10; glycine: 0.90 to 1.10; leucine: 0.90 to 1.10; isoleucine: 0.90 to 1.10; tyrosine: 0.7 to 1.05; half cystine: 1.4 to 2.1.
Not more than traces of other amino acids are present.

TESTS

pH (*2.2.3*)
3.0 to 5.0.

Related substances
Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution The preparation to be examined.

Resolution solution Dissolve the contents of a vial of *oxytocin/desmopressin validation mixture CRS* in 1 mL of a 15.6 g/L solution of *sodium dihydrogen phosphate R*.

Column:
— *size*: l = 0.125 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: 15.6 g/L solution of *sodium dihydrogen phosphate R*;
— *mobile phase B*: *acetonitrile for chromatography R*, *water R* (50:50 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	70 → 40	30 → 60
30 - 30.1	40 → 70	60 → 30
30.1 - 45	70	30

Flow rate 1 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 50 µL.

Retention time Oxytocin = about 7.5 min; desmopressin = about 10 min.

System suitability Resolution solution:
— *resolution*: minimum 5.0 between the peaks due to desmopressin and oxytocin.

Limits:
— *any impurity*: maximum 1.5 per cent;
— *total*: maximum 5 per cent;
— *disregard limit*: 0.1 per cent.

Bacterial endotoxins (*2.6.14*)
Less than 300 IU in the volume that contains 1 mg of oxytocin, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Reference solution Dissolve the contents of a vial of *oxytocin CRS* in a 15.6 g/L solution of *sodium dihydrogen phosphate R* to obtain a concentration of 0.25 mg/mL.

Injection 25 µL.

Calculate the content of oxytocin ($C_{43}H_{66}N_{12}O_{12}S_2$) from the declared content of $C_{43}H_{66}N_{12}O_{12}S_2$ in *oxytocin CRS*.

STORAGE

At a temperature of 2 °C to 8 °C, protected from light. If the substance is sterile, store in a sterile, airtight, tamper-evident container.

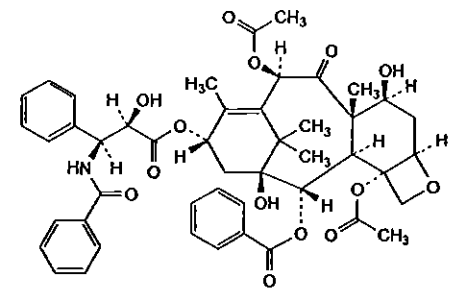
LABELLING

The label states the oxytocin peptide content in milligrams of $C_{43}H_{66}N_{12}O_{12}S_2$ per millilitre.

Ph Eur

Paclitaxel

(Ph. Eur. monograph 1794)



$C_{47}H_{51}NO_{14}$ 854 *33069-62-4*

Action and use
Taxane cytotoxic.

Ph Eur

DEFINITION

4,10β-Bis(acetyloxy)-13α-[[(2*R*,3*S*)-3-benzamido-2-hydroxy-3-phenylpropanoyl]oxy]-1,7β-dihydroxy-9-oxo-5β,20-epoxytax-11-en-2α-yl benzoate.

It is isolated from natural sources or produced by fermentation or by a semi-synthetic process.

Content
97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, soluble in methanol and freely soluble in methylene chloride.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *paclitaxel CRS*.

If the spectra obtained in the solid state show differences, dissolve 10 mg of the substance to be examined and the reference substance separately in 0.4 mL of *methylene chloride R*, evaporate to dryness and record new spectra using the residues.

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 0.1 g in 10 mL of *methanol R*.

Specific optical rotation (*2.2.7*)
−55.0 to −49.0 (anhydrous substance).

Dissolve 0.250 g in *methanol R* and dilute to 25.0 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

A. Paclitaxel isolated from natural sources or produced by fermentation.

Test solution (a) Dissolve 20.0 mg of the substance to be examined in *acetonitrile R1* and dilute to 10.0 mL with the same solvent.

Test solution (b) Dilute 1.0 mL of test solution (a) to 20.0 mL with *acetonitrile R1*.

Reference solution (a) Dilute 1.0 mL of test solution (a) to 10.0 mL with *acetonitrile R1*. Dilute 1.0 mL of this solution to 100.0 mL with *acetonitrile R1*.

Reference solution (b) Dissolve 5.0 mg of *paclitaxel CRS* in *acetonitrile R1* and dilute to 5.0 mL with the same solvent. Dilute 2.0 mL of this solution to 20.0 mL with *acetonitrile R1*.

Reference solution (c) Dissolve 2.0 mg of *paclitaxel impurity C CRS* in *acetonitrile R1* and dilute to 20.0 mL with the same solvent.

Reference solution (d) Dilute 1.0 mL of reference solution (c) to 50.0 mL with *acetonitrile R1*.

Reference solution (e) To 1 mL of reference solution (b) add 1 mL of reference solution (c).

Reference solution (f) Dissolve 5 mg of *paclitaxel natural for peak identification CRS* (containing impurities A, B, C, D, E, F, H, O, P, Q and R) in *acetonitrile R1* and dilute to 5 mL with the same solvent.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *diisopropylcyanosilyl silica gel for chromatography R* (5 µm) with a specific surface area of 180 m^2/g and a pore size of 8 nm;
- *temperature*: 20 ± 1 °C.

Mobile phase:

- *mobile phase A*: *methanol R1, water for chromatography R* (20:80 *V/V*);
- *mobile phase B*: *methanol R1, acetonitrile for chromatography R* (20:80 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 60	85 → 56	15 → 44
60 - 61	56 → 85	44 → 15
61 - 75	85	15

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 227 nm.

Injection 10 µL of test solution (a) and reference solutions (a), (d), (e) and (f).

Identification of impurities Use the chromatogram supplied with *paclitaxel natural for peak identification CRS* and the chromatogram obtained with reference solution (f) to identify the peaks due to impurities A, B, C, D, E, F, H, O, P, Q and R.

Relative retention With reference to paclitaxel (retention time = about 50 min): impurities A and B = about 0.90; impurity R = about 0.93; impurity H = about 0.96; impurities Q and P = about 1.02; impurity C = about 1.05; impurity D = about 1.07; impurities O and E = about 1.15; impurity F = about 1.20.

System suitability Reference solution (e):

- *resolution*: minimum 3.5 between the peaks due to paclitaxel and impurity C.

Limits:

- *sum of impurities E and O*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *impurity R*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *sum of impurities A and B*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);
- *impurity C*: not more than 3 times the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.3 per cent);
- *impurity D*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *sum of impurities P and Q*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *impurity F*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.1 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 15 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

B. Paclitaxel produced by a semi-synthetic process.

Test solution Dissolve 10.0 mg of the substance to be examined in *acetonitrile R1* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 10.0 mL with *acetonitrile R1*. Dilute 1.0 mL of this solution to 100.0 mL with *acetonitrile R1*.

Reference solution (b) Dissolve 5.0 mg of *paclitaxel CRS* in *acetonitrile R1* and dilute to 5.0 mL with the same solvent.

Reference solution (c) Dissolve 5 mg of *paclitaxel semi-synthetic for peak identification CRS* (containing impurities A, G, I and L) in *acetonitrile R1* and dilute to 5 mL with the same solvent.

Reference solution (d) Dissolve the contents of a vial of *paclitaxel semi-synthetic for system suitability CRS* (containing impurities E, H and N) in 1 mL of *acetonitrile R1*.

Column:

- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm) with a specific surface area of 300 m^2/g and a pore size of 12 nm;
- temperature: 35 °C.

Mobile phase:

- *mobile phase A*: *acetonitrile for chromatography R, water for chromatography R* (40:60 *V/V*);
- *mobile phase B*: *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	100	0
20 - 60	100 → 10	0 → 90
60 - 62	10 → 100	90 → 0
62 - 70	100	0

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 227 nm.

Injection 15 µL of the test solution and reference solutions (a), (c) and (d).

Identification of impurities Use the chromatogram supplied with *paclitaxel semi-synthetic for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, G, I and L; use the chromatogram supplied with *paclitaxel semi-synthetic for system suitability CRS* and the chromatogram obtained with reference solution (d) to identify the peaks due to impurities E, H and N.

Relative retention With reference to paclitaxel (retention time = about 23 min): impurity N = about 0.2; impurity G = about 0.5; impurity A = about 0.8; impurities M, J and H = about 0.9; impurity E = about 1.3; impurity I = about 1.4; impurity L = about 1.5; impurity K = about 2.2.

System suitability Reference solution (d):
- *resolution:* minimum 1.5 between the peaks due to impurity H and paclitaxel.

Limits:
- *correction factor*: for the calculation of content, multiply the peak area of impurity N by 1.29;
- *impurity A*: not more than 7 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.7 per cent);
- *impurity L*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *impurities E, I*: for each impurity, not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);
- *sum of impurities H, J and M*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);
- *impurities G, K, N*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 12 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.2 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.32*)
Maximum 3.0 per cent, determined on 0.050 g.

Microbial contamination
TAMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

Bacterial endotoxins (*2.6.14*)
Less than 0.4 IU/mg.

ASSAY

A. Paclitaxel isolated from natural sources or produced by fermentation.

Liquid chromatography (*2.2.29*) as described in test A for related substances with the following modification.

Injection Test solution (b) and reference solution (b).

Calculate the percentage content of $C_{47}H_{51}NO_{14}$ from the declared content of *paclitaxel CRS*.

B. Paclitaxel produced by a semi-synthetic process.

Liquid chromatography (*2,2.29*) as described in test B for related substances with the following modification.

Injection 10 µL of the test solution and reference solution (b).

Calculate the percentage content of $C_{47}H_{51}NO_{14}$ from the declared content of *paclitaxel CRS*.

STORAGE

In an airtight container, protected from light.

LABELLING

The label states the origin of the substance:
- — isolated from natural sources;
- — produced by fermentation;
- — produced by a semi-synthetic process.

IMPURITIES

Test A for related substances

A, B, C, D, E, F, H, O, P, Q, R.

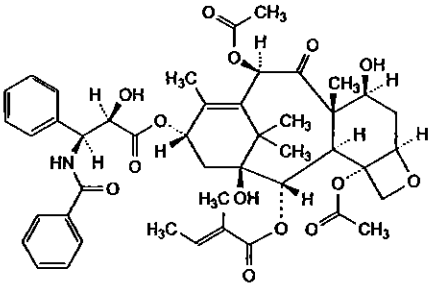
Specified impurities *A, B, C, D, E, F, O, P, Q, R.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *H.*

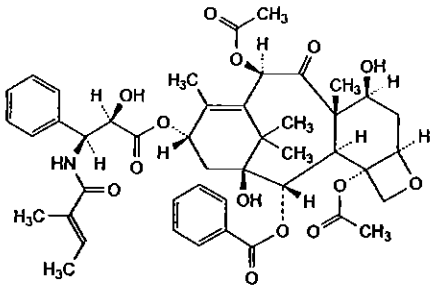
Test B for related substances

A, E, G, H, I, J, K, L, M, N.

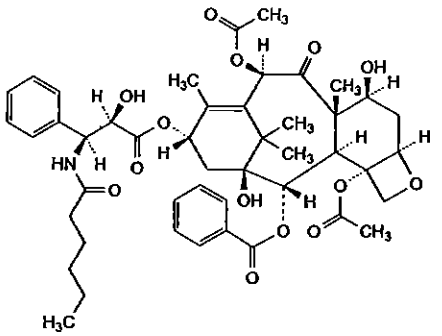
Specified impurities *A, E, G, H, I, J, K, L, M, N.*



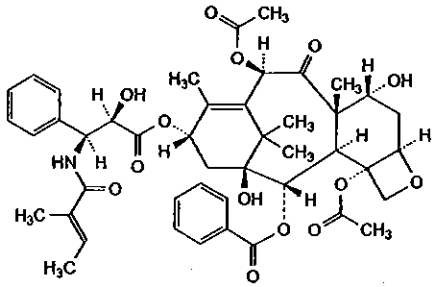
A. 4,10β-bis(acetyloxy)-13α-[[(2*R*,3*S*)-3-benzamido-2-hydroxy-3-phenylpropanoyl]oxy]-1,7β-dihydroxy-9-oxo-5β,20-epoxytax-11-en-2α-yl (2*E*)-2-methylbut-2-enoate (2-*O*-debenzoyl-2-*O*-tigloylpaclitaxel),



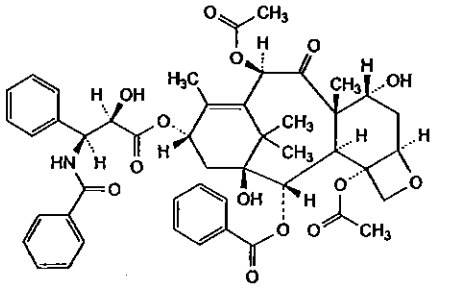
B. 4,10β-bis(acetyloxy)-1,7β-dihydroxy-13α-[[(2*R*,3*S*)-2-hydroxy-3-[[(2*E*)-2-methylbut-2-enoyl]amino]-3-phenylpropanoyl]oxy]-9-oxo-5β,20-epoxytax-11-en-2α-yl benzoate (*N*-debenzoyl-*N*-tigloylpaclitaxel; cephalomannine),



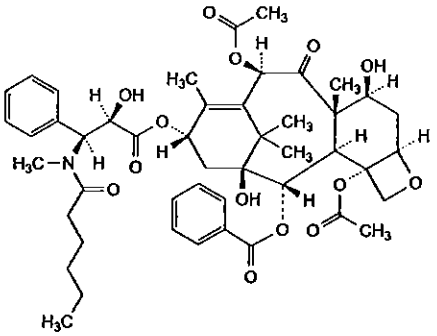
C. 4,10β-bis(acetyloxy)-13α-[[(2*R*,3*S*)-3-(hexanoylamino)-2-hydroxy-3-phenylpropanoyl]oxy]-1,7β-dihydroxy-9-oxo-5β,20-epoxytax-11-en-2α-yl benzoate (*N*-debenzoyl-*N*-hexanoylpaclitaxel; paclitaxel C),



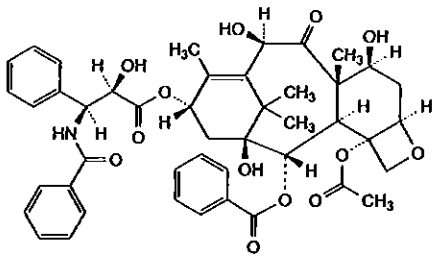
D. 4,10β-bis(acetyloxy)-1,7α-dihydroxy-13α-[[(2*R*,3*S*)-2-hydroxy-3-[[(2*E*)-2-methylbut-2-enoyl]amino]-3-phenylpropanoyl]oxy]-9-oxo-5β,20-epoxytax-11-en-2α-yl benzoate (*N*-debenzoyl-*N*-tigloyl-7-*epi*-paclitaxel; 7-*epi*-cephalomannine),



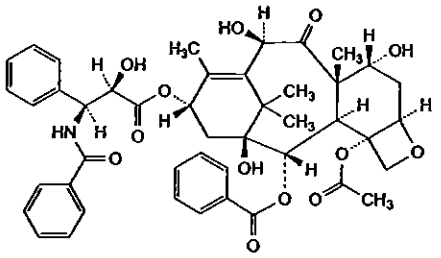
E. 4,10β-bis(acetyloxy)-13α-[[(2*R*,3*S*)-3-benzamido-2-hydroxy-3-phenylpropanoyl]oxy]-1,7α-dihydroxy-9-oxo-5β,20-epoxytax-11-en-2α-yl benzoate (7-*epi*-paclitaxel),



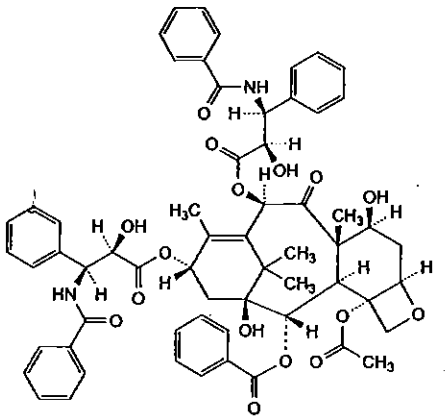
F. 4,10β-bis(acetyloxy)-13α-[[(2*R*,3*S*)-3-[hexanoyl(methyl)amino]-2-hydroxy-3-phenylpropanoyl]oxy]-1,7β-dihydroxy-9-oxo-5β,20-epoxytax-11-en-2α-yl benzoate (*N*-debenzoyl-*N*-hexanoyl-*N*-methylpaclitaxel; *N*-methylpaclitaxel C),



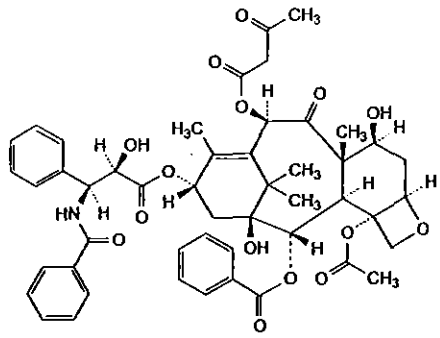
G. 4-(acetyloxy)-13α-[[(2*R*,3*S*)-3-benzamido-2-hydroxy-3-phenylpropanoyl]oxy]-1,7β,10β-trihydroxy-9-oxo-5β,20-epoxytax-11-en-2α-yl benzoate (10-*O*-deacetylpaclitaxel),



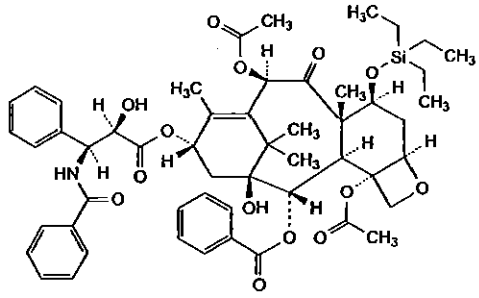
H. 4-(acetyloxy)-13α-[[(2*R*,3*S*)-3-benzamido-2-hydroxy-3-phenylpropanoyl]oxy]-1,7α,10β-trihydroxy-9-oxo-5β,20-epoxytax-11-en-2α-yl benzoate (10-*O*-deacetyl-7-*epi*-paclitaxel),



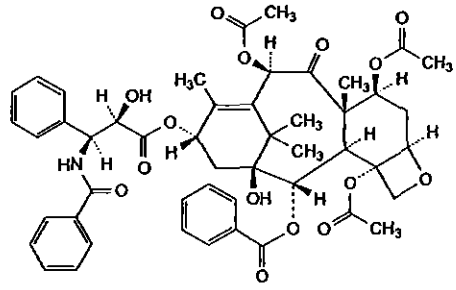
I. 4-(acetyloxy)-10β,13α-bis[[(2*R*,3*S*)-3-benzamido-2-hydroxy-3-phenylpropanoyl]oxy]-1,7β-dihydroxy-9-oxo-5β,20-epoxytax-11-en-2α-yl benzoate (10-*O*-[(2*R*,3*S*)-3-benzamido-2-hydroxy-3-phenylpropanoyl]-10-*O*-deacetylpaclitaxel),



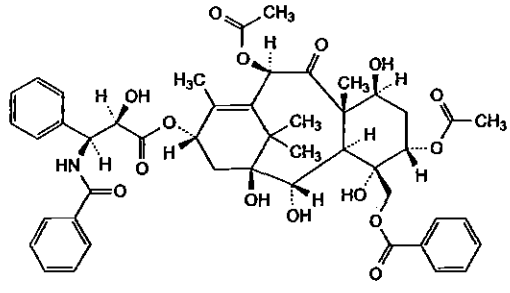
J. 4-(acetyloxy)-13α-[[(2*R*,3*S*)-3-benzamido-2-hydroxy-3-phenylpropanoyl]oxy]-1,7β-dihydroxy-9-oxo-10β-[(3-oxobutanoyl)oxy]-5β,20-epoxytax-11-en-2α-yl benzoate (10-*O*-deacetyl-10-*O*-(3-oxobutanoyl)paclitaxel),



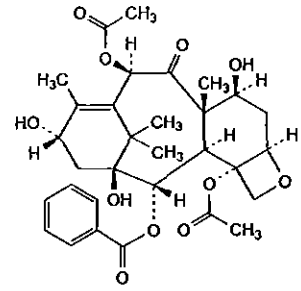
K. 4,10β-bis(acetyloxy)-13α-[[(2*R*,3*S*)-3-benzamido-2-hydroxy-3-phenylpropanoyl]oxy]-1-hydroxy-9-oxo-7β-[(triethylsilyl)oxy]-5β,20-epoxytax-11-en-2α-yl benzoate (7-*O*-(triethylsilanyl)paclitaxel),



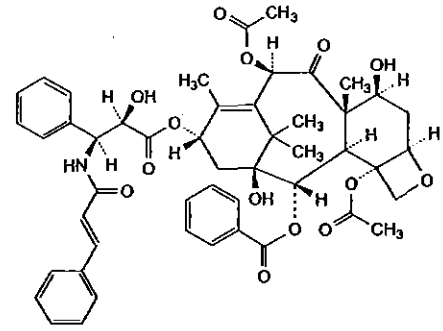
L. 4,7β,10β-tris(acetyloxy)-13α-[[(2*R*,3*S*)-3-benzamido-2-hydroxy-3-phenylpropanoyl]oxy]-1-hydroxy-9-oxo-5β,20-epoxytax-11-en-2α-yl benzoate (7-*O*-acetylpaclitaxel),



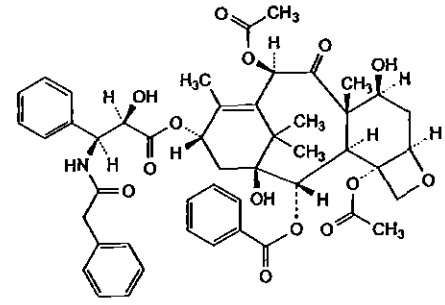
M. 5α,10β-bis(acetyloxy)-13α-[[(2*R*,3*S*)-3-benzamido-2-hydroxy-3-phenylpropanoyl]oxy]-1,2α,4,7β-tetrahydroxy-9-oxotax-11-en-20-yl benzoate,



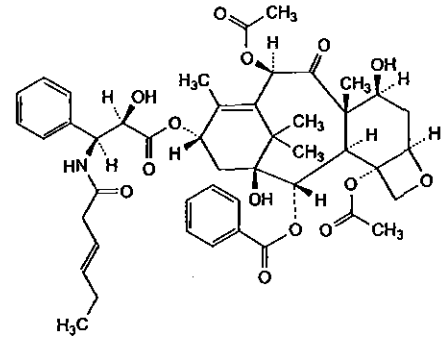
N. 4,10β-bis(acetyloxy)-1,7β,13α-trihydroxy-9-oxo-5β,20-epoxytax-11-en-2α-yl benzoate (13-*O*-de[(2*R*,3*S*)-3-benzamido-2-hydroxy-3-phenylpropanoyl]paclitaxel; baccatin III),



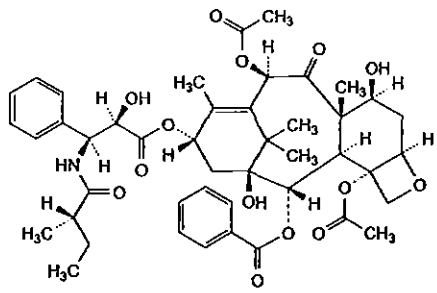
O. 4,10β-bis(acetyloxy)-1,7β-dihydroxy-13α-[[(2*R*,3*S*)-2-hydroxy-3-phenyl-3-[[(2*E*)-3-phenylprop-2-enoyl]amino]propanoyl]oxy]-9-oxo-5β,20-epoxytax-11-en-2α-yl benzoate (*N*-cinnamoyl-*N*-debenzoylpaclitaxel),



P. 4,10β-bis(acetyloxy)-1,7β-dihydroxy-13α-[[(2*R*,3*S*)-2-hydroxy-3-phenyl-3-(2-phenylacetamido)propanoyl]oxy]-9-oxo-5β,20-epoxytax-11-en-2α-yl benzoate (*N*-debenzoyl-*N*-(phenylacetyl)paclitaxel),



Q. 4,10β-bis(acetyloxy)-13α-[[(2*R*,3*S*)-3-[[(3*E*)-hex-3-enoyl]amino]-2-hydroxy-3-phenylpropanoyl]oxy]-1,7β-dihydroxy-9-oxo-5β,20-epoxytax-11-en-2α-yl benzoate (*N*-debenzoyl-*N*-[(3*E*)-hex-3-enoyl]paclitaxel),



R. 4,10β-bis(acetyloxy)-1,7β-dihydroxy-13α-[[(2*R*,3*S*)-2-hydroxy-3-[[(2*S*)-2-methylbutanoyl]amino]-3-phenylpropanoyl]oxy]-9-oxo-5β,20-epoxytax-11-en-2α-yl benzoate (*N*-debenzoyl-*N*-[(2*S*)-2-methylbutanoyl] paclitaxel).

Ph Eur

Palmitic Acid

(Ph. Eur. monograph 1904)

57-10-3

Action and use
Excipient.

Ph Eur

DEFINITION
Hexadecanoic acid ($C_{16}H_{32}O_2$; M_r 256.4), obtained from fats or oils of vegetable or animal origin.

Content
Minimum 92.0 per cent.

CHARACTERS

Appearance
White or almost white, waxy solid.

Solubility
Practically insoluble in water, soluble in ethanol (96 per cent).

IDENTIFICATION
A. Freezing point (see Tests).

B. Acid value (*2.5.1*): 216 to 220, determined on 0.1 g.

C. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with the reference solution.

TESTS

Appearance
Heat the substance to be examined to about 75 °C. The resulting liquid is not more intensely coloured than reference solution Y_7 or BY_7 (*2.2.2, Method I*).

Acidity
Melt 5.0 g, stir for 2 min in 10 mL of hot *carbon dioxide-free water R*, cool slowly and filter. To the filtrate add 0.05 mL of *methyl orange solution R*. No red colour develops.

Freezing point (*2.2.18*)
60 °C to 66 °C.

Iodine value (*2.5.4*)
Maximum 1.

Stearic acid
Maximum 6.0 per cent, determined as prescribed in the assay.

ASSAY
Gas chromatography (*2.4.22, Method C*) with the following modifications. Prepare the solutions as described in the method but omitting the initial hydrolysis.

Reference solution Prepare the reference solution in the same manner as the test solution using a mixture of 50 mg of *palmitic acid R* and 50 mg of *stearic acid R* instead of the substance to be examined.

Relative retention With reference to methyl stearate: methyl palmitate = about 0.9.

System suitability:
— *resolution*: minimum 5.0 between the peaks due to methyl stearate and methyl palmitate.

Ph Eur

Fractionated Palm Kernel Oil

The standards of this monograph encompass several different suppository bases. The selection of a basis for a particular suppository formulation should be appropriate to the product concerned and it may be necessary to apply more restrictive standards for a particular application.

Action and use
Suppository basis.

DEFINITION
Fractionated Palm Kernel Oil is obtained by expression of the natural oil from the kernels of *Elaeis guineensis* Jacq. followed by selective solvent fractionation and hydrogenation.

CHARACTERISTICS
A white, solid, brittle fat; odourless or almost odourless.

Practically insoluble in water; miscible with ether and with petroleum spirit (boiling range, 40° to 60°); practically insoluble in ethanol (96%).

TESTS

Acid value
Not more than 0.2, Appendix X B.

Iodine value
Not more than 6.0 *(iodine bromide method)*, Appendix X E.

Melting point
31° to 36°, Appendix V A, Method IV. Prepare the substance being examined in the following manner. Melt about 30 g in an oven at a temperature of 55° to 60° and filter through a suitable dry filter paper, maintaining the temperature between 53° and 60°. Cool with occasional stirring until the temperature falls to between 32° and 34°, stir continuously with a mechanical stirrer until the first signs of cloudiness appear and continue to stir by hand until the substance has the consistence of a paste. Immediately transfer to a vessel previously kept at a temperature of 15° to 22° and allow to stand at this temperature for 24 hours before carrying out the test.

Refractive index
At 50°, 1.445 to 1.447, Appendix V E.

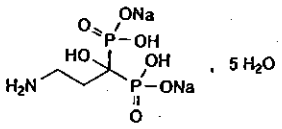
Saponification value
246 to 250, Appendix X G.

Peroxides
Dissolve 5 g in 15 mL of *chloroform*, add 20 mL of *glacial acetic acid* and 0.5 mL of a saturated solution of *potassium iodide*, mix well, allow to stand in the dark for exactly 1 minute, add 30 mL of *water* and titrate with 0.01M *sodium thiosulfate VS* using *starch mucilage* as indicator. Not more than 0.5 mL of 0.01M *sodium thiosulfate VS* is required.

Pamidronate Disodium Pentahydrate

Disodium Pamidronate

(Ph. Eur. monograph 1779)



$C_3H_9NNa_2O_7P_2,5H_2O$ 369.1 *109552-15-0*

Action and use
Bisphosphonate; treatment of osteolytic lesions; Paget's disease; hypercalcaemia of malignancy.

Preparation
Pamidronate Disodium Infusion

Ph Eur

DEFINITION
Disodium dihydrogen (3-amino-1-hydroxypropylidene)bisphosphonate pentahydrate.

Content
98.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Soluble in water, practically insoluble in methylene chloride. It is sparingly soluble in dilute mineral acids and dissolves in dilute alkaline solutions.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *pamidronate disodium pentahydrate CRS.*

B. Dissolve 0.5 g in 10 mL of *water R*. The solution gives reaction (a) of sodium (*2.3.1*).

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Dissolve 0.20 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

pH (*2.2.3*)
7.8 to 8.8.

Dissolve 0.100 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Impurity A
Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 30 mg of the substance to be examined in *water R* and dilute to 10.0 mL with the same solvent.

Reference solution Dissolve 15 mg of *3-aminopropionic acid R* in *water R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of this solution to 10.0 mL with *water R*.

Plate *TLC silica gel plate R.*

Mobile phase *concentrated ammonia R, di-isopropyl ether R, methanol R* (4:8:9 *V/V/V*).

Application 10 µL.

Development Over 2/3 of the plate.

Drying In a current of warm air.

Detection Spray with a *ninhydrin solution R*. Heat at 100-105 °C for 15 min.

Limit:
— *impurity A*: any spot due to impurity A is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Impurities B and C
Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in *water R* and dilute to 10.0 mL with the same solvent.

Reference solution To 2.0 mL of a 0.3 g/L solution of *phosphoric acid R* add 2.0 mL of a 0.25 g/L solution of *phosphorous acid R* and dilute to 50.0 mL with *water R*.

Column:
— *size*: *l* = 0.10 m, Ø = 4.6 mm,
— *stationary phase*: *anion-exchange resin R* (5 µm),
— *temperature*: 35 °C.

Mobile phase To 0.5 mL of *anhydrous formic acid R* add 2500 mL of *water R*; adjust to pH 3.5 with an 80 g/L solution of *sodium hydroxide R*.

Flow rate 1.0 mL/min.

Detection Refractometer.

Injection 100 µL.

Relative retention With reference to pamidronate (retention time = about 13 min): impurity B = about 1.3; impurity C = about 1.6.

System suitability Reference solution:
— *resolution*: minimum 2.5 between the peaks due to impurities B and C.

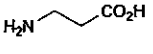
Limits:
— *impurities B, C*: for each impurity, not more than the area of the corresponding peaks in the chromatogram obtained with the reference solution (0.5 per cent).

Water (*2.5.12*)
23.0 per cent to 27.0 per cent, determined on 0.100 g.

ASSAY
Dissolve 0.250 g in 70 mL of *water R*. Titrate with *0.1 M hydrochloric acid* determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M hydrochloric acid* is equivalent to 27.91 mg of $C_3H_9NNa_2O_7P_2$.

IMPURITIES
Specified impurities *A, B, C.*



A. 3-aminopropanoic acid (β-alanine),
B. H_3PO_4: phosphoric acid,
C. H_3PO_3: phosphorous acid.

Ph Eur

Pancreatic Extract

(Pancreas Powder, Ph. Eur. monograph 0350)

Ph Eur

DEFINITION

Pancreas powder is prepared from the fresh or frozen pancreases of mammals. It contains various enzymes having proteolytic, lipolytic and amylolytic activities.

1 mg of pancreas powder contains not less than 1.0 Ph. Eur. U. of total proteolytic activity, 15 Ph. Eur. U. of lipolytic activity and 12 Ph. Eur. U. of amylolytic activity.

PRODUCTION

The animals from which pancreas powder is derived must fulfil the requirements for the health of animals suitable for human consumption.

CHARACTERS

Appearance

Slightly brown, amorphous powder.

Solubility

Partly soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Triturate 0.5 g with 10 mL of *water R* and adjust to pH 8 with *0.1 M sodium hydroxide*, using 0.1 mL of *cresol red solution R* as indicator. Divide the suspension into 2 equal parts (suspension (a) and suspension (b)). Boil suspension (a). To each suspension add 10 mg of *fibrin congo red R*, heat to 38-40 °C and maintain at this temperature for 1 h. Suspension (a) is colourless or slightly pink and suspension (b) is distinctly more red.

B. Triturate 0.25 g with 10 mL of *water R* and adjust to pH 8 with *0.1 M sodium hydroxide*, using 0.1 mL of *cresol red solution R* as indicator. Divide the suspension into 2 equal parts (suspension (a) and suspension (b)). Boil suspension (a). Dissolve 0.1 g of *soluble starch R* in 100 mL of boiling *water R*, boil for 2 min, cool and dilute to 150 mL with *water R*. To 75 mL of the starch solution add suspension (a) and to the remaining 75 mL add suspension (b). Heat each mixture to 38-40 °C and maintain at this temperature for 5 min.

To 1 mL of each mixture add 10 mL of *iodine solution R2*. The mixture obtained with suspension (a) has an intense blue-violet colour; the mixture obtained with suspension (b) has the colour of the iodine solution.

TESTS

Fat content

Maximum 5.0 per cent.

In an extraction apparatus, treat 1.0 g with *light petroleum R1* for 3 h. Evaporate the solvent and dry the residue at 100-105 °C for 2 h. The residue weighs a maximum of 50 mg.

Loss on drying (*2.2.32*)

Maximum 5.0 per cent, determined on 0.50 g by drying at 60 °C at a pressure not exceeding 670 Pa for 4 h.

Microbial contamination

TAMC: acceptance criterion 10^4 CFU/g (*2.6.12*).

TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

Absence of *Escherichia coli* (*2.6.13*).

Absence of *Salmonella* (*2.6.13*).

ASSAY

Total proteolytic activity

The total proteolytic activity of pancreas powder is determined by comparing the quantity of peptides non-precipitable by a 50 g/L solution of *trichloroacetic acid R* released per minute from a substrate of casein solution with the quantity of such peptides released by *pancreas powder (protease) BRP* from the same substrate in the same conditions.

Casein solution Suspend a quantity of *casein BRP* equivalent to 1.25 g of dried substance in 5 mL of *water R*, add 10 mL of *0.1 M sodium hydroxide* and stir for 1 min. (Determine the water content of *casein BRP* prior to the test by heating at 60 °C *in vacuo* for 4 h.) Add 60 mL of *water R* and stir with a magnetic stirrer until the solution is practically clear. Adjust to pH 8.0 with *0.1 M sodium hydroxide* or *0.1 M hydrochloric acid*. Dilute to 100.0 mL with *water R*. Use the solution on the day of preparation.

Enterokinase solution Dissolve 50 mg of *enterokinase BRP* in *0.02 M calcium chloride solution R* and dilute to 50.0 mL with the same solvent. Use the solution on the day of preparation.

To avoid absorption of water formed by condensation, allow the preparation to be examined and the reference preparation to reach room temperature before opening the containers.

For the test suspension and the reference suspension, prepare the suspension and carry out the dilution at 0-4 °C.

Test suspension Triturate 0.100 g of the substance to be examined for 5 min adding gradually 25 mL of *0.02 M calcium chloride solution R*. Transfer completely to a volumetric flask and dilute to 100.0 mL with *0.02 M calcium chloride solution R*. To 10.0 mL of this suspension add 10.0 mL of the enterokinase solution and heat on a water-bath at 35 ± 0.5 °C for 15 min. Cool and dilute with *borate buffer solution pH 7.5 R* at 5 ± 3 °C to a final concentration of about 0.065 Ph. Eur. U. of total proteolytic activity per millilitre calculated on the basis of the stated activity.

Reference suspension Prepare a suspension of *pancreas powder (protease) BRP* as described for the test suspension but without the addition of enterokinase so as to obtain a known final concentration of about 0.065 Ph. Eur. U. per millilitre calculated on the basis of the stated activity.

Designate tubes in duplicate T, T_b, S_1, S_{1b}, S_2, S_{2b}, S_3, S_{3b}; designate a tube B.

Add *borate buffer solution pH 7.5 R* to the tubes as follows:

B: 3.0 mL;

S_1 and S_{1b}: 2.0 mL;

S_2, S_{2b}, T and T_b: 1.0 mL.

Add the reference suspension to the tubes as follows:

S_1 and S_{1b}: 1.0 mL;

S_2 and S_{2b}: 2.0 mL;

S_3 and S_{3b}: 3.0 mL.

Add 2.0 mL of the test suspension to tubes T and T_b.

Add 5.0 mL of a 50 g/L solution of *trichloroacetic acid R* to tubes B, S_{1b}, S_{2b}, S_{3b} and T_b. Mix by shaking.

Place the tubes and the casein solution in a water-bath at 35 ± 0.5 °C. Place a glass rod in each tube. When temperature equilibrium is reached, add 2.0 mL of the casein solution to tubes B, S_{1b}, S_{2b}, S_{3b} and T_b. Mix. At time zero, add 2.0 mL of casein solution successively and at intervals of 30 s to tubes S_1, S_2, S_3 and T. Mix immediately after each addition. Exactly 30 min after addition of the casein solution, taking into account the regular interval adopted, add 5.0 mL of a 50 g/L solution of *trichloroacetic acid R* to tubes S_1, S_2,

S_3 and T. Mix. Withdraw the tubes from the water-bath and allow to stand at room temperature for 20 min.

Filter the contents of each tube twice through the same suitable filter paper previously washed with a 50 g/L solution of *trichloroacetic acid R*, then with *water R* and dried.

A suitable filter paper complies with the following test: filter 5 mL of a 50 g/L solution of *trichloroacetic acid R* on a 7 cm disc of white filter paper; the absorbance (*2.2.25*) of the filtrate, measured at 275 nm using unfiltered trichloroacetic acid solution as the compensation liquid, is less than 0.04.

A schematic presentation of the above operations is shown in Table 0350.-1.

Table 0350.-1

	Tubes								
	S_1	S_{1b}	S_2	S_{2b}	S_3	S_{3b}	T	T_b	B
Buffer solution	2	2	1	1			1	1	3
Reference suspension	1	1	2	2	3	3			
Test suspension							2	2	
Trichloroacetic acid solution		5		5		5		5	5
Mix		+		+		+		+	+
Water-bath 35 °C	+	+	+	+	+	+	+	+	+
Casein solution		2		2		2		2	2
Mix		+		+		+		+	+
Casein solution	2		2		2		2		
Mix	+		+		+		+		
Water-bath 35 °C 30 min	+	+	+	+	+	+	+	+	+
Trichloroacetic acid solution	5		5		5		5		
Mix	+		+		+		+		
Room temperature 20 min	+	+	+	+	+	+	+	+	+
Filter	+	+	+	+	+	+	+	+	+

Measure the absorbance (*2.2.25*) of the filtrates at 275 nm using the filtrate obtained from tube B as the compensation liquid.

Correct the average absorbance values for the filtrates obtained from tubes S_1, S_2 and S_3 by subtracting the average values obtained for the filtrates from tubes S_{1b}, S_{2b} and S_{3b} respectively. Draw a calibration curve of the corrected values against the volume of reference suspension used.

Determine the activity of the substance to be examined using the corrected absorbance for the test suspension ($T - T_b$) and the calibration curve and taking into account the dilution factors.

The test is not valid unless the corrected absorbance values are between 0.15 and 0.60.

Lipolytic activity

The lipolytic activity is determined by comparing the rate at which a suspension of pancreas powder hydrolyses a substrate of olive oil emulsion with the rate at which a suspension of *pancreas powder (lipase) BRP* hydrolyses the same substrate under the same conditions. *The test is carried out under nitrogen.*

Olive oil stock emulsion In an 800 mL beaker 9 cm in diameter, place 40 mL of *olive oil R*, 330 mL of *acacia solution R* and 30 mL of *water R*. Place an electric mixer at the bottom of the beaker. Place the beaker in a vessel containing *ethanol (96 per cent) R* and a sufficient quantity of ice as a cooling mixture. Emulsify using the mixer at an average speed of 1000-2000 r/min. Cool to 5-10 °C. Increase the mixing speed to 8000 r/min. Mix for 30 min keeping the temperature below 25 °C by the continuous addition of crushed ice into the cooling mixture. (A mixture of calcium chloride and crushed ice is also suitable). Store the stock emulsion in a refrigerator and use within 14 days.

The emulsion must not separate into 2 distinct layers. Check the diameter of the globules of the emulsion under a microscope. At least 90 per cent have a diameter below 3 µm and none has a diameter greater than 10 µm. Shake the emulsion thoroughly before preparing the emulsion substrate.

Olive oil emulsion For 10 determinations, mix the following solutions in the order indicated: 100 mL of the stock emulsion, 80 mL of *tris(hydroxymethyl)aminomethane solution R1*, 20 mL of a freshly prepared 80 g/L of *sodium taurocholate BRP* and 95 mL of *water R*. Use on the day of preparation.

Apparatus Use a reaction vessel of about 50 mL capacity provided with:

— a device that will maintain a temperature of 37 ± 0.5 °C;
— a magnetic stirrer;
— a lid with holes for the insertion of electrodes, the tip of a burette, a tube for the admission of nitrogen and the introduction of reagents.

An automatic or manual titration apparatus may be used. In the latter case, the burette is graduated in 0.005 mL and the pH-meter is provided with a wide reading scale and glass-silver-silver chloride or other suitable electrodes. After each test the reaction vessel is evacuated by suction and washed several times with *water R*, the washings being removed each time by suction.

To avoid absorption of water formed by condensation, allow the preparation to be examined and the reference preparation to reach room temperature before opening the containers.

For the test suspension and the reference suspension, prepare the suspension and carry out the dilution at 0-4 °C.

Test suspension In a small mortar cooled to 0-4 °C, triturate carefully a quantity of the substance to be examined equivalent to about 2500 Ph. Eur. U. of lipolytic activity with 1 mL of maleate buffer solution pH 7.0 R (lipase solvent) until a very fine suspension is obtained. Dilute the suspension with maleate buffer solution pH 7.0 R, transfer quantitatively to a volumetric flask and dilute to 100.0 mL with the buffer solution. Keep the flask containing the test suspension in iced water during the titration.

Reference suspension Prepare a suspension of *pancreas powder (lipase) BRP* as described for the test suspension using a quantity equivalent to about 2500 Ph. Eur. U.

Carry out the titrations immediately after preparation of the test suspension and the reference suspension. Place 29.5 mL of olive oil emulsion in the reaction vessel equilibrated at 37 ± 0.5 °C. Fit the vessel with the electrodes, a stirrer and the burette (the tip being immersed in the olive oil emulsion).

Put the lid in place and switch on the apparatus. Carefully add *0.1 M sodium hydroxide* with stirring to adjust to pH 9.2. Using a rapid-flow graduated pipette transfer about 0.5 mL of the previously homogenised reference suspension, start the chronometer and add continuously *0.1 M sodium hydroxide* to maintain the pH at 9.0. After exactly 1 min, note the volume of *0.1 M sodium hydroxide* used. Carry out the measurement a further 4 times. Discard the first reading and determine the average of the 4 others (S_1). Make 2 further determinations (S_2 and S_3). Calculate the average of the values S_1, S_2 and S_3. The average volume of *0.1 M sodium hydroxide* used should be about 0.12 mL per minute with limits of 0.08 mL to 0.16 mL.

Carry out 3 determinations in the same manner for the test suspension (T_1, T_2 and T_3). If the quantity of *0.1 M sodium hydroxide* used is outside the limits of 0.08 mL to 0.16 mL per minute, the assay is repeated with a quantity of test suspension that is more suitable but situated between 0.4 mL and 0.6 mL. Otherwise the quantity of the substance to be examined is adjusted to comply with the conditions of the test. Calculate the average of the values T_1, T_2 and T_3.

Calculate the activity in European Pharmacopoeia Units per milligram using the following expression:

$$\frac{n \times m_1}{n_1 \times m} \times A$$

n	=	average volume of *0.1 M sodium hydroxide* used per minute during the titration of the test suspension, in millilitres;
n_1	=	average volume of *0.1 M sodium hydroxide* used per minute during the titration of the reference suspension, in millilitres;
m	=	mass of the substance to be examined, in milligrams;
m_1	=	mass of the reference preparation, in milligrams;
A	=	activity of *pancreas powder (lipase) BRP*, in European Pharmacopoeia Units per milligram.

Amylolytic activity

The amylolytic activity is determined by comparing the rate at which a suspension of pancreas powder hydrolyses a substrate of starch solution with the rate at which a suspension of *pancreas powder (amylase) BRP* hydrolyses the same substrate under the same conditions.

Starch solution To a quantity of *starch BRP* equivalent to 2.0 g of the dried substance add 10 mL of *water R* and mix. (Determine the water content of *starch BRP* prior to the test by heating at 120 °C for 4 h). Add this suspension, whilst stirring continuously, to 160 mL of boiling *water R*. Wash the container several times with successive quantities, each of 10 mL, of *water R* and add the washings to the hot starch solution. Heat to boiling, stirring continuously. Cool to room temperature and dilute to 200 mL with *water R*. Use the solution on the day of preparation.

To avoid absorption of water formed by condensation, allow the preparation to be examined and the reference preparation to reach room temperature before opening the containers.

For the test suspension and the reference suspension, prepare the suspension and carry out the dilution at 0-4 °C.

Test suspension Triturate a quantity of the substance to be examined equivalent to about 1500 Ph. Eur. U. of amylolytic activity with 60 mL of phosphate buffer solution pH 6.8 R1 for 15 min. Transfer quantitatively to a volumetric flask and dilute to 100.0 mL with phosphate buffer solution pH 6.8 R1.

Reference suspension Prepare a suspension of *pancreas powder (amylase) BRP* as described for the test suspension, using a quantity equivalent to about 1500 Ph. Eur. U.

In a test-tube 200 mm long and 22 mm in diameter, fitted with a ground-glass stopper, place 25.0 mL of starch solution, 10.0 mL of phosphate buffer solution pH 6.8 R1 and 1.0 mL of an 11.7 g/L solution of *sodium chloride R*. Close the tube, shake and place in a water-bath at 25.0 ± 0.1 °C. When the temperature equilibrium has been reached, add 1.0 mL of the test suspension and start the chronometer. Mix and place the tube in the water-bath. After exactly 10 min, add 2 mL of *1 M hydrochloric acid*. Transfer the mixture quantitatively to a 300 mL conical flask fitted with a ground-glass stopper. Whilst shaking continuously, add 10.0 mL of *0.05 M iodine* immediately followed by 45 mL of *0.1 M sodium hydroxide*. Allow to stand in the dark at a temperature between 15 °C and 25 °C for 15 min.

Add 4 mL of a mixture of 1 volume of *sulfuric acid R* and 4 volumes of *water R*. Titrate the excess of iodine with *0.1 M sodium thiosulfate* using a microburette. Carry out a blank titration adding the 2 mL of *1 M hydrochloric acid* before introducing the test suspension. Carry out the titration of the reference suspension in the same manner.

The test is not valid unless both n'-n and n'_1-n_1 are between 1.9 mL and 3.6 mL.

Calculate the amylolytic activity in European Pharmacopoeia Units per milligram using the following expression:

$$\frac{(n' - n)m_1}{(n'_1 - n_1)m} \times A$$

n	=	volume of *0.1 M sodium thiosulfate* used in the titration of the test suspension, in millilitres;
n_1	=	volume of *0.1 M sodium thiosulfate* used in the titration of the reference suspension, in millilitres;
n'	=	volume of *0.1 M sodium thiosulfate* used in the blank titration of the test suspension, in millilitres;
n'_1	=	volume of *0.1 M sodium thiosulfate* used in the blank titration of the reference suspension, in millilitres;
m	=	mass of the substance to be examined, in milligrams;
m_1	=	mass of the reference preparation, in milligrams;
A	=	activity of *pancreas powder (amylase) BRP*, in European Pharmacopoeia Units per milligram.

STORAGE

In an airtight container.

Ph Eur

Pancreatin

Action and use

Enzyme; treatment of pancreatic exocrine deficiency.

Preparations

Pancreatin Gastro-resistant Capsules

Pancreatin Granules

Pancreatin Gastro-resistant Tablets

DEFINITION

Pancreatin is a preparation of mammalian pancreas containing enzymes having protease, lipase and amylase activity. It may contain Sodium Chloride.

Potency

Pancreatin contains in 1 mg not less than 1.4 Units of free protease activity, not less than 20 Units of lipase activity and not less than 24 Units of amylase activity.

PRODUCTION

Pancreatin is prepared in conditions designed to minimise the degree of microbial contamination.

CHARACTERISTICS

A white or buff amorphous powder.

Soluble or partly soluble in *water* forming a slightly turbid solution; practically insoluble in *ethanol (96%)* and in *ether*.

IDENTIFICATION

A. It demonstrates free protease activity in the Assay for Free protease activity.

B. Triturate 0.25 g with 10 mL of *water* and adjust to pH 8.0 by the addition of 1M *sodium hydroxide* using *cresol red solution* as indicator. Divide the resulting solution into two equal portions. Boil one portion [solution (1)] and leave the other untreated [solution (2)]. Dissolve 0.1 g of *soluble starch* in 100 mL of boiling *water*, boil for 2 minutes, cool and

dilute to 150 mL with *water*. Add solution (1) to half the starch mucilage and solution (2) to the remainder and maintain the mixtures at 38° to 40° for 5 minutes. To 1 mL of each mixture add 10 mL of *iodinated potassium iodide solution*. The liquid containing solution (2) retains the colour of the solution of iodine and the liquid containing solution (1) acquires an intense blue colour.

TESTS

Fat

Extract 1 g with *petroleum spirit (boiling range, 40° to 60°)* for 3 hours in an apparatus for the *continuous extraction of drugs*, Appendix XI F, evaporate the extract and dry the residue at 105° for 2 hours. The residue weighs not more than 30 mg.

Loss on drying

When dried at 60° at a pressure not exceeding 0.7 kPa for 4 hours, loses not more than 5.0% of its weight. Use 0.5 g.

Microbial contamination

1 g is free from *Escherichia coli*; 10 g is free from *Salmonella*, Appendix XVI B1.

ASSAY

Carry out the *Assay of pancreatin*, Appendix XIV I.

STORAGE

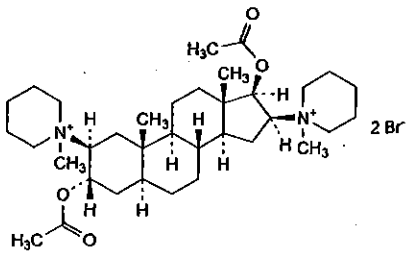
Pancreatin should be stored at a temperature not exceeding 15°.

LABELLING

The label states (1) the minimum number of Units of activity of free protease, lipase and amylase per mg; (2) the name of any added substance; (3) the date after which the material is not intended to be used; (4) the conditions under which it should be stored.

Pancuronium Bromide

(Ph. Eur. monograph 0681)



$C_{35}H_{60}Br_2N_2O_4$ 733 *15500-66-0*

Action and use

Non-depolarizing neuromuscular blocker.

Preparation

Pancuronium Injection

Ph Eur

DEFINITION

1,1′-[3α,17β-Bis(acetyloxy)-5α-androstane-2β,16β-diyl]bis(1-methylpiperidinium) dibromide.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White, yellowish-white or slightly pink, crystalline powder, hygroscopic.

Solubility

Very soluble or freely soluble in water, very soluble in methylene chloride, freely soluble in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *pancuronium bromide CRS*.

B. It gives reaction (a) of bromides (*2.3.1*).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 50 mg in *water R* and dilute to 25 mL with the same solvent.

Specific optical rotation (*2.2.7*)

+ 38.0 to + 42.0 (anhydrous substance).

Dissolve 0.75 g in *water R* and dilute to 25.0 mL with the same solvent.

Related substances

Thin-layer chromatography (*2.2.27*). *Prepare the solutions immediately before use.*

Test solution Dissolve 50.0 mg of the substance to be examined in *methylene chloride R* and dilute to 5.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 50.0 mL with *methylene chloride R*. Dilute 1.0 mL of this solution to 20.0 mL with *methylene chloride R*.

Reference solution (b) Dissolve 10.0 mg of *pancuronium bromide for system suitability CRS* (containing 1.0 per cent of impurity D) in 1.0 mL of *methylene chloride R*.

Plate *TLC silica gel plate R* (2-10 µm).

Mobile phase 400 g/L solution of *sodium iodide R*, *acetonitrile R*, *2-propanol R* (5:10:85 *V/V/V*).

Application 5 µL.

Development In an unlined and unsaturated tank over a path of 8 cm.

Drying In a current of air at room temperature.

Detection Spray with a 20 g/L solution of *sodium nitrite R* and allow to dry for 5 min. Then spray with *potassium iodobismuthate solution R5*. Cover the plate with a transparent glass cover.

System suitability:

- the chromatogram obtained with reference solution (b) shows 2 clearly separated spots due to pancuronium bromide (R_F = about 0.5) and impurity D (R_F = about 0.6);
- the chromatogram obtained with reference solution (a) shows a clearly visible spot.

Note Impurity A if present will co-migrate with impurity D.

Limits:

- *impurities A, D*: any spot due to impurities A and/or D is not more intense than the spot due to impurity D in the chromatogram obtained with reference solution (b) (1.0 per cent),
- *unspecified impurities*: any other spot is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.10 per cent).

Water (*2.5.12*)
Maximum 8.0 per cent, determined on 0.300 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 50 mL of *acetic anhydride R*, heating if necessary. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 36.63 mg of $C_{35}H_{60}Br_2N_2O_4$.

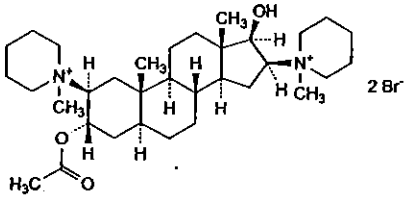
STORAGE

In an airtight container, protected from light.

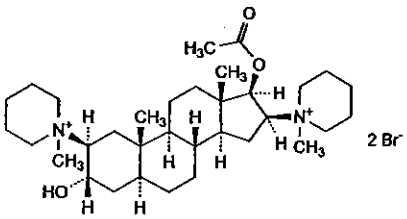
IMPURITIES

Specified impurities *A, D.*

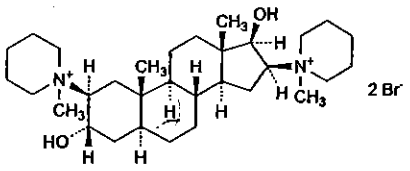
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, E.*



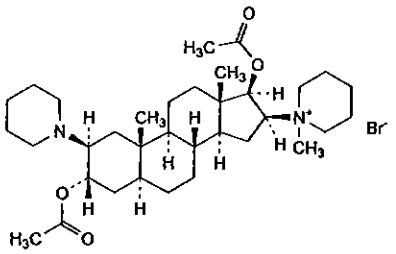
A. 1,1′-[3α-(acetyloxy)-17β-hydroxy-5α-androstane-2β,16β-diyl]bis(1-methylpiperidinium) dibromide (dacuronium bromide),



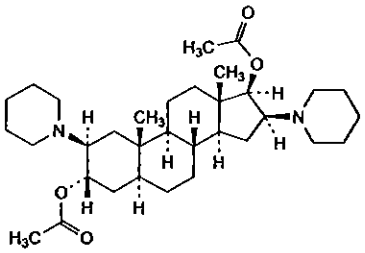
B. 1,1′-[17β-(acetyloxy)-3α-hydroxy-5α-androstane-2β,16β-diyl]bis(1-methylpiperidinium) dibromide,



C. 1,1′-(3α,17β-dihydroxy-5α-androstane-2β,16β-diyl)bis(1-methylpiperidinium) dibromide,



D. 1-[3α,17β-bis(acetyloxy)-2β-(piperidin-1-yl)-5α-androstan-16β-yl]-1-methylpiperidinium bromide (vecuronium bromide),

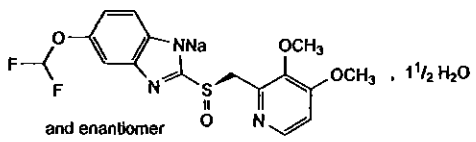


E. 2β,16β-bis(piperidin-1-yl)-5α-androstane-3α,17β-diyl diacetate.

Ph Eur

Pantoprazole Sodium Sesquihydrate

(Ph. Eur. monograph 2296)



$C_{16}H_{14}F_2N_3NaO_4S,1½H_2O$ 432.4 *164579-32-2*

Action and use

Proton pump inhibitor; treatment of peptic ulcer disease.

Preparations

Pantoprazole for Injection

Pantoprazole Gastro-resistant Tablets

Ph Eur

DEFINITION

Sodium 5-(difluoromethoxy)-2-[(*RS*)-[(3,4-dimethoxypyridin-2-yl)methyl]sulfinyl]benzimidazol-1-ide sesquihydrate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

PRODUCTION

It is produced by methods of manufacture designed to guarantee the proper hydrate form and it complies, if tested, with a suitable test that demonstrates its sesquihydrate nature (for example near-infrared spectroscopy (*2.2.40*) or X-ray powder diffraction (*2.9.33*)).

CHARACTERS

Appearance

White or almost white powder.

Solubility
Freely soluble in water and in ethanol (96 per cent), practically insoluble in hexane.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *pantoprazole sodium sesquihydrate CRS.*

B. It gives reaction (a) of sodium (*2.3.1*).

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution B_6 (*2.2.2, Method II*).

Dissolve 0.20 g in *water R* and dilute to 20.0 mL with the same solvent.

Optical rotation (*2.2.7*)
−0.4° to + 0.4°.

Dissolve 0.2 g in 10 mL of *water R*. Adjust to pH 11.5-12.0 with an 8 g/L solution of *sodium hydroxide R*. Dilute to 20.0 mL with *water R*.

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R*, 40 mg/L solution of *sodium hydroxide R* (50:50 *V/V*).

Test solution Dissolve 23 mg of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 2.5 mg of *pantoprazole for system suitability CRS* (containing impurities A, B, C, D and E) in the solvent mixture and dilute to 5.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.125 m, Ø = 4 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: 1.74 g/L solution of *dipotassium hydrogen phosphate R* adjusted to pH 7.00 ± 0.05 with a 330 g/L solution of *phosphoric acid R*;
— *mobile phase B*: *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 40	80 → 20	20 → 80
40 - 45	20 → 80	80 → 20

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 290 nm and, for impurity C, at 305 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram supplied with *pantoprazole for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C, D + F and E.

Relative retention With reference to pantoprazole (retention time = about 11 min): impurity C = about 0.6; impurity A = about 0.9; impurities D and F = about 1.2; impurity E = about 1.3; impurity B = about 1.5.

System suitability Reference solution (b):
— *resolution*: minimum 1.5 between the peaks due to impurities E and D + F;
— the chromatogram obtained is similar to the chromatogram supplied with *pantoprazole for system suitability CRS*.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity C by 0.3;
— *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *sum of impurities D and F*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *impurities B, C, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)
5.9 per cent to 6.9 per cent, determined on 0.150 g.

ASSAY

Dissolve 0.200 g in 80 mL of *anhydrous acetic acid R*, add 5 mL of *acetic anhydride R* and mix for at least 10 min. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

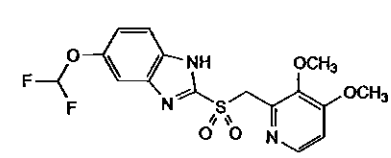
1 mL of *0.1 M perchloric acid* is equivalent to 20.27 mg of $C_{16}H_{14}F_2N_3NaO_4S$.

STORAGE

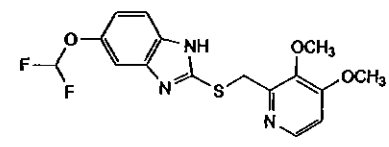
Protected from light.

IMPURITIES

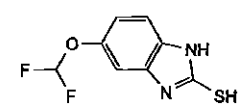
Specified impurities *A, B, C, D, E, F.*



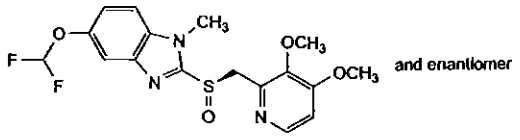
A. 5-(difluoromethoxy)-2-{[(3,4-dimethoxypyridin-2-yl) methyl]sulfonyl}-1*H*-benzimidazole,



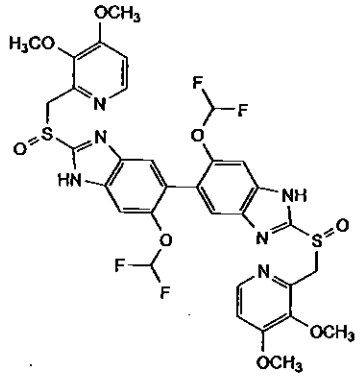
B. 5-(difluoromethoxy)-2-[[(3,4-dimethoxypyridin-2-yl) methyl]sulfanyl]-1*H*-benzimidazole,



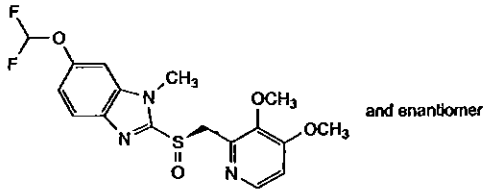
C. 5-(difluoromethoxy)-1*H*-benzimidazole-2-thiol,



D. 5-(difluoromethoxy)-2-[(*RS*)-[(3,4-dimethoxypyridin-2-yl)methyl]sulfinyl]-1-methyl-1*H*-benzimidazole,



E. mixture of the stereoisomers of 6,6′-bis(difluoromethoxy)-2,2′-bis[[(3,4-dimethoxypyridin-2-yl)methyl]sulfinyl]-1*H*,1′*H*-5,5′-bibenzimidazolyl,



F. 6-(difluoromethoxy)-2-[(*RS*)-[(3,4-dimethoxypyridin-2-yl)methyl]sulfinyl]-1-methyl-1*H*-benzimidazole.

Ph Eur

Papaveretum

Action and use
Opioid receptor agonist; analgesic.

DEFINITION

Papaveretum is a mixture of 253 parts of Morphine Hydrochloride ($C_{17}H_{19}NO_3$,HCl,3H_2O, 375.9), 23 parts of Papaverine Hydrochloride ($C_{20}H_{21}NO_4$,HCl, 375.9) and 20 parts of Codeine Hydrochloride ($C_{18}H_{21}NO_3$,HCl,2H_2O, 371.9). It contains not less than 80.0% and not more than 88.4% of $C_{17}H_{19}NO_3$,HCl, not less than 8.3% and not more than 9.2% of $C_{20}H_{21}NO_4$,HCl and not less than 6.6% and not more than 7.4% of $C_{18}H_{21}NO_3$,HCl, calculated with reference to the dried material.

CHARACTERISTICS

A white or almost white, crystalline powder.

Soluble in *water*; sparingly soluble in *ethanol (96%)*.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *water*.

(1) 1.5% w/v of the substance being examined.

(2) 1.28% w/v of *morphine sulfate BPCRS*.

(3) 0.115% w/v of *papaverine hydrochloride BPCRS*.

(4) 0.11% w/v of *codeine phosphate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel precoated plate (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry at 100° to 105° for 15 minutes, allow it to cool and spray with *potassium iodobismuthate solution* and then with a 0.4% v/v solution of *sulfuric acid*.

MOBILE PHASE

2 volumes of 13.5M *ammonia*, 6 volumes of *ethanol (96%)*, 40 volumes of *acetone* and 40 volumes of *toluene*.

CONFIRMATION

The chromatogram obtained with solution (1) shows three principal spots corresponding in position and colour to the principal spots in the chromatograms obtained with solutions (2), (3) and (4).

B. Yields the reactions characteristics of *chlorides*, Appendix VI.

TESTS

Acidity
pH of a 1.5% w/v solution, 3.7 to 4.7, Appendix V L.

Clarity and colour of solution
A 1.5% w/v solution in *water* is *clear*, Appendix IV A, and not more intensely coloured than *reference solution* BY_5, Appendix IV B, Method II.

Loss on drying
When dried to constant weight at 130°, loses not less than 10.0% and not more than 14.0% of its weight. Use 0.5 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *methanol (60%)*.

(1) 0.15% w/v of the substance being examined.

(2) 0.128% w/v of *morphine sulfate BPCRS*.

(3) 0.128% w/v of *morphine sulfate BPCRS*, 0.0115% w/v of *papaverine hydrochloride BPCRS* and 0.011% w/v of *codeine phosphate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 285 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

0.01M *sodium acetate* and 0.005M *dioctyl sodium sulfosuccinate* in *methanol (60%)* adjusted to pH 5.5 with *glacial acetic acid*.

SYSTEM SUITABILITY

If necessary, adjust the proportion of methanol in the mobile phase in the range 55% v/v to 65% v/v so that the retention time of morphine in solution (2) is 4 to 5 minutes. Adjust the pH of the mobile phase with either *glacial acetic acid* or 2M *sodium hydroxide* in order to obtain optimum separation of the three principal components in solution (3). The retention

times of codeine and papaverine relative to that of morphine are about 1.3 and 1.7 respectively.

DETERMINATION OF CONTENT

For anhydrous morphine hydrochloride
Calculate the content of $C_{17}H_{19}NO_3,HCl$ using the declared content of $C_{17}H_{19}NO_3$ in *morphine sulfate BPCRS*. Each mg of $C_{17}H_{19}NO_3$ is equivalent to 1.13 mg of $C_{17}H_{19}NO_3,HCl$.

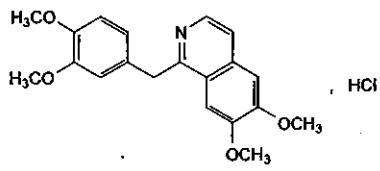
For papaverine hydrochloride
Calculate the content of $C_{20}H_{21}NO_4,HCl$ using the declared content of $C_{20}H_{21}NO_4,HCl$ in *papaverine hydrochloride BPCRS*.

For anhydrous codeine hydrochloride
Calculate the content of $C_{18}H_{21}NO_3,HCl$ using the declared content of $C_{18}H_{21}NO_3$ in *codeine phosphate BPCRS*. Each mg of $C_{18}H_{21}NO_3$ is equivalent to 1.09 mg of $C_{18}H_{21}NO_3,HCl$.

STORAGE
Papaveretum should be protected from light.

Papaverine Hydrochloride

(Ph. Eur. monograph 0102)



$C_{20}H_{22}ClNO_4$ 375.9 *61-25-6*

Action and use
Phosphodiesterase inhibitor; smooth muscle relaxant.

Preparation
Papaverine Injection

Ph Eur

DEFINITION
1-(3,4-Dimethoxybenzyl)-6,7-dimethoxyisoquinoline hydrochloride.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder, or white or almost white crystals.

Solubility
Sparingly soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION
First identification: A, D.
Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *papaverine hydrochloride CRS.*

B. Thin-layer chromatography (*2.2.27*).
Test solution Dissolve 5 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.
Reference solution Dissolve 5 mg of *papaverine hydrochloride CRS* in *methanol R* and dilute to 10 mL with the same solvent.
Plate *TLC silica gel GF_{254} plate R.*
Mobile phase *diethylamine R, ethyl acetate R, toluene R* (10:20:70 *V/V/V*).
Application 10 µL.
Development Over 2/3 of the plate.
Drying At 100-105 °C for 2 h.
Detection Examine in ultraviolet light at 254 nm.
Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. To 10 mL of solution S (see Tests) add 5 mL of *ammonia R* dropwise and allow to stand for 10 min. The precipitate, washed and dried, melts (*2.2.14*) at 146 °C to 149 °C.

D. It gives reaction (a) of chlorides (*2.3.1*).

TESTS
Solution S
Dissolve 0.4 g in *carbon dioxide-free water R*, heating gently if necessary, and dilute to 20 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

pH (*2.2.3*)
3.0 to 4.0 for solution S.

Related substances
Liquid chromatography (*2.2.29*).
Solvent mixture *acetonitrile R*, mobile phase A (20:80 *V/V*).
Test solution Dissolve 20.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.
Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.
Reference solution (b) Dissolve 12 mg of *noscapine CRS* in 1.0 mL of the test solution and dilute to 100.0 mL with the solvent mixture.
Column:
— *size: l* = 0.25 m, Ø = 4.0 mm;
— *stationary phase: base-deactivated octylsilyl silica gel for chromatography R* (5 µm).
Mobile phase:
— *mobile phase A*: 3.4 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 3.0 with *dilute phosphoric acid R*;
— *mobile phase B: acetonitrile R*;
— *mobile phase C: methanol R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Mobile phase C (per cent *V/V*)
0 - 5	85	5	10
5 - 12	85 → 60	5	10 → 35
12 - 20	60	5	35
20 - 24	60 → 40	5 → 20	35 → 40
24 - 27	40	20	40
27 - 32	40 → 85	20 → 5	40 → 10

Flow rate 1 mL/min.
Detection Spectrophotometer at 238 nm.
Injection 10 µL.

Relative retention With reference to papaverine (retention time = about 24 min): impurity E = about 0.7; impurity C = about 0.75; impurity B = about 0.8; impurity A = about 0.9; impurity F = about 1.1; impurity D = about 1.2.

System suitability Reference solution (b):
— *resolution*: minimum 1.5 between the peaks due to impurity A and papaverine.

Limits:
— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 6.2; impurity C = 2.7; impurity D = 0.5;
— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

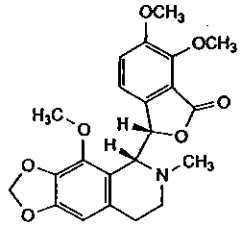
Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on the residue from the test for loss on drying.

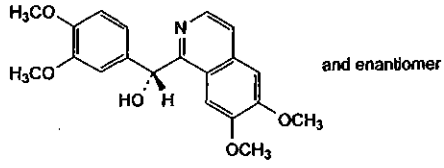
ASSAY
Dissolve 0.300 g in a mixture of 5.0 mL of *0.01 M hydrochloric acid* and 50 mL of *alcohol R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 37.59 mg of $C_{20}H_{22}ClNO_4$.

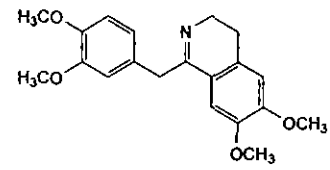
IMPURITIES



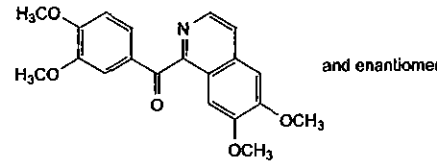
A. (3*S*)-6,7-dimethoxy-3-[(5*R*)-4-methoxy-6-methyl-5,6,7,8-tetrahydro-1,3-dioxolo[4,5-*g*]isoquinolin-5-yl]isobenzofuran-1(3*H*)-one (noscapine),



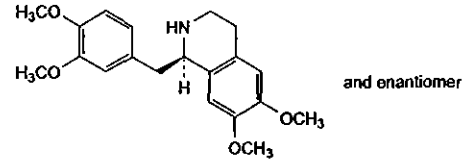
B. (*RS*)-(3,4-dimethoxyphenyl)(6,7-dimethoxyisoquinolin-1-yl)methanol (papaverinol),



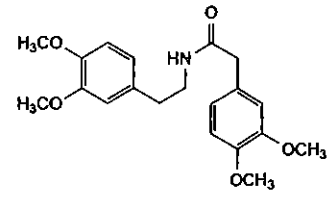
C. 1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinoline (dihydropapaverine),



D. (3,4-dimethoxyphenyl)(6,7-dimethoxyisoquinolin-1-yl)methanone (papaveraldine),



E. (1*RS*)-1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (tetrahydropapaverine),

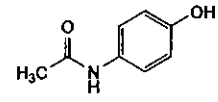


F. 2-(3,4-dimethoxyphenyl)-*N*-[2-(3,4-dimethoxyphenyl)ethyl]acetamide.

Ph Eur

Paracetamol

(Ph. Eur. monograph 0049)



$C_8H_9NO_2$ 151.2 *103-90-2*

Action and use
Analgesic; antipyretic.

Preparations
Co-codamol Tablets
Co-codamol Capsules
Co-codamol Effervescent Tablets
Paracetamol Effervescent Tablets
Co-dydramol Tablets
Co-proxamol Tablets
Paracetamol Capsules
Paediatric Paracetamol Oral Solution
Paediatric Paracetamol Oral Suspension
Paracetamol Oral Suspension

Paracetamol Suppositories
Paracetamol Tablets
Paracetamol and Caffeine Tablets
Paracetamol and Caffeine Soluble Tablets
Paracetamol Dispersible Tablets
Paracetamol Soluble Tablets
Paracetamol, Codeine Phosphate and Caffeine Capsules
Paracetamol, Codeine Phosphate and Caffeine Tablets

Ph Eur

DEFINITION

N-(4-Hydroxyphenyl)acetamide.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Sparingly soluble in water, freely soluble in ethanol (96 per cent), very slightly soluble in methylene chloride.

IDENTIFICATION

First identification: B.

Second identification: A.

A. Melting point (*2.2.14*).

Determination A Determine the melting point of the substance to be examined.

Result A 168 °C to 172 °C.

Determination B Mix equal parts of the substance to be examined and *paracetamol CRS* and determine the melting point of the mixture.

Result B The absolute difference between the melting point of the mixture and the value obtained in determination A is not greater than 2 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *paracetamol CRS.*

TESTS

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture *methanol R, water R* (15:85 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 5.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 20.0 mL with the solvent mixture.

Reference solution (b) *Prepare immediately before use.* Dissolve 5.0 mg of *paracetamol impurity K CRS* and 5 mg of *paracetamol CRS* in the solvent mixture and dilute to 100.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 100.0 mL with the solvent mixture.

Reference solution (c) Dissolve 5.0 mg of *paracetamol impurity J CRS* in the solvent mixture and dilute to 250.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 200.0 mL with the solvent mixture.

Precolumn:
— *size: l* = 0.005 m, Ø = 2.1 mm;
— *stationary phase: end-capped solid core octadecylsilyl silica gel for chromatography R* (2.7 µm).

Column:
— *size: l* = 0.10 m, Ø = 2.1 mm;
— *stationary phase: end-capped solid core octadecylsilyl silica gel for chromatography R* (2.7 µm);
— *temperature:* 30 °C.

Mobile phase:
— *mobile phase A:* dissolve 1.7 g of *potassium dihydrogen phosphate R* and 1.8 g of *dipotassium hydrogen phosphate R* in *water for chromatography R* and dilute to 1000 mL with the same solvent;
— *mobile phase B: methanol R;*

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 1	95	5
1 - 10	95 → 90	5 → 10
10 - 20	90	10
20 - 40	90 → 66	10 → 34
40 - 50	66	34

Flow rate 0.3 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 5 µL.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity K; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity J.

Relative retention With reference to paracetamol (retention time = about 4 min): impurity K = about 0.4; impurity J = about 10.1.

System suitability Reference solution (b):
— *resolution:* minimum 5.0 between the peaks due to impurity K and paracetamol.

Calculation of percentage contents:
— for impurity J, use the concentration of impurity J in reference solution (c);
— for impurity K, use the concentration of impurity K in reference solution (b);
— for impurities other than J and K, use the concentration of paracetamol in reference solution (a).

Limits:
— *impurity K:* maximum 50 ppm;
— *impurity J:* maximum 10 ppm;
— *unspecified impurities:* for each impurity, maximum 0.05 per cent;
— *total:* maximum 0.2 per cent;
— *reporting threshold:* 0.03 per cent, except for impurities J and K.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in a mixture of 10 mL of *water R* and 30 mL of *dilute sulfuric acid R*. Boil under a reflux condenser for 1 h, cool and dilute to 100.0 mL with *water R*.
To 20.0 mL of the solution add 40 mL of *water R*, 40 g of ice, 15 mL of *dilute hydrochloric acid R* and 0.1 mL of *ferroin R*. Titrate with *0.1 M cerium sulfate* until a greenish-yellow colour is obtained. Carry out a blank titration.

1 mL of *0.1 M cerium sulfate* is equivalent to 7.56 mg of $C_8H_9NO_2$.

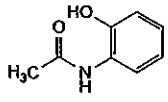
STORAGE

Protected from light.

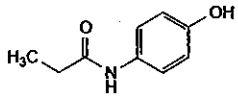
IMPURITIES

Specified impurities J, K.

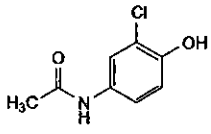
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) A, B, C, D, E, F, G, H, I, L, M, N.



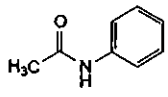
A. *N*-(2-hydroxyphenyl)acetamide,



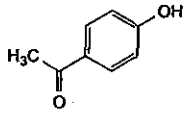
B. *N*-(4-hydroxyphenyl)propanamide,



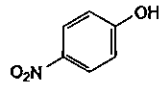
C. *N*-(3-chloro-4-hydroxyphenyl)acetamide,



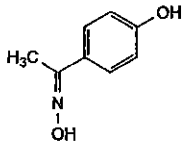
D. *N*-phenylacetamide,



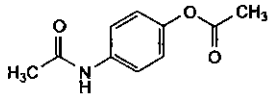
E. 1-(4-hydroxyphenyl)ethan-1-one,



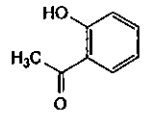
F. 4-nitrophenol,



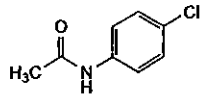
G. [1-(4-hydroxyphenyl)ethylidene]hydroxylamine,



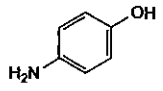
H. 4-acetamidophenyl acetate,



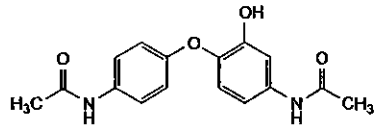
I. 1-(2-hydroxyphenyl)ethan-1-one,



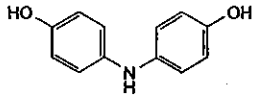
J. *N*-(4-chlorophenyl)acetamide (chloroacetanilide),



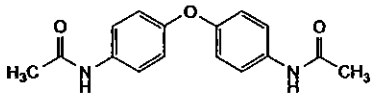
K. 4-aminophenol,



L. *N*-[4-(4-acetamido-2-hydroxyphenoxy)phenyl]acetamide,



M. 4,4′-azanediyldiphenol,



N. *N*,*N*′-[oxydi(4,1-phenylene)]diacetamide.

Ph Eur

Hard Paraffin

(Ph. Eur. monograph 1034)

Ph Eur

DEFINITION

A purified mixture of solid saturated hydrocarbons generally obtained from petroleum. It may contain a suitable antioxidant.

CHARACTERS

Appearance

Colourless or white or almost white mass; the melted substance is free from fluorescence in daylight.

Solubility

Practically insoluble in water, freely soluble in methylene chloride, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

First identification: A, C.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison hard paraffin CRS.

Preparation Place about 2 mg on a sodium chloride plate, heat in an oven at 100 °C for 10 min, spread the melted

substance with another sodium chloride plate and remove one of the plates.

B. Acidity or alkalinity (see Tests).

C. Melting point (*2.2.16*): 50 °C to 61 °C.

TESTS

Acidity or alkalinity

To 15 g add 30 mL of boiling *water R* and shake vigorously for 1 min. Allow to cool and to separate. To 10 mL of the aqueous layer add 0.1 mL of *phenolphthalein solution R*. The solution is colourless. Not more than 1.0 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to red. To a further 10 mL of the aqueous layer add 0.1 mL of *methyl red solution R*. The solution is yellow. Not more than 0.5 mL of *0.01 M hydrochloric acid* is required to change the colour of the indicator to red.

Polycyclic aromatic hydrocarbons

Use reagents for ultraviolet absorption spectrophotometry

Dissolve 0.50 g in 25 mL of *heptane R* and place in a 125 mL separating funnel with unlubricated ground-glass parts (stopper, stopcock). Add 5.0 mL of *dimethyl sulfoxide R*. Shake vigorously for 1 min and allow to stand until 2 clear layers are formed. Transfer the lower layer to a 2nd separating funnel, add 2 mL of *heptane R* and shake the mixture vigorously. Allow to stand until 2 clear layers are formed. Separate the lower layer and measure its absorbance (*2.2.25*) between 265 nm and 420 nm using as the compensation liquid the clear lower layer obtained by vigorously shaking 5.0 mL of *dimethyl sulfoxide R* with 25 mL of *heptane R* for 1 min. Prepare a 7.0 mg/L reference solution of *naphthalene R* in *dimethyl sulfoxide R* and measure the absorbance of this solution at the absorption maximum at 278 nm using *dimethyl sulfoxide R* as the compensation liquid. At wavelengths from 265 nm to 420 nm, the absorbance of the test solution is not greater than one-third that of the reference solution at 278 nm.

Sulfates (*2.4.13*)

Maximum 150 ppm.

Introduce 2.0 g of the melted substance to be examined into a 50 mL ground-glass-stoppered separating funnel. Add 30 mL of boiling *distilled water R*, shake vigorously for 1 min and filter.

STORAGE

Protected from light.

Ph Eur

Light Liquid Paraffin

(Ph. Eur. monograph 0240)

Preparation

Light Liquid Paraffin Eye Drops

Ph Eur

DEFINITION

Purified mixture of liquid saturated hydrocarbons obtained from petroleum.

CHARACTERS

Appearance

Colourless, transparent, oily liquid, free from fluorescence in daylight.

Solubility

Practically insoluble in water, slightly soluble in ethanol (96 per cent), miscible with hydrocarbons.

IDENTIFICATION

First identification: A, C.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of liquid paraffin.

B. In a test tube cautiously boil 1 mL with 1 mL of *0.1 M sodium hydroxide*, with continuous shaking, for about 30 s. On cooling to room temperature, 2 phases separate. To the aqueous phase add 0.1 mL of *phenolphthalein solution R*. The solution becomes red.

C. Viscosity (see Tests).

TESTS

Acidity or alkalinity

To 10 mL add 20 mL of boiling *water R* and shake vigorously for 1 min. Separate the aqueous layer and filter. To 10 mL of the filtrate, add 0.1 mL of *phenolphthalein solution R*. The solution is colourless. Not more than 0.1 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

Relative density (*2.2.5*)

0.810 to 0.875.

Viscosity (*2.2.9*)

25 mPa·s to 80 mPa·s.

Polycyclic aromatic hydrocarbons

Use reagents for ultraviolet spectrophotometry.

Introduce 25.0 mL into a 125 mL separating funnel with unlubricated ground-glass parts (stopper, stopcock). Add 25 mL of *hexane R* which has been previously shaken twice with one-fifth its volume of *dimethyl sulfoxide R*. Mix and add 5.0 mL of *dimethyl sulfoxide R*. Shake vigorously for 1 min and allow to stand until 2 clear layers are formed. Transfer the lower layer to a 2nd separating funnel, add 2 mL of *hexane R* and shake the mixture vigorously. Allow to stand until 2 clear layers are formed. Separate the lower layer and measure its absorbance (*2.2.25*) between 260 nm and 420 nm, using as the compensation liquid the clear lower layer obtained by vigorously shaking 5.0 mL of *dimethyl sulfoxide R* with 25 mL of *hexane R* for 1 min. Prepare a 7.0 mg/L reference solution of *naphthalene R* in *trimethylpentane R* and measure the absorbance of the solution at the absorption maximum at 275 nm, using *trimethylpentane R* as the compensation liquid. At no wavelength between 260 nm and 420 nm does the absorbance of the test solution exceed one-third that of the reference solution at 275 nm.

Readily carbonisable substances

Use a ground-glass-stoppered tube about 125 mm long and 18 mm in internal diameter, graduated at 5 mL and 10 mL; wash with hot *water R* (temperature at least 60 °C), *acetone R*, *heptane R* and finally with *acetone R*, dry at 100-110 °C. Cool in a desiccator. Introduce 5 mL of the substance to be examined and add 5 mL of *nitrogen-free sulfuric acid R1*. Insert the stopper and shake as vigorously as possible, in the longitudinal direction of the tube, for 5 s. Loosen the stopper, immediately place the tube in a water-bath, avoiding contact of the tube with the bottom or side of the bath, and heat for 10 min. After 2 min, 4 min, 6 min and 8 min, remove the tube from the bath and shake as vigorously as possible, in the longitudinal direction of the tube for 5 s. At the end of 10 min of heating, remove the tube from the water-bath and allow to stand for 10 min. Centrifuge at 2000 *g* for 5 min. The lower layer is not more intensely coloured (*2.2.2, Method I*) than a mixture of 0.5 mL of blue primary solution, 1.5 mL of red primary

solution, 3.0 mL of yellow primary solution and 2 mL of a 10 g/L solution of *hydrochloric acid R*.

Solid paraffins
Dry a suitable quantity of the substance to be examined by heating at 100 °C for 2 h and cool in a desiccator over *sulfuric acid R*. Place in a glass tube with an internal diameter of about 25 mm, close the tube and immerse in a bath of iced water. After 4 h, the liquid is sufficiently clear for a black line, 0.5 mm wide, to be easily seen against a white background held vertically behind the tube.

STORAGE
Protected from light.

FUNCTIONALITY-RELATED CHARACTERISTICS
This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristic may be relevant for light liquid paraffin used as emollient in ointments, as vehicle in eye preparations or as lubricant in tablets and capsules.

Viscosity
(see Tests).

Ph Eur

Liquid Paraffin

(Ph. Eur. monograph 0239)

Action and use
Faecal softener.

Preparations
Liquid Paraffin Oral Emulsion

Liquid Paraffin and Magnesium Hydroxide Oral Emulsion

Ph Eur

DEFINITION
Purified mixture of liquid saturated hydrocarbons obtained from petroleum.

CHARACTERS

Appearance
Colourless, transparent, oily liquid, free from fluorescence in daylight.

Solubility
Practically insoluble in water, slightly soluble in ethanol (96 per cent), miscible with hydrocarbons.

IDENTIFICATION
First identification: A, C.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of liquid paraffin.

B. In a test tube cautiously boil 1 mL with 1 mL of *0.1 M sodium hydroxide*, with continuous shaking, for about 30 s. On cooling to room temperature, 2 phases separate. To the aqueous phase add 0.1 mL of *phenolphthalein solution R*. The solution becomes red.

C. Viscosity (see Tests).

TESTS

Acidity or alkalinity
To 10 mL add 20 mL of boiling *water R* and shake vigorously for 1 min. Separate the aqueous layer and filter. To 10 mL of the filtrate, add 0.1 mL of *phenolphthalein solution R*. The solution is colourless. Not more than 0.1 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

Relative density (*2.2.5*)
0.827 to 0.890.

Viscosity (*2.2.9*)
110 mPa·s to 230 mPa·s.

Polycyclic aromatic hydrocarbons
Use reagents for ultraviolet spectrophotometry.

Introduce 25.0 mL into a 125 mL separating funnel with unlubricated ground-glass parts (stopper, stopcock). Add 25 mL of *hexane R* which has been previously shaken twice with one-fifth its volume of *dimethyl sulfoxide R*. Mix and add 5.0 mL of *dimethyl sulfoxide R*. Shake vigorously for 1 min and allow to stand until 2 clear layers are formed. Transfer the lower layer to a 2^{d} separating funnel, add 2 mL of *hexane R* and shake the mixture vigorously. Allow to stand until 2 clear layers are formed. Separate the lower layer and measure its absorbance (*2.2.25*) between 260 nm and 420 nm, using as the compensation liquid the clear lower layer obtained by vigorously shaking 5.0 mL of *dimethyl sulfoxide R* with 25 mL of *hexane R* for 1 min. Prepare a 7.0 mg/L reference solution of *naphthalene R* in *trimethylpentane R* and measure the absorbance of the solution at the absorption maximum at 275 nm, using *trimethylpentane R* as the compensation liquid. At no wavelength between 260 nm and 420 nm does the absorbance of the test solution exceed one-third that of the reference solution at 275 nm.

Readily carbonisable substances
Use a ground-glass-stoppered tube about 125 mm long and 18 mm in internal diameter, graduated at 5 mL and 10 mL; wash with hot *water R* (temperature at least 60 °C), *acetone R*, *heptane R* and finally with *acetone R*, dry at 100-110 °C. Cool in a desiccator. Introduce 5 mL of the substance to be examined and add 5 mL of *nitrogen-free sulfuric acid R1*. Insert the stopper and shake as vigorously as possible, in the longitudinal direction of the tube, for 5 s. Loosen the stopper, immediately place the tube in a water-bath, avoiding contact of the tube with the bottom or side of the bath, and heat for 10 min. After 2 min, 4 min, 6 min and 8 min, remove the tube from the bath and shake as vigorously as possible, in the longitudinal direction of the tube for 5 s. At the end of 10 min of heating, remove the tube from the water-bath and allow to stand for 10 min. Centrifuge at 2000 *g* for 5 min. The lower layer is not more intensely coloured (*2.2.2, Method I*) than a mixture of 0.5 mL of blue primary solution, 1.5 mL of red primary solution, 3.0 mL of yellow primary solution and 2 mL of a 10 g/L solution of *hydrochloric acid R*.

Solid paraffins
Dry a suitable quantity of the substance to be examined by heating at 100 °C for 2 h and cool in a desiccator over

sulfuric acid R. Place in a glass tube with an internal diameter of about 25 mm, close the tube and immerse in a bath of iced water. After 4 h, the liquid is sufficiently clear for a black line, 0.5 mm wide, to be easily seen against a white background held vertically behind the tube.

STORAGE

Protected from light.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristic may be relevant for liquid paraffin used as emollient in ointments or as lubricant in tablets and capsules.

Viscosity

(see Tests).

Ph Eur

White Soft Paraffin

White Petroleum Jelly

(Ph. Eur. monograph 1799)

Ph Eur

DEFINITION

Purified and wholly or nearly decolorised mixture of semi-solid hydrocarbons, obtained from petroleum. It may contain a suitable antioxidant. White soft paraffin described in this monograph is not suitable for oral use.

CHARACTERS

Appearance

White or almost white, translucent, soft unctuous mass, slightly fluorescent in daylight when melted.

Solubility

Practically insoluble in water, slightly soluble in methylene chloride, practically insoluble in ethanol (96 per cent) and in glycerol.

IDENTIFICATION

First identification: A, B, D.

Second identification: A, C, D.

A. The drop point is between 35 °C and 70 °C and does not differ by more than 5 °C from the value stated on the label, according to method (*2.2.17*) with the following modification to fill the cup: heat the substance to be examined at a temperature not exceeding 80 °C, with stirring to ensure uniformity. Warm the metal cup at a temperature not exceeding 80 °C in an oven, remove it from the oven, place on a clean plate or ceramic tile and pour a sufficient quantity of the melted sample into the cup to fill it completely. Allow the filled cup to cool for 30 min on the plate or the ceramic tile and place it in a water bath at 24-26 °C for 30-40 min. Level the surface of the sample with a single stroke of a knife or razor blade, avoiding compression of the sample.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Place about 2 mg on a *sodium chloride R* plate, spread the substance with another *sodium chloride R* plate and remove 1 of the plates.

Comparison Repeat the operations using *white soft paraffin CRS*.

C. Melt 2 g and when a homogeneous phase is obtained, add 2 mL of *water R* and 0.2 mL of *0.05 M iodine*. Shake. Allow to cool. The solid upper layer is violet-pink or brown.

D. Appearance (see Tests).

TESTS

Appearance

The substance is white. Melt 12 g on a water-bath. The melted mass is not more intensely coloured than a mixture of 1 volume of yellow primary solution and 9 volumes of a 10 g/L solution of *hydrochloric acid R* (*2.2.2, Method II*).

Acidity or alkalinity

To 10 g add 20 mL of boiling *water R* and shake vigorously for 1 min. Allow to cool and decant. To 10 mL of the aqueous layer add 0.1 mL of *phenolphthalein solution R*. The solution is colourless. Not more than 0.5 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to red.

Consistency (*2.9.9*)

60 to 300.

Polycyclic aromatic hydrocarbons

Use reagents for ultraviolet spectrophotometry Dissolve 1.0 g in 50 mL of *hexane R* which has been previously shaken twice with 10 mL of *dimethyl sulfoxide R*. Transfer the solution to a 125 mL separating funnel with unlubricated ground-glass parts (stopper, stopcock). Add 20 mL of *dimethyl sulfoxide R*. Shake vigorously for 1 min and allow to stand until 2 clear layers are formed. Transfer the lower layer to a second separating funnel. Repeat the extraction with a further 20 mL of *dimethyl sulfoxide R*. Shake vigorously the combined lower layers with 20 mL of *hexane R* for 1 min. Allow to stand until 2 clear layers are formed. Separate the lower layer and dilute to 50.0 mL with *dimethyl sulfoxide R*. Measure the absorbance (*2.2.25*) over the range 260 nm to 420 nm using a path length of 4 cm and as compensation liquid the clear lower layer obtained by vigorously shaking 10 mL of *dimethyl sulfoxide R* with 25 mL of *hexane R* for 1 min. Prepare a reference solution in *dimethyl sulfoxide R* containing 6.0 mg of *naphthalene R* per litre and measure the absorbance of the solution at the maximum at 278 nm using a path length of 4 cm and *dimethyl sulfoxide R* as compensation liquid. At no wavelength in the range 260 nm to 420 nm does the absorbance of the test solution exceed that of the reference solution at 278 nm.

Sulfated ash (*2.4.14*)

Maximum 0.05 per cent, determined on 2.0 g.

STORAGE

Protected from light.

LABELLING

The label states the nominal drop point.

Ph Eur

Yellow Soft Paraffin

Yellow Petroleum Jelly

(Ph. Eur. monograph 1554)

Action and use
Excipient.

Ph Eur

DEFINITION
Purified mixture of semi-solid hydrocarbons, obtained from petroleum. It may contain a suitable antioxidant.

CHARACTERS

Appearance
Yellow, translucent, unctuous mass, slightly fluorescent in daylight when melted.

Solubility
Practically insoluble in water, slightly soluble in methylene chloride, practically insoluble in ethanol (96 per cent) and in glycerol.

IDENTIFICATION
First identification: A, B, D.

Second identification: A, C, D.

A. The drop point (*2.2.17*) is 40 °C to 60 °C and does not differ by more than 5 °C from the value stated on the label, with the following modification to fill the cup: heat the substance to be examined at 118-122 °C, with stirring to ensure uniformity, then cool to 100-107 °C. Warm the metal cup at 103-107 °C in an oven, remove it from the oven, place on a clean plate or ceramic tile and pour a sufficient quantity of the melted sample into the cup to fill it completely. Allow the filled cup to cool for 30 min on the ceramic tile and place it in a water-bath at 24-26 °C for a further 30-40 min. Level the surface of the sample with a single stroke of a knife or razor blade, avoiding compression of the sample.

B. Examine by infrared absorption spectrophotometry (*2.2.24*).

Preparation Place about 2 mg on a *sodium chloride R* plate, spread the substance with another *sodium chloride R* plate and remove 1 of the plates.

Comparison Repeat the operations using *yellow soft paraffin CRS*.

C. Melt 2 g and when a homogeneous phase is obtained, add 2 mL of *water R* and 0.2 mL of *0.05 M iodine*. Shake. Allow to cool. The solid upper layer is violet-pink or brown.

D. Appearance (see Tests).

TESTS

Appearance
The substance is yellow. Melt 12 g on a water-bath. The melted mass is not more intensely coloured than a mixture of 7.6 volumes of yellow primary solution and 2.4 volumes of red primary solution (*2.2.2, Method II*).

Acidity or alkalinity
To 10 g add 20 mL of boiling *water R* and shake vigorously for 1 min. Allow to cool and decant. To 10 mL of the aqueous layer add 0.1 mL of *phenolphthalein solution R*. The solution is colourless. Not more than 0.5 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to red.

Consistency (*2.9.9*)
100 to 300.

Polycyclic aromatic hydrocarbons
Use reagents for ultraviolet absorption spectrophotometry
Dissolve 1.0 g in 50 mL of *hexane R* which has been previously shaken twice with one-fifth its volume of *dimethyl sulfoxide R*. Transfer the solution to a 125 mL separating funnel with unlubricated ground-glass parts (stopper, stopcock). Add 20 mL of *dimethyl sulfoxide R*. Shake vigorously for 1 min and allow to stand until two clear layers are formed. Transfer the lower layer to a 2nd separating funnel. Repeat the extraction with a further 20 mL of *dimethyl sulfoxide R*. Shake vigorously the combined lower layers with 20 mL of *hexane R* for 1 min. Allow to stand until 2 clear layers are formed. Separate the lower layer and dilute to 50.0 mL with *dimethyl sulfoxide R*. Measure the absorbance (*2.2.25*) between 260 nm and 420 nm using a path length of 4 cm and using as the compensation liquid the clear lower layer obtained by vigorously shaking 10 mL of *dimethyl sulfoxide R* with 25 mL of *hexane R* for 1 min. Prepare a 9.0 mg/L reference solution of *naphthalene R* in *dimethyl sulfoxide R* and measure the absorbance of this solution at the maximum at 278 nm using a path length of 4 cm and using *dimethyl sulfoxide R* as the compensation liquid. At no wavelength in the range of 260 nm to 420 nm does the absorbance of the test solution exceed that of the reference solution at 278 nm.

Sulfated ash (*2.4.14*)
Not more than 0.05 per cent, determined on 2.0 g.

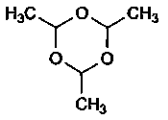
STORAGE
Store protected from light.

LABELLING
The label states the nominal drop point.

Ph Eur

Paraldehyde

(Ph. Eur. monograph 0351)



$C_6H_{12}O_3$ 132.2 *123-63-7*

Action and use
Anticonvulsant.

Ph Eur

DEFINITION
2,4,6-Trimethyl-1,3,5-trioxane (cyclic trimer of acetaldehyde).

It may contain a suitable quantity of an antioxidant.

CHARACTERS

Appearance
Colourless or slightly yellow, transparent liquid. It solidifies on cooling to form a crystalline mass.

Solubility
Soluble in water, but less soluble in boiling water, miscible with ethanol (96 per cent) and with essential oils.

IDENTIFICATION

A. Solution S (see Tests) is clear (*2.2.1*) but becomes turbid on warming.

B. To 5 mL add 0.1 mL of *dilute sulfuric acid R* and heat. Acetaldehyde, recognisable by its odour, is evolved.

C. To 5 mL of solution S in a test-tube add 5 mL of *ammoniacal silver nitrate solution R* and heat in a water-bath. Silver is deposited as a mirror on the wall of the tube.

TESTS

Solution S

Dissolve 20.0 mL in *carbon dioxide-free water R* and dilute to 200.0 mL with the same solvent.

Acidity

To 50.0 mL of solution S add 0.05 mL of *phenolphthalein solution R*. Not more than 1.5 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Refractive index (*2.2.6*)

1.403 to 1.406.

Relative density (*2.2.5*)

0.991 to 0.996.

Distillation range (*2.2.11*)

A maximum of 10 per cent distils below 123 °C and a minimum of 95 per cent distils below 126 °C.

Freezing point (*2.2.18*)

10 °C to 13 °C.

Acetaldehyde

To 5.0 mL add a mixture of 0.2 mL of *methyl orange solution R*, 5 mL of *ethanol (60 per cent V/V) R* and 5 mL of *alcoholic hydroxylamine solution R* and shake. Not more than 0.8 mL of *0.5 M sodium hydroxide* is required to change the colour of the indicator to pure yellow.

Peroxides

Place 50.0 mL of solution S in a ground-glass-stoppered flask, add 5 mL of *dilute sulfuric acid R* and 10 mL of *potassium iodide solution R*, close the flask and allow to stand protected from light for 15 min. Titrate with *0.1 M sodium thiosulfate* using 1 mL of *starch solution R* as indicator. Allow to stand for 5 min and, if necessary complete the titration. Not more than 2.0 mL of *0.1 M sodium thiosulfate* is required.

Non-volatile residue

Maximum 0.6 g/L.

Heat 5.0 mL in a tared evaporating dish on a water-bath and dry at 105 °C for 1 h. The residue weighs a maximum of 3 mg.

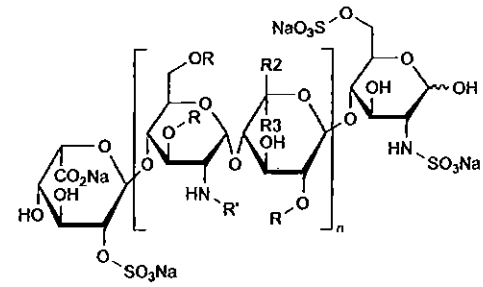
STORAGE

In a small, well-filled, airtight container, protected from light. If the substance has solidified the whole contents of the container must be liquefied before use.

Ph Eur

Parnaparin Sodium

(Ph. Eur. monograph 1252)



n = 1 to 21 , R = H or SO_3Na , R' = SO_3Na or CO-CH_3
R2 = H and R3 = CO_2Na or R2 = CO_2Na and R3 = H

Action and use

Low molecular weight heparin.

Ph Eur

DEFINITION

Sodium salt of a low-molecular-mass heparin that is obtained by radical-catalysed depolymerisation, with hydrogen peroxide and with a cupric salt, of heparin from porcine intestinal mucosa. The majority of the components have a 2-*O*-sulfo-α-L-idopyranuronic acid structure at the non-reducing end and a 2-*N*,6-*O*-disulfo-D-glucosamine structure at the reducing end of their chain.

Parnaparin sodium complies with the monograph Low-molecular-mass heparins (0828), with the modifications and additional requirements below.

The mass-average relative molecular mass ranges between 4000 and 6000 with a characteristic value of about 5000.

The degree of sulfatation is 2.0 to 2.6 per disaccharide unit.

The potency is not less than 75 IU and not more than 110 IU of anti-factor Xa activity per milligram calculated with reference to the dried substance. The ratio of anti-factor Xa activity to anti-factor IIa activity is between 1.5 and 3.0.

IDENTIFICATION

Carry out identification test A as described in the monograph *Low-molecular-mass heparins (0828)* using *parnaparin sodium CRS*.

Carry out identification test C as described in the monograph *Low-molecular-mass heparins (0828)*. In order to verify the suitability of the system in the lower molecular mass ranges (for example M_r 2000), a suitable reference preparation is used. The following requirements apply.

The mass-average relative molecular mass ranges between 4000 and 6000. The mass percentage of chains lower than 3000 is not more than 30 per cent. The mass percentage of chains between 3000 and 8000 ranges between 50 per cent and 60 per cent.

TESTS

Appearance of solution

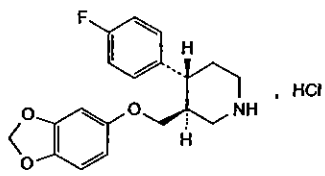
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5 (*2.2.2, Method II*).

Dissolve 1.5 g in 10 mL of *water R*.

Ph Eur

Paroxetine Hydrochloride

Anhydrous Paroxetine Hydrochloride
(Ph. Eur. monograph 2283)



$C_{19}H_{21}ClFNO_3$ 365.8 *78246-49-8*

Action and use
Selective serotonin reuptake inhibitor; antidepressant.

Preparation
Paroxetine Tablets

Ph Eur

DEFINITION
(3*S*,4*R*)-3-[[(1,3-Benzodioxol-5-yl)oxy]methyl]-4-(4-fluorophenyl)piperidine hydrochloride.

Content
97.5 per cent to 102.0 per cent (anhydrous substance).

PRODUCTION
Impurity G
Maximum 1 ppm, determined by a suitable validated method.

CHARACTERS
Appearance
White or almost white, hygroscopic, crystalline powder.

Solubility
Slightly soluble in water, freely soluble in methanol, sparingly soluble in anhydrous ethanol and in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *anhydrous paroxetine hydrochloride CRS.*

If the spectra obtained in the solid state show differences, mix 1 part of the substance to be examined and 1 part of the reference substance separately with 30 parts of anhydrous *acetone R* and heat to boiling to dissolve. Recrystallise and record new spectra using the residues.

B. Water (see Tests).

C. Dissolve 20 mg in 2 mL of *methanol R*. The solution gives reaction (a) of chlorides (*2.3.1*).

D. Enantiomeric purity (see Tests).

TESTS
Enantiomeric purity
Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in 5 mL of *methanol R* and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 5.0 mg of *paroxetine impurity D CRS* in 2 mL of *methanol R* and dilute to 50.0 mL with the mobile phase.

Reference solution (b) Dilute 1 mL of reference solution (a) to 10 mL with the test solution.

Reference solution (c) Dilute 1.0 mL of reference solution (a) to 100.0 mL with the mobile phase.

Column:
— *size:* l = 0.10 m, Ø = 4.0 mm;
— *stationary phase:* α_1*-acid-glycoprotein silica gel for chiral separation R* (5 µm);
— *temperature:* 30 °C.

Mobile phase Dissolve 8.7 g of *dipotassium hydrogen phosphate R* in 1000 mL of *water for chromatography R* and adjust to pH 6.5 with *phosphoric acid R*; mix 930 mL of this solution and 70 mL of *acetonitrile R*.

Flow rate 0.9 mL/min.

Detection Spectrophotometer at 295 nm.

Injection 20 µL of the test solution and reference solutions (b) and (c).

Run time 2.5 times the retention time of paroxetine.

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peak due to impurity D.

Relative retention With reference to paroxetine (retention time = about 13 min): impurity D = about 0.7.

System suitability Reference solution (b):
— *peak-to-valley ratio:* minimum 2.0, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to paroxetine.

Limit:
— *impurity D:* not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent).

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture *tetrahydrofuran R, water R* (10:90 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dilute 5.0 mL of the test solution to 50.0 mL with the solvent mixture.

Reference solution (b) Dissolve 5.0 mg of *anhydrous paroxetine hydrochloride impurity H CRS* in 25 mL of *tetrahydrofuran R* and dilute to 50.0 mL with *water R*.

Reference solution (c) Dissolve 5.0 mg of *anhydrous paroxetine hydrochloride impurity C CRS* in 25 mL of *tetrahydrofuran R* and dilute to 50.0 mL with *water R*.

Reference solution (d) To 5.0 mL of reference solution (a) add 1.0 mL of reference solution (b) and dilute to 100.0 mL with the solvent mixture.

Reference solution (e) To 5.0 mL of reference solution (a) add 5.0 mL of reference solution (b) and 5.0 mL of reference solution (c). Dilute to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (f) Dissolve 2.5 mg of *paroxetine impurity E CRS* in the solvent mixture, add 2.5 mL of the test solution and dilute to 100 mL with the solvent mixture.

Reference solution (g) Dissolve 5 mg of *paroxetine impurity A CRS* in the solvent mixture and dilute to 50 mL with the solvent mixture. Use this solution to identify the peak due to impurity A.

Column:
— *size:* l = 0.25 m, Ø = 4.6 mm;
— *stationary phase:* *end-capped octylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: *trifluoroacetic acid R, tetrahydrofuran R, water for chromatography R* (0.5:10:90 *V/V/V*);
— *mobile phase B*: *trifluoroacetic acid R, tetrahydrofuran R, acetonitrile R* (0.5:10:90 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	80	20
30 - 50	80 → 20	20 → 80
50 - 55	20	80

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 295 nm.

Injection 20 µL of the test solution and reference solutions (d), (e), (f) and (g).

Relative retention With reference to paroxetine (retention time = about 28 min): impurity A = about 0.8; impurity E = about 0.9; impurity C = about 1.5.

Relative retention With reference to impurity C: impurity F = about 0.97; impurity J = about 1.02.

System suitability:
— *resolution*: minimum 3.5 between the peaks due to impurity E and paroxetine in the chromatogram obtained with reference solution (f);
— *signal-to-noise ratio*: minimum 3 for the peak due to paroxetine in the chromatogram obtained with reference solution (e).

Limits:
— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity C = 1.6; impurity F = 1.7; impurity J = 1.3;
— *impurity A*: not more than 0.6 times the area of the peak due to paroxetine in the chromatogram obtained with reference solution (d) (0.3 per cent);
— *impurities C, F, J*: for each impurity, not more than 0.2 times the area of the peak due to paroxetine in the chromatogram obtained with reference solution (d) (0.1 per cent);
— *unspecified impurities*: for each impurity, not more than 0.2 times the area of the peak due to paroxetine in the chromatogram obtained with reference solution (d) (0.10 per cent);
— *total*: not more than the area of the peak due to paroxetine in the chromatogram obtained with reference solution (d) (0.5 per cent);
— *disregard limit*: the area of the peak due to paroxetine in the chromatogram obtained with reference solution (e) (0.05 per cent).

Impurities H and I

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Detection Spectrophotometer at 263 nm.

Injection Test solution and reference solutions (d) and (e).

Relative retention With reference to paroxetine (retention time = about 28 min): impurity I = about 0.2; impurity H = about 0.4.

System suitability Reference solution (e):
— *signal-to-noise ratio*: minimum 3 for the peak due to impurity H.

Limits:
— *impurities H, I*: for each impurity, not more than the area of the peak due to impurity H in the chromatogram obtained with reference solution (d) (0.1 per cent).

Acetone (*2.4.24, System B*)

Maximum 3.5 per cent.

2-Propanol (*2.4.24, System B*)

Maximum 4.3 per cent.

Water (*2.5.12*)

Maximum 1.5 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 100.0 mL with the same solvent.

Reference solution (a) Dissolve 51.2 mg of *paroxetine hydrochloride hemihydrate CRS* in *water R* and dilute to 100.0 mL with the same solvent.

Reference solution (b) Dissolve 5 mg of *paroxetine hydrochloride hemihydrate CRS* and 5 mg of *paroxetine impurity A CRS* in *water R* and dilute to 10 mL with the same solvent.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *trimethylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 3.85 g of *ammonium acetate R* in *water for chromatography R*, adjust to pH 5.5 with *anhydrous acetic acid R* and dilute to 600 mL with *water for chromatography R*; add 400 mL of *acetonitrile R*; slowly add, with stirring, 10 mL of *triethylamine R* and adjust to pH 5.5 with *anhydrous acetic acid R*.

Flow rate 1 mL/min.

Detection Spectrophotometer at 295 nm.

Injection 10 µL.

Run time Twice the retention time of paroxetine.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity A.

Relative retention With reference to paroxetine (retention time = about 8 min): impurity A = about 0.9.

System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the peaks due to impurity A and paroxetine.

Calculate the percentage content of $C_{19}H_{21}ClFNO_3$ using the chromatogram obtained with reference solution (a) and taking into account the assigned content of *paroxetine hydrochloride hemihydrate CRS*.

STORAGE

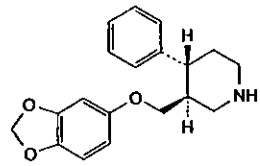
In an airtight container, at a temperature not exceeding 25 °C.

IMPURITIES

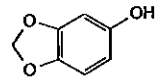
Specified impurities *A, C, D, F, G, H, I, J.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is

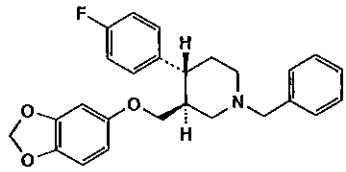
therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) B, E.



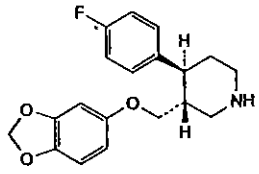
A. (3*S*,4*R*)-3-[[(1,3-benzodioxol-5-yl)oxy]methyl]-4-phenylpiperidine (defluoroparoxetine),



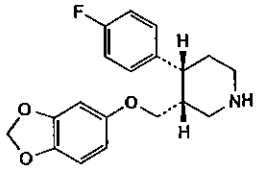
B. 1,3-benzodioxol-5-ol (sesamol),



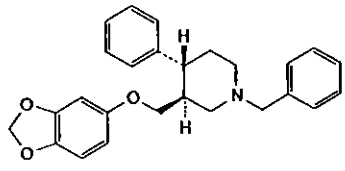
C. (3*S*,4*R*)-3-[[(1,3-benzodioxol-5-yl)oxy]methyl]-1-benzyl-4-(4-fluorophenyl)piperidine (*N*-benzylparoxetine),



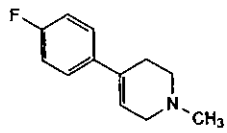
D. (3*R*,4*S*)-3-[[(1,3-benzodioxol-5-yl)oxy]methyl]-4-(4-fluorophenyl)piperidine ((+)-*trans*-paroxetine),



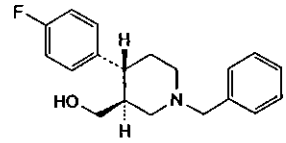
E. (3*RS*,4*RS*)-3-[[(1,3-benzodioxol-5-yl)oxy]methyl]-4-(4-fluorophenyl)piperidine (*cis*-paroxetine),



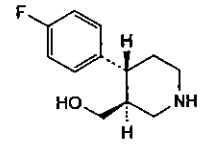
F. (3*S*,4*R*)-3-[[(1,3-benzodioxol-5-yl)oxy]methyl]-1-benzyl-4-phenylpiperidine (*N*-benzyldefluoroparoxetine),



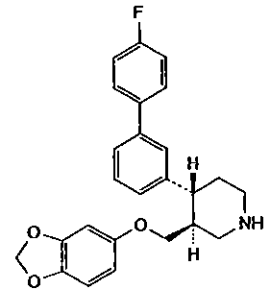
G. 4-(4-fluorophenyl)-1-methyl-1,2,3,6-tetrahydropyridine,



H. [(3*S*,4*R*)-1-benzyl-4-(4-fluorophenyl)piperidin-3-yl]methanol,



I. [(3*S*,4*R*)-4-(4-fluorophenyl)piperidin-3-yl]methanol,

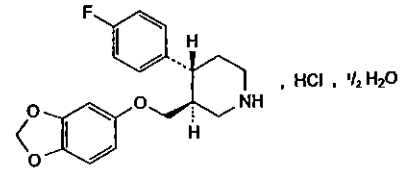


J. (3*S*,4*R*)-3-[[(1,3-benzodioxol-5-yl)oxy]methyl]-4-(4′-fluoro[1,1′-biphenyl]-3-yl)piperidine.

Ph Eur

Paroxetine Hydrochloride Hemihydrate

(Ph. Eur. monograph 2018)



$C_{19}H_{21}ClFNO_3,½H_2O$ 374.8 *110429-35-1*

Action and use
Selective serotonin reuptake inhibitor; antidepressant.

Ph Eur

DEFINITION
(3*S*,4*R*)-3-[[(1,3-Benzodioxol-5-yl)oxy]methyl]-4-(4-fluorophenyl)piperidine hydrochloride hemihydrate.

Content
97.5 per cent to 102.0 per cent (anhydrous substance).

PRODUCTION

Impurity G
Maximum 1 ppm, determined by a suitable, validated method.

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Slightly soluble in water, freely soluble in methanol, sparingly soluble in ethanol (96 per cent) and in methylene chloride.

It shows pseudopolymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *paroxetine hydrochloride hemihydrate CRS.*

If the spectra obtained show differences, dissolve 1 part of the substance to be examined and 1 part of the reference substance separately in 10 parts of a mixture of 1 volume of *water R* and 9 volumes of *2-propanol R* and heat to 70 °C to dissolve. Recrystallise and record new spectra using the residues.

B. Enantiomeric purity (see Tests).

C. Water (see Tests).

D. Dissolve 21 mg in 2 mL of *methanol R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Enantiomeric purity
Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in 20 mL of *methanol R* and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 5 mg of *paroxetine impurity D CRS* and 5 mg of *paroxetine hydrochloride hemihydrate CRS* in 2 mL of *methanol R* and dilute to 100 mL with the mobile phase.

Column:
— *size*: *l* = 0.10 m, Ø = 4.0 mm;
— *stationary phase*: *α_1-acid-glycoprotein silica gel for chiral separation R* (5 µm).

Mobile phase Mix 2 volumes of *methanol R* and 8 volumes of a 5.8 g/L solution of *sodium chloride R*.

Flow rate 0.5 mL/min.

Detection Spectrophotometer at 295 nm.

Injection 10 µL.

Run time 2.5 times the retention time of paroxetine.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity D.

Relative retention With reference to paroxetine (retention time = about 30 min): impurity D = about 0.4.

System suitability Reference solution (b):
— *resolution*: minimum 2.2 between the peaks due to impurity D and paroxetine.

Limit:
— *impurity D*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent).

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture *tetrahydrofuran R, water R* (10:90 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dilute 5.0 mL of the test solution to 50.0 mL with the solvent mixture. Dilute 2.0 mL of this solution to 200.0 mL with the solvent mixture.

Reference solution (b) Dissolve the contents of a vial of *paroxetine for system suitability CRS* (containing impurity C) in 1 mL of the solvent mixture.

Reference solution (c) Dissolve 2 mg of *paroxetine impurity A CRS* in the solvent mixture and dilute to 20 mL with the solvent mixture.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: *trifluoroacetic acid R, tetrahydrofuran R, water for chromatography R* (0.5:10:90 *V/V/V*);
— *mobile phase B*: *trifluoroacetic acid R, tetrahydrofuran R, acetonitrile R* (0.5:10:90 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	80	20
30 - 50	80 → 20	20 → 80
50 - 60	20	80

Flow rate 1 mL/min.

Detection Spectrophotometer at 295 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peak due to impurity A; use the chromatogram supplied with *paroxetine for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity C.

Relative retention With reference to paroxetine (retention time = about 28 min): impurity A = about 0.8; impurity C = about 1.2.

System suitability Reference solution (b):
— *resolution*: minimum 3.5 between the peaks due to paroxetine and impurity C.

Limits:
— *impurity A*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)
2.2 per cent to 2.7 per cent, determined on 0.300 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 100.0 mL with the same solvent.

Reference solution (a) Dissolve 50.0 mg of *paroxetine hydrochloride hemihydrate CRS* in *water R* and dilute to 100.0 mL with the same solvent.

Reference solution (b) Dissolve 5 mg of *paroxetine hydrochloride hemihydrate CRS* and 5 mg of *paroxetine impurity A CRS* in *water R* and dilute to 10 mL with the same solvent.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *trimethylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 3.85 g of *ammonium acetate R* in *water for chromatography R*, adjust to pH 5.5 with *anhydrous acetic acid R* and dilute to 600 mL with *water for chromatography R*; add 400 mL of *acetonitrile R*; slowly add, with stirring, 10 mL of *triethylamine R* and readjust to pH 5.5 with *anhydrous acetic acid R*.

Flow rate 1 mL/min.

Detection Spectrophotometer at 295 nm.

Injection 10 µL.

Run time Twice the retention time of paroxetine.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity A.

Relative retention With reference to paroxetine (retention time = about 8 min): impurity A = about 0.9.

System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the peaks due to impurity A and paroxetine.

Calculate the percentage content of $C_{19}H_{21}ClFNO_3$ using the chromatogram obtained with reference solution (a) and taking into account the assigned content of *paroxetine hydrochloride hemihydrate CRS*.

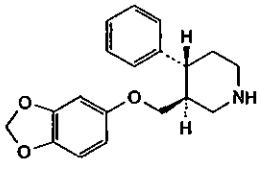
STORAGE

Protected from light.

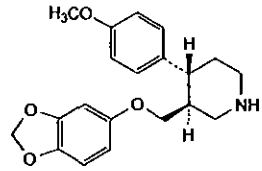
IMPURITIES

Specified impurities *A, D, G.*

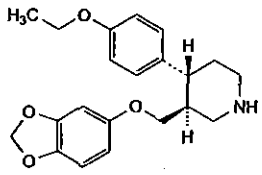
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, E, F.*



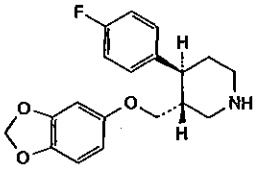
A. (3*S*,4*R*)-3-[[(1,3-benzodioxol-5-yl)oxy]methyl]-4-phenylpiperidine (defluoroparoxetine),



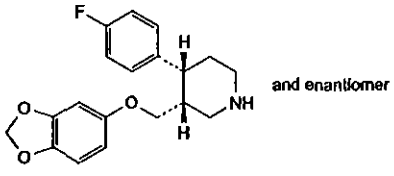
B. (3*S*,4*R*)-3-[[(1,3-benzodioxol-5-yl)oxy]methyl]-4-(4-methoxyphenyl)piperidine,



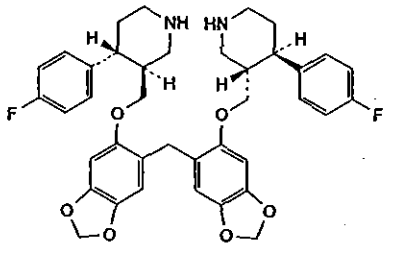
C. (3*S*,4*R*)-3-[[(1,3-benzodioxol-5-yl)oxy]methyl]-4-(4-ethoxyphenyl)piperidine,



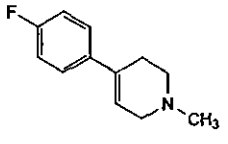
D. (3*R*,4*S*)-3-[[(1,3-benzodioxol-5-yl)oxy]methyl]-4-(4-fluorophenyl)piperidine ((+)-*trans*-paroxetine),



E. (3*RS*,4*RS*)-3-[[(1,3-benzodioxol-5-yl)oxy]methyl]-4-(4-fluorophenyl)piperidine (*cis*-paroxetine),



F. 3,3′-[methylenebis(1,3-benzodioxole-6,5-diyloxymethylene)]bis[(3*S*,4*R*)-4-(4-fluorophenyl)piperidine],

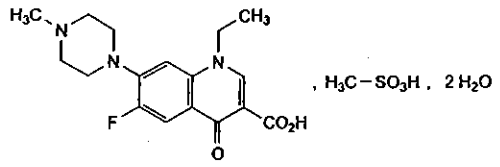


G. 4-(4-fluorophenyl)-1-methyl-1,2,3,6-tetrahydropyridine.

Ph Eur

Pefloxacin Mesilate

(Perfloxacin Mesilate Dihydrate, Ph. Eur. monograph 1460)



$C_{18}H_{24}FN_3O_6S,2H_2O$ 465.5 *149676-40-4*

Action and use
Antibacterial.

Ph Eur

DEFINITION

1-Ethyl-6-fluoro-7-(4-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid methanesulfonate dihydrate.

Content
98.5 per cent to 101.5 per cent (anhydrous substance).

PRODUCTION

It is considered that alkyl methanesulfonate esters are genotoxic and are potential impurities in pefloxacin mesilate dihydrate. The manufacturing process should be developed taking into consideration the principles of quality risk management, together with considerations of the quality of starting materials, process capability and validation.
The general methods *2.5.37. Methyl, ethyl and isopropyl methanesulfonate in methanesulfonic acid, 2.5.38. Methyl, ethyl and isopropyl methanesulfonate in active substances* and *2.5.39. Methanesulfonyl chloride in methanesulfonic acid* are available to assist manufacturers.

CHARACTERS

Appearance
Fine, white or almost white powder.

Solubility
Freely soluble in water, slightly soluble in ethanol (96 per cent), very slightly soluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Dissolve 0.1 g in 10 mL of *water R*. Add 5 mL of *1 M sodium hydroxide*. Adjust to pH 7.4 ± 0.1 with *phosphoric acid R* and shake with 2 quantities, each of 30 mL, of *methylene chloride R*. Combine the organic layers and dry over *anhydrous sodium sulfate R*. Evaporate to dryness. Examine the residue as a disc of *potassium bromide R*.

Comparison Repeat the operations using 0.1 g of *pefloxacin mesilate dihydrate CRS*.

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 40 mg in *water R* and dilute to 1 mL with the same solvent.

Reference solution Dissolve 60 mg of *methanesulfonic acid R* in *water R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel plate R*.

Mobile phase *water R, ammonia R, butanol R, acetone R* (5:10:20:65 *V/V/V/V*).

Application 10 µL.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with a 0.4 g/L solution of *bromocresol purple R* in *ethanol (50 per cent V/V) R*, adjusted to pH 10 using *1 M sodium hydroxide*.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Solution S
Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 10.0 mL with the same solvent.

Appearance of solution
Examined within 1 h after its preparation, solution S is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than intensity 3 of the range of reference solutions of the most appropriate colour (*2.2.2, Method II*).

pH (*2.2.3*)
3.5 to 4.5.

Dilute 1 mL of solution S to 10 mL with *carbon dioxide-free water R*.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dissolve 5.0 mg of *pefloxacin impurity B CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 100.0 mL with the mobile phase. In 2.0 mL of this solution, dissolve the contents of a vial of *pefloxacin impurity C CRS*.

Reference solution (b) Dissolve 10.0 mg of *norfloxacin impurity A CRS* (impurity F) in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 100.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.15 m, Ø = 6 mm;
— *stationary phase*: *octadecylsilyl vinyl polymer for chromatography R* (5 µm).

Mobile phase Mix 30 volumes of *acetonitrile R*, 70 volumes of a solution containing 2.70 g/L of *cetyltrimethylammonium bromide R* and 6.18 g/L of *boric acid R* (exactly adjusted to pH 8.30 with *1 M sodium hydroxide*), and 0.2 volumes of thiodiethylene glycol R.

Flow rate 1 mL/min.

Detection Spectrophotometer at 258 nm and at 273 nm.

Injection 20 µL.

Run time 4 times the retention time of pefloxacin (about 60 min).

Relative retentions and correction factors:

	Approximate relative retention	Correction factor
Impurity E	0.2	–
Impurity D	0.3	–
Impurity A	0.5	–
Impurity G	0.8	1.4
Pefloxacin	1	–
Impurity C	1.7	2.4
Impurity B	1.8	–
Impurity H	2.4	1.8
Impurity F	3.5	–

System suitability Reference solution (a) at 273 nm:
— *resolution*: minimum 1.5 between the peaks due to impurities C and B.

From the chromatogram obtained at 258 nm with the test solution, calculate the percentage content of impurities C, F, G and H using the area of the principal peak in the chromatogram obtained at 258 nm with reference solution (b) (external standardisation) taking into account the correction factors indicated in the table.

From the chromatogram obtained at 273 nm with the test solution, calculate the percentage content of impurities A, B, D and E and of any other impurity from the areas of the peaks in the chromatogram obtained with the test solution by the normalisation procedure.

Limits:
— *impurities A, B, D, E and any other impurity at 273 nm and impurities C, F, G, H at 258 nm*: for each impurity, maximum 0.5 per cent and not more than 3 impurities have a content between 0.2 per cent and 0.5 per cent;
— *total*: maximum 1.0 per cent;
— *disregard limit at 273 nm*: 0.0005 times the area of the principal peak in the chromatogram obtained with the test solution (0.05 per cent).

Water (*2.5.12*)
7.0 per cent to 8.5 per cent, determined on 50.0 mg using a mixture of 10 volumes of *methanol R* and 50 volumes of *methylene chloride R*.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 15.0 mL of *anhydrous acetic acid R* and add 75.0 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

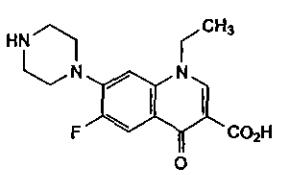
1 mL of *0.1 M perchloric acid* is equivalent to 21.48 mg of $C_{18}H_{24}FN_3O_6S$.

STORAGE

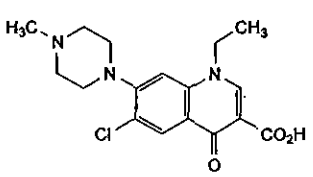
In an airtight container, protected from light.

IMPURITIES

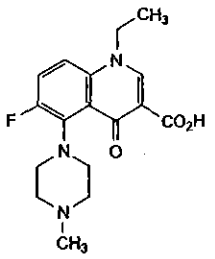
Specified impurities *A, B, C, D, E, F, G, H.*



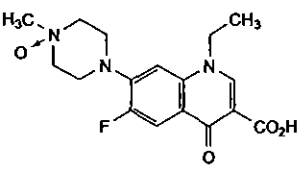
A. 1-ethyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (demethylated pefloxacin or norfloxacin),



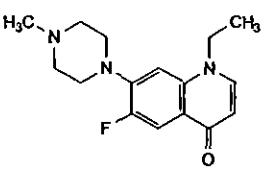
B. 6-chloro-1-ethyl-7-(4-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (chlorinated homologue of pefloxacin),



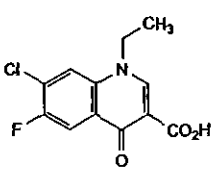
C. 1-ethyl-6-fluoro-5-(4-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (isopefloxacin),



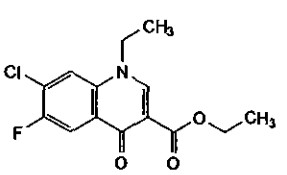
D. 4-(3-carboxy-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinolin-7-yl)-1-methylpiperazine 1-oxide (*N*-oxide of pefloxacin),



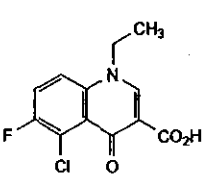
E. 1-ethyl-6-fluoro-7-(4-methylpiperazin-1-yl)quinoline-4(1*H*)-one (decarboxylated pefloxacin),



F. 7-chloro-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (*N*-ethyl acid) (norfloxacin impurity A),



G. ethyl 7-chloro-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (*N*-ethyl ester),

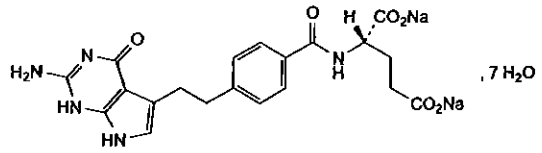


H. 5-chloro-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (iso-*N*-ethyl acid).

Ph Eur

Pemetrexed Disodium Heptahydrate

(Ph. Eur. monograph 2637)



$C_{20}H_{19}N_5Na_2O_6,7H_2O$ 597.5 *357166-29-1*

Action and use
Thymidylate synthetase inhibitor; cytostatic.

Ph Eur

DEFINITION

Disodium (2*S*)-2-[[4-[2-(2-amino-4-oxo-4,7-dihydro-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]amino]pentanedioate heptahydrate.

Content
97.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Freely soluble in water, very slightly soluble in anhydrous ethanol, practically insoluble in methylene chloride.

IDENTIFICATION

Carry out either tests A, C, D, E or tests B, C, D, E.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *pemetrexed disodium heptahydrate CRS.*

B. Nuclear magnetic resonance spectrometry (*2.2.33*).

Preparation 25-50 mg/mL solution in *deuterium oxide R.*

Comparison Solution of equal concentration of *pemetrexed disodium heptahydrate CRS* in *deuterium oxide R.*

Results The ^{1}H NMR spectrum obtained is qualitatively similar to the ^{1}H NMR spectrum obtained with *pemetrexed disodium heptahydrate CRS*; disregard the peak located at approximately 5.0 ppm for the comparison.

C. It gives reaction (a) of sodium (*2.3.1*).

D. Enantiomeric purity (see Tests).

E. Water (see Tests).

TESTS

Solution S
Dissolve 0.56 g in *carbon dioxide-free water R* and dilute to 10.0 mL with the same solvent.

Appearance of solution
Solution S is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution GY_4 or Y_4 (*2.2.2, Method II*).

pH (*2.2.3*)
7.5 to 8.4 for solution S.

Enantiomeric purity
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use or store them at 2-8 °C for not more than 24 h.*

Solution A Dissolve 8 g of *β-cyclodextrin R* in 900 mL of *water for chromatography R.* Add 15 mL of *triethylamine R* then 6 mL of *phosphoric acid R* and adjust to pH 6.0 with *phosphoric acid R.* Dilute to 1000 mL with *water for chromatography R.*

Test solution Dissolve 12 mg of the substance to be examined in *water for chromatography R* and dilute to 50.0 mL with the same solvent.

Reference solution (a) Dissolve 6 mg of *pemetrexed for system suitability CRS* (containing impurity E) in *water for chromatography R* and dilute to 25.0 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *water for chromatography R.* Dilute 3.0 mL of this solution to 10.0 mL with *water for chromatography R.*

Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm) with a pore size of 12 nm;
— *temperature:* 40 °C.

Mobile phase *acetonitrile R*, solution A (5:95 *V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 50 µL.

Run time 1.5 times the retention time of pemetrexed.

Relative retention With reference to pemetrexed (retention time = about 30 min): impurity E = about 0.94.

System suitability:
— *symmetry factor:* maximum 2.0 for the principal peak in the chromatogram obtained with reference solution (b);
— *peak-to-valley ratio:* minimum 5.0, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to pemetrexed in the chromatogram obtained with reference solution (a).

Calculation of percentage contents:
— for impurity E, use the concentration of pemetrexed disodium heptahydrate in reference solution (b).

Limit:
— *impurity E:* maximum 0.3 per cent.

Column rinse: the following program is given for information only.
Use a gradient column rinse before column storage or after 30 sample injections to avoid build-up on the column. If a drifting baseline is observed, allow additional time for equilibration with the mobile phase. If a blank chromatogram exhibits broad humps, perform a gradient column rinse.

Rinsing solution A *water for chromatography R.*

Rinsing solution B *acetonitrile R1.*

Time (min)	Mobile phase (per cent *V/V*)	Rinsing solution A (per cent *V/V*)	Rinsing solution B (per cent *V/V*)
0 - 4	100 → 0	0 → 50	0 → 50
4 - 9	0	50	50
9 - 14	0	50 → 10	50 → 90
14 - 54	0	10	90
54 - 69	0	10 → 95	90 → 5
69 - 100	0	95	5

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use or store them at 2-8 °C for not more than 24 h.*

Solution A 1.45 g/L solution of *ammonium formate R* in *water for chromatography R*, adjusted to pH 3.5 with *anhydrous formic acid R*.

Test solution Dissolve 20 mg of the substance to be examined in *water for chromatography R* and dilute to 100.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *water for chromatography R*. Dilute 1.0 mL of this solution to 10.0 mL with *water for chromatography R*.

Reference solution (b) In order to prepare impurities B and C *in situ*, dissolve 30 mg of the substance to be examined in 10.0 mL of a 4.0 g/L solution of *sodium hydroxide R*, heat at 70 °C for 40 min and allow to cool. Dilute 1.0 mL of the solution to 10.0 mL with *water for chromatography R*.

Reference solution (c) Dissolve the contents of a vial of *pemetrexed impurity mixture CRS* (impurities A and D) in 1.0 mL of *water for chromatography R*.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated octylsilyl silica gel for chromatography R* (3.5 µm).

Mobile phase:
— *mobile phase A*: *acetonitrile R*, solution A (5:95 *V/V*);
— *mobile phase B*: *acetonitrile R*, solution A (30:70 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	100	0
3 - 45	100 → 0	0 → 100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 250 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram supplied with *pemetrexed impurity mixture CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A and D; use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B and C.

Relative retention With reference to pemetrexed (retention time = about 26 min): impurity A = about 0.82; impurity B = about 0.87; impurity C = about 0.88; impurity D = about 0.90.

System suitability Reference solution (b):
— *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity B and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity C.

Calculation of percentage contents:
— for each impurity, use the concentration of pemetrexed disodium heptahydrate in reference solution (a).

Limits:
— *impurities A, D*: for each impurity, maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.6 per cent;
— *reporting threshold*: 0.05 per cent.

Water (*2.5.12*)
19.5 per cent to 22.1 per cent, determined on 0.050 g.

Bacterial endotoxins (*2.6.14*)
Less than 0.17 IU/mg.

ASSAY

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use or store them at 2-8 °C for not more than 24 h.*

Acetate buffer Mix 1.7 mL of *glacial acetic acid R* and 900 mL of *water for chromatography R*, adjust to pH 5.3 with a 760 g/L solution of *sodium hydroxide R* in *water for chromatography R* and dilute to 1000 mL with *water for chromatography R*.

Test solution Dissolve 30.0 mg of the substance to be examined in *water for chromatography R* and dilute to 200.0 mL with the same solvent.

Reference solution Dissolve 30.0 mg of *pemetrexed disodium heptahydrate CRS* in *water for chromatography R* and dilute to 200.0 mL with the same solvent.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated octylsilyl silica gel for chromatography R* (3.5 µm);
— *temperature*: 30 °C.

Mobile phase *acetonitrile R*, acetate buffer (11:89 *V/V*).

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 285 nm.

Injection 20 µL.

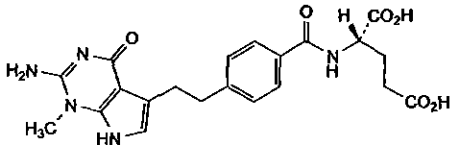
Run time Twice the retention time of pemetrexed (retention time = about 3 min).

Calculate the percentage content of $C_{20}H_{19}N_5Na_2O_6$ taking into account the assigned content of *pemetrexed disodium heptahydrate CRS*.

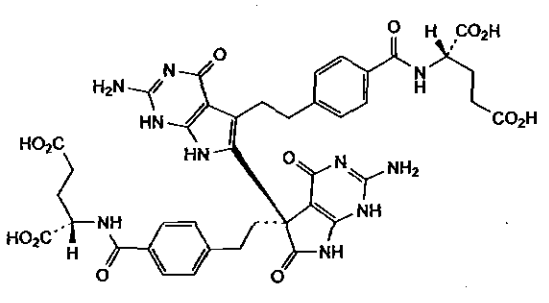
IMPURITIES

Specified impurities *A, D, E.*

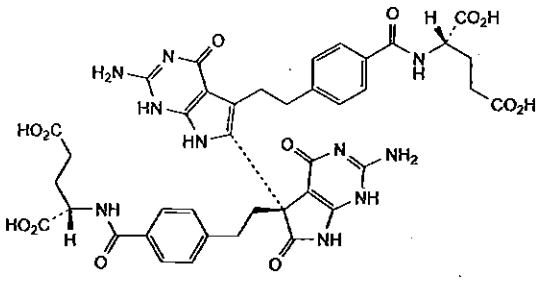
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C.*



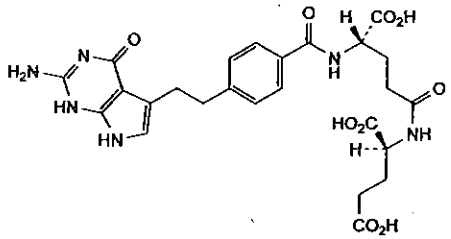
A. (2*S*)-2-[[4-[2-(2-amino-1-methyl-4-oxo-4,7-dihydro-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]amino]-pentanedioic acid,



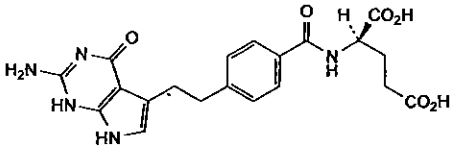
B. (2*S*,2′*S*)-2,2′-[[(5*R*)-2,2′-diamino-4,4′,6-trioxo-1,4,4′,6,7,7′-hexahydro-1′*H*,5*H*-5,6′-bipyrrolo[2,3-*d*]pyrimidine-5,5′-diyl]bis(ethylenebenzene-4,1-diylcarbonylimino)]dipentanedioic acid,



C. (2*S*,2′*S*)-2,2′-[[(5*S*)-2,2′-diamino-4,4′,6-trioxo-1,4,4′,6,7,7′-hexahydro-1′*H*,5*H*-5,6′-bipyrrolo[2,3-*d*]pyrimidine-5,5′-diyl]bis(ethylenebenzene-4,1-diylcarbonylimino)]dipentanedioic acid,



D. (2*S*)-2-[[(4*S*)-4-[[4-[2-(2-amino-4-oxo-4,7-dihydro-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]amino]-4-carboxybutanoyl]amino]pentanedioic acid,

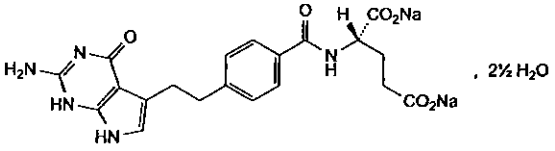


E. (2*R*)-2-[[4-[2-(2-amino-4-oxo-4,7-dihydro-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]amino]pentanedioic acid.

Ph Eur

Pemetrexed Disodium 2.5-Hydrate

(Ph. Eur. monograph 3046)



$C_{20}H_{19}N_5Na_2O_6,2½H_2O$ 516.4 *357166-30-4*

Action and use
Thymidylate synthetase inhibitor; antineoplastic

Ph Eur

DEFINITION

Disodium (2*S*)-2-[4-[2-(2-amino-4-oxo-4,7-dihydro-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzamido]pentanedioate 2.5-hydrate.

Content
97.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, hygroscopic powder.

Solubility
Freely soluble in water, very slightly soluble in anhydrous ethanol, practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *pemetrexed disodium 2.5-hydrate CRS.*

B. It gives reaction (a) of sodium (*2.3.1*).

C. Enantiomeric purity (see Tests).

D. Water (see Tests).

TESTS

Solution S
Dissolve 0.48 g in *carbon dioxide-free water R* and dilute to 10.0 mL with the same solvent.

Appearance of solution
Solution S is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution GY_4 or Y_4 (*2.2.2, Method II*).

pH (*2.2.3*)
7.5 to 8.4 for solution S.

Enantiomeric purity
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use or store them at 2-8 °C and use them within 24 h.*

Solution A Dissolve 8 g of *β-cyclodextrin R* in 900 mL of *water for chromatography R*. Add 15 mL of *triethylamine R* then 6 mL of *phosphoric acid R* and adjust to pH 6.0 with *phosphoric acid R*. Dilute to 1000 mL with *water for chromatography R*.

Test solution Dissolve 12 mg of the substance to be examined in *water R* and dilute to 50.0 mL with the same solvent.

Reference solution (a) Dissolve 6 mg of *pemetrexed for system suitability CRS* (containing impurity E) in *water R* and dilute to 25 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *water R*. Dilute 3.0 mL of this solution to 10.0 mL with *water R*.

Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 40 °C.

Mobile phase *acetonitrile for chromatography R*, solution A (5:95 *V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 50 µL.

Run time 1.5 times the retention time of pemetrexed.

Relative retention With reference to pemetrexed (retention time = about 30 min): impurity E = about 0.94.

System suitability:
— *symmetry factor*: maximum 2.0 for the principal peak in the chromatogram obtained with reference solution (b);
— *peak-to-valley ratio*: minimum 5.0, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to pemetrexed in the chromatogram obtained with reference solution (a).

Calculation of percentage contents:
— for impurity E, use the concentration of pemetrexed disodium 2.5-hydrate in reference solution (b).

Limit:
— *impurity E*: maximum 0.3 per cent.

Column rinse: the following programme is given for information only.

Use a gradient column rinse before column storage or after 30 sample injections to avoid build-up on the column. If a drifting baseline is observed, allow additional time for equilibration with the mobile phase. If a blank chromatogram exhibits broad humps, perform a gradient column rinse.

Rinsing solution A *water for chromatography R.*

Rinsing solution B *acetonitrile for chromatography R.*

Time (min)	Mobile phase (per cent *V/V*)	Rinsing solution A (per cent *V/V*)	Rinsing solution B (per cent *V/V*)
0 - 4	100 → 0	0 → 50	0 → 50
4 - 9	0	50	50
9 - 14	0	50 → 10	50 → 90
14 - 54	0	10	90
54 - 69	0	10 → 95	90 → 5
69 - 100	0	95	5

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use or store them at 2-8 °C and use them within 24 h.*

Solution A 1.45 g/L solution of *ammonium formate R* in *water for chromatography R*, adjusted to pH 3.5 with *anhydrous formic acid R*.

Test solution Dissolve 20 mg of the substance to be examined in *water R* and dilute to 100.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *water R*. Dilute 1.0 mL of this solution to 10.0 mL with *water R*.

Reference solution (b) In order to prepare impurities B and C *in situ*, dissolve 30 mg of the substance to be examined in 10 mL of a 4.0 g/L solution of *sodium hydroxide R*, heat at 70 °C for 40 min and allow to cool. Dilute 1 mL of the solution to 10 mL with *water R*.

Reference solution (c) Dissolve the contents of a vial of *pemetrexed impurity mixture CRS* (impurities A and D) in 1 mL of *water R*.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *octylsilyl silica gel for chromatography R* (3.5 µm).

Mobile phase:
— *mobile phase A*: *acetonitrile R*, solution A (5:95 *V/V*);
— *mobile phase B*: *acetonitrile R*, solution A (30:70 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	100	0
3 - 45	100 → 0	0 → 100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 250 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram supplied with *pemetrexed impurity mixture CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A and D; use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B and C.

Relative retention With reference to pemetrexed (retention time = about 26 min): impurity A = about 0.82; impurity B = about 0.87; impurity C = about 0.88; impurity D = about 0.90.

System suitability Reference solution (b):
— *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity B and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity C.

Calculation of percentage contents:
— *correction factor*: multiply the peak area of impurity D by 1.5;
— for each impurity, use the concentration of pemetrexed disodium 2.5-hydrate in reference solution (a).

Limits:
— *impurities A, D*: for each impurity, maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.6 per cent;
— *reporting threshold*: 0.05 per cent.

Water (*2.5.12*)

8.0 per cent to 11.0 per cent, determined on 0.050 g.

ASSAY

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use or store them at 2-8 °C and use them within 24 h.*

Acetate buffer Mix 1.7 mL of *glacial acetic acid R* and 900 mL of *water for chromatography R*, adjust to pH 5.3 with a 760 g/L solution of *sodium hydroxide R* in *water for chromatography R* and dilute to 1000 mL with *water for chromatography R*.

Test solution Dissolve 25.9 mg of the substance to be examined in *water R* and dilute to 200.0 mL with the same solvent.

Reference solution Dissolve 30.0 mg of *pemetrexed disodium heptahydrate CRS* in *water R* and dilute to 200.0 mL with the same solvent.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *octylsilyl silica gel for chromatography R* (3.5 µm);
— *temperature*: 30 °C.

Mobile phase *acetonitrile R*, acetate buffer (11:89 *V/V*).

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 285 nm.

Injection 20 µL.

Run time Twice the retention time of pemetrexed (retention time = about 3 min).

Calculate the percentage content of $C_{20}H_{19}N_5Na_2O_6$ taking into account the assigned content of *pemetrexed disodium heptahydrate CRS*.

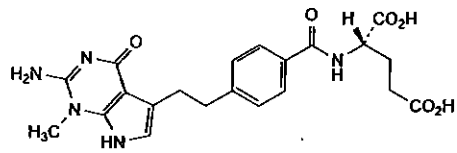
STORAGE

In an airtight container at a temperature of 2 °C to 8 °C.

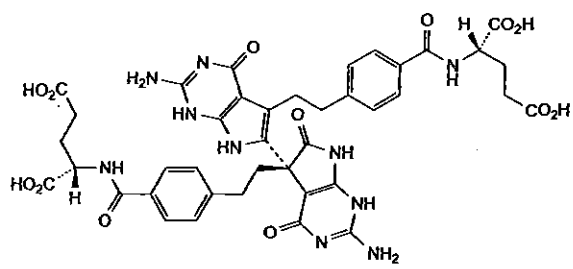
IMPURITIES

Specified impurities *A, D, E.*

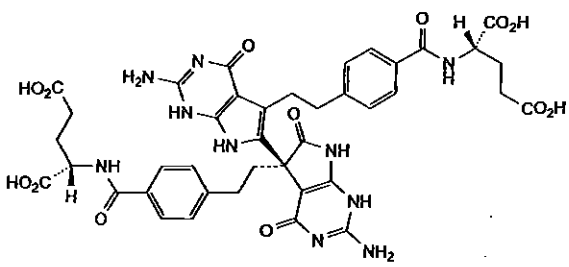
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C.*



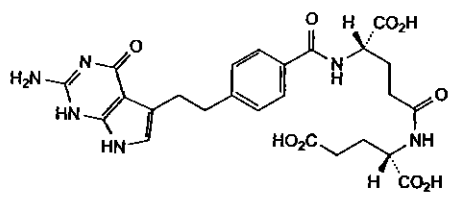
A. (2*S*)-2-[4-[2-(2-amino-1-methyl-4-oxo-4,7-dihydro-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzamido] pentanedioic acid,



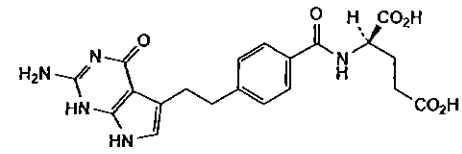
B. (2*S*,2′*S*)-2,2′-[(4^5*R*)-4^2,5^2-diamino-4^4,4^6,5^4-trioxo-4^1,4^4,4^6,4^7,5^4,5^7-hexahydro-4^5*H*,5^1*H*-4(5,5),5(6,5)-dipyrrolo[2,3-*d*]pyrimidina-1,8(1)-dibenzenaoctaphane-1^4,8^4-dicarboxamido]dipentanedioic acid,



C. (2*S*,2′*S*)-2,2′-[(4^5*S*)-4^2,5^2-diamino-4^4,4^6,5^4-trioxo-4^1,4^4,4^6,4^7,5^4,5^7-hexahydro-4^5*H*,5^1*H*-4(5,5),5(6,5)-dipyrrolo[2,3-*d*]pyrimidina-1,8(1)-dibenzenaoctaphane-1^4,8^4-dicarboxamido]dipentanedioic acid,



D. (2*S*)-2-[(4*S*)-4-[4-[2-(2-amino-4-oxo-4,7-dihydro-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzamido]-4-carboxybutanamido]pentanedioic acid,



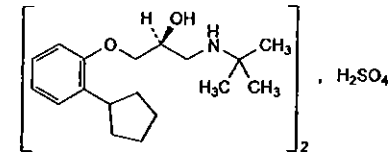
E. (2*R*)-2-[4-[2-(2-amino-4-oxo-4,7-dihydro-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzamido]pentanedioic acid.

Ph Eur

Penbutolol Sulfate

Penbutolol Sulphate

(Ph. Eur. monograph 1461)



$C_{36}H_{60}N_2O_8S$ 681 *38363-32-5*

Action and use
Beta-adrenoceptor antagonist.

Ph Eur

DEFINITION
Di[(2*S*)-1-(2-cyclopentylphenoxy)-3-[(1,1-dimethylethyl)amino]propan-2-ol] sulfate.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Slightly soluble in water, soluble in methanol, practically insoluble in cyclohexane.

IDENTIFICATION
First identification: A, C, D.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *penbutolol sulfate CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 40 mg of the substance to be examined in 1 mL of *methanol R.*

Reference solution Dissolve 40 mg of *penbutolol sulfate CRS* in 1 mL of *methanol R.*

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *glacial acetic acid R, water R, butanol R, ethyl acetate R* (10:20:35:35 *V/V/V/V*).

Application 5 µL.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. Dissolve 50 mg in a mixture of 5 mL of *water R* and 1 mL of *0.1 M hydrochloric acid*. The solution gives reaction (a) of sulfates (*2.3.1*).

D. Specific optical rotation (see Tests).

TESTS

Solution S

Dissolve 1.00 g in *methanol R* and dilute to 20.0 mL with the same solvent.

Acidity or alkalinity

To 4 mL of solution S add 4 mL of *carbon dioxide-free water R*. Add 0.1 mL of *methyl red solution R* and 0.2 mL of *0.01 M sodium hydroxide*; the solution is yellow. Add 0.4 mL of *0.01 M hydrochloric acid*; the solution is red.

Specific optical rotation (*2.2.7*)

−23 to −25 (dried substance), determined on solution S.

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture Mobile phase B, mobile phase A (40:60 *V/V*).

Test solution Dissolve 40.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (a) Dissolve 4.0 mg of the substance to be examined and 1.0 mg of *penbutolol impurity A CRS* in 5.0 mL of the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 200.0 mL with the solvent mixture.

Reference solution (c) Dilute 1.0 mL of reference solution (b) to 10.0 mL with the solvent mixture.

Reference solution (d) Dissolve 5.0 mg of *penbutolol impurity A CRS* in the solvent mixture and dilute to 50.0 mL with the solvent mixture. Dilute 2.0 mL of this solution to 10.0 mL with the solvent mixture.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: *acetonitrile for chromatography R, methanol R* (39:61 *V/V*);

— *mobile phase B*: dissolve 11 g of *sodium heptanesulfonate R* in 1000 mL of *water R*, add 5.0 mL of *triethylamine R* and adjust to pH 2.7 with *phosphoric acid R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	60	40
15 - 35	60 → 80	40 → 20

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 270 nm.

Injection 10 µL.

System suitability Reference solution (a):

— *resolution*: minimum 3.0 between the 2 principal peaks.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.5 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *sum of impurities other than A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.500 g in 40 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

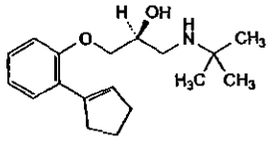
1 mL of *0.1 M perchloric acid* is equivalent to 68.10 mg of $C_{36}H_{60}N_2O_8S$.

STORAGE

Protected from light.

IMPURITIES

Specified impurities *A*.

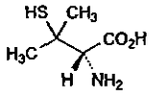


A. (2*S*)-1-[2-(cyclopent-1-enyl)phenoxy]-3-[(1,1-dimethylethyl)amino]propan-2-ol.

Ph Eur

Penicillamine

(Ph. Eur. monograph 0566)



$C_5H_{11}NO_2S$ 149.2 *52-67-5*

Action and use

Disease-modifying antirheumatic drug; chelating agent; treatment of Wilson's disease; heavy metal poisoning; cystinuria.

Preparation

Penicillamine Tablets

Ph Eur

DEFINITION

(2*S*)-2-Amino-3-methyl-3-sulfanylbutanoic acid.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: A, B, D.

Second identification: A, C, D.

A. Dissolve 0.5 g in a mixture of 0.5 mL of *hydrochloric acid R* and 4 mL of warm *acetone R*, cool in iced water and initiate crystallisation by scratching the wall of the tube with a glass rod. A white precipitate is formed. Filter with the aid of vacuum, wash with *acetone R* and dry with suction. A 10 g/L solution of the precipitate is dextrorotatory.

B. Examine the chromatograms obtained in the test for impurity A.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in 4 mL of *water R*.

Reference solution Dissolve 10 mg of *penicillamine CRS* in 4 mL of *water R*.

Plate *TLC silica gel G plate R.*

Mobile phase *glacial acetic acid R, water R, butanol R* (18:18:72 *V/V/V*).

Application 2 µL.

Development Over a path of 10 cm.

Drying At 100-105 °C for 5-10 min.

Detection Expose to iodine vapour for 5-10 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve 40 mg in 4 mL of *water R* and add 2 mL of *phosphotungstic acid solution R*. Allow to stand for 5 min. A blue colour develops.

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than intensity 6 of the range of reference solutions of the most appropriate colour (*2.2.2, Method II*).

pH (*2.2.3*)

4.5 to 5.5.

Dilute 1 mL of solution S to 10 mL with *carbon dioxide-free water R*.

Specific optical rotation (*2.2.7*)

−65.0 to −61.0 (dried substance).

Dissolve 0.500 g in a 40 g/L solution of *sodium hydroxide R* and dilute to 10.0 mL with the same solution.

Ultraviolet-absorbing substances

Maximum 0.5 per cent of penilloic acid.

Dissolve 0.100 g in *water R* and dilute to 50.0 mL with the same solvent. The absorbance (*2.2.25*) of the solution at 268 nm is not greater than 0.07.

Impurity A

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 40.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dissolve 40 mg of *penicillamine CRS* in the mobile phase and dilute to 10 mL with the mobile phase.

Reference solution (b) Dissolve 20.0 mg of *penicillamine disulfide CRS* (impurity A) in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 2.0 mL of the solution to 20.0 mL with the mobile phase.

Reference solution (c) Dissolve 40 mg of the substance to be examined in reference solution (b) and dilute to 10 mL with the same solution.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: octylsilyl silica gel for chromatography R* (10 µm).

Mobile phase Solution containing 0.1 g/L of *sodium edetate R* and 2 g/L of *methanesulfonic acid R*.

Flow rate 1.7 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µL.

Run time Twice the retention time of penicillamine.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity A.

Relative retention With reference to penicillamine (retention time = about 6 min): impurity A = about 1.8.

System suitability Reference solution (c):

— *resolution*: minimum 4.0 between the peaks due to penicillamine and impurity A.

Limit:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (1 per cent).

Impurity B

Maximum 0.1 ppm.

Carry out all the operations in a penicillin-free atmosphere and with equipment reserved for this test. Sterilise the equipment at 180 °C for 3 h and the buffer solutions at 121 °C for 20 min before use.

Test solution (a) Dissolve 1.000 g in 8 mL of *buffer solution pH 2.5 R* and add 8 mL of *ether R*. Shake vigorously for 1 min. Repeat the extraction and combine the ether layers. Add 8 mL of *buffer solution pH 2.5 R*. Shake for 1 min, allow to settle and quantitatively separate the upper layer, taking care to eliminate the aqueous phase completely (*penicillin is unstable at pH 2.5; carry out operations at this pH within 6-7 min*). Add 8 mL of *phosphate buffer solution pH 6.0 R2* to the ether phase, shake for 5 min, allow to settle, then separate the aqueous layer and check that the pH is 6.0.

Test solution (b) To 2 mL of test solution (a) add 20 µL of *penicillinase solution R* and incubate at 37 °C for 1 h.

Reference solution (a) Dissolve 5 mg of *benzylpenicillin sodium R* in 500 mL of *phosphate buffer solution pH 6.0 R2*. Dilute 0.25 mL of the solution to 200.0 mL with *buffer solution pH 2.5 R*. Carry out the extraction using 8 mL of this solution as described for test solution (a).

Reference solution (b) To 2 mL of reference solution (a) add 20 µL of *penicillinase solution R* and incubate at 37 °C for 1 h.

Blank solution Prepare the solution as described for test solution (a) but omitting the substance to be examined.

Liquefy a suitable nutrient medium such as that described below and inoculate it at a suitable temperature with a culture of *Kocuria rhizophila* (ATCC 9341), to give 5×10^4 micro-organisms per millilitre or a different quantity

if necessary to obtain the required sensitivity and formation of clearly defined inhibition zones of suitable diameter. Immediately pour the inoculated medium into 5 Petri dishes 10 cm in diameter to give uniform layers 2-5 mm deep. The medium may alternatively consist of 2 layers, only the upper layer being inoculated. Store the dishes so that no appreciable growth or death of the micro-organisms occurs before use and so that the surface of the agar is dry at the time of use. In each dish, place 5 stainless steel hollow cylinders 6 mm in diameter on the surface of the agar evenly spaced on a circle with a radius of about 25 mm and concentric with the dish. For each dish, place in separate cylinders 0.15 mL of test solutions (a) and (b), reference solutions (a) and (b) and the blank solution. Maintain at 30 °C for at least 24 h. Measure the diameters of the inhibition zones to the nearest 0.1 mm. The test is valid if reference solution (a) gives a clear inhibition zone and if reference solution (b) and the blank solution give no inhibition zone. If test solution (a) gives an inhibition zone, this is caused by penicillin if test solution (b) gives no inhibition zone. If this is so, the average diameter of the inhibition zones given by test solution (a) for the 5 Petri dishes is less than the average diameter of the inhibition zones given by reference solution (a) measured in the same conditions.

Nutrient medium (pH 6.0)

Peptone	5 g
Yeast extract	1.5 g
Meat extract	1.5 g
Sodium chloride	3.5 g
Agar	15 g
Distilled water R	1000 mL

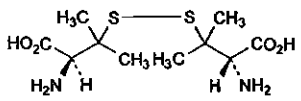
Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

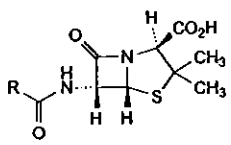
ASSAY
Dissolve 0.1000 g in 30 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 14.92 mg of $C_5H_{11}NO_2S$.

IMPURITIES
Specified impurities *A, B.*



A. 3,3′-(disulfanediyl)bis[(2*S*)-2-amino-3-methylbutanoic] acid (penicillamine disulfide),

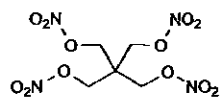


B. penicillin.

Ph Eur

Diluted Pentaerythrityl Tetranitrate

(Ph. Eur. monograph 1335)



$C_5H_8N_4O_{12}$ 316.1

Action and use
Vasodilator.

Ph Eur

DEFINITION
Dry mixture of 2,2-bis(hydroxymethyl)propane-1,3-diol tetranitrate (pentaerythrityl tetranitrate) and *Lactose monohydrate (0187)* or *Mannitol (0559)*.

Content
95.0 per cent *m/m* to 105.0 per cent *m/m* of the declared content of pentaerythrityl tetranitrate.

CAUTION: undiluted pentaerythrityl tetranitrate may explode if subjected to percussion or excessive heat. Appropriate precautions must be taken and only very small quantities handled.

CHARACTERS
Appearance of pentaerythrityl tetranitrate White or slightly yellowish powder.

Solubility of pentaerythrityl tetranitrate Practically insoluble in water, soluble in acetone, slightly soluble in ethanol (96 per cent).

The solubility of diluted pentaerythrityl tetranitrate depends on the diluent and its concentration.

IDENTIFICATION
First identification: A, C.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Separately shake a quantity of the substance to be examined and a quantity of the reference substance, each corresponding to 25 mg of pentaerythrityl tetranitrate, with 10 mL of *acetone R* for 5 min; filter, evaporate to dryness at a temperature below 40 °C, and dry the residue over *diphosphorus pentoxide R* at a pressure of 0.7 kPa for 16 h. Examine the residues prepared as discs.

Comparison *diluted pentaerythrityl tetranitrate CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Shake a quantity of the substance to be examined corresponding to 10 mg of pentaerythrityl tetranitrate with 10 mL of *ethanol (96 per cent) R* for 5 min and filter.

Reference solution Shake a quantity of *diluted pentaerythrityl tetranitrate CRS* corresponding to 10 mg of pentaerythrityl tetranitrate with 10 mL of *ethanol (96 per cent) R* for 5 min and filter.

Plate *TLC silica gel plate R.*

Mobile phase *ethyl acetate R, toluene R* (20:80 *V/V*).

Application 10 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with freshly prepared *potassium iodide and starch solution R*, expose to ultraviolet light at 254 nm for 15 min and examine in daylight.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. Thin-layer chromatography (*2.2.27*).

Test solution Shake a quantity of the substance to be examined corresponding to 0.10 g of lactose or mannitol with 10 mL of *water R*. Filter if necessary.

Reference solution (a) Dissolve 0.10 g of *lactose monohydrate R* in *water R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 0.10 g of *mannitol R* in *water R* and dilute to 10 mL with the same solvent.

Reference solution (c) Mix equal volumes of reference solutions (a) and (b).

Plate *TLC silica gel G plate R.*

Mobile phase *water R, methanol R, anhydrous acetic acid R, ethylene chloride R* (10:15:25:50 *V/V/V/V*). Measure the volumes accurately since a slight excess of water produces cloudiness.

Application 1 µL; thoroughly dry the points of application.

Development A Over 2/3 of the plate.

Drying A In a current of warm air.

Development B Immediately, over 2/3 of the plate, after renewing the mobile phase.

Drying B In a current of warm air.

Detection Spray with *4-aminobenzoic acid solution R*, dry in a current of cold air until the acetone is removed, then heat at 100 °C for 15 min; allow to cool, spray with a 2 g/L solution of *sodium periodate R*, dry in a current of cold air and heat at 100 °C for 15 min.

System suitability Reference solution (c):
— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a) for lactose or to the principal spot in the chromatogram obtained with reference solution (b) for mannitol.

TESTS

Impurity A

Thin-layer chromatography (*2.2.27*).

Test solution Shake a quantity of the substance to be examined corresponding to 0.10 g of pentaerythrityl tetranitrate with 5 mL of *ethanol (96 per cent) R* and filter.

Reference solution Dissolve 10 mg of *potassium nitrate R* in 1 mL of *water R* and dilute to 100 mL with *ethanol (96 per cent) R*.

Plate *TLC silica gel plate R.*

Mobile phase *glacial acetic acid R, acetone R, toluene R* (15:30:60 *V/V/V*).

Application 10 µL.

Development Over 2/3 of the plate.

Drying In a current of air until the acetic acid is completely removed.

Detection Spray copiously with freshly prepared *potassium iodide and starch solution R*, expose the plate to ultraviolet light at 254 nm for 15 min and examine in daylight.

Limit:
— *nitrate*: any spot due to nitrate is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent, calculated as potassium nitrate).

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Sonicate for 15 min a quantity of the substance to be examined corresponding to 25.0 mg of pentaerythrityl tetranitrate in 20 mL of the mobile phase and dilute to 25.0 mL with the mobile phase. Filter.

Test solution (b) Dilute 1.0 mL of test solution (a) to 10.0 mL with the mobile phase.

Reference solution (a) Sonicate for 15 min a quantity of *diluted pentaerythrityl tetranitrate CRS* corresponding to 25.0 mg of pentaerythrityl tetranitrate in 20 mL of the mobile phase and dilute to 25.0 mL with the mobile phase. Filter.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 10.0 mL with the mobile phase.

Reference solution (c) Dilute 0.3 mL of reference solution (b) to 10.0 mL with the mobile phase.

Reference solution (d) Dilute 200 µL of *glyceryl trinitrate solution CRS* to 25.0 mL with the mobile phase.

Reference solution (e) To 1 mL of reference solution (b) add 1 mL of reference solution (d) and dilute to 10 mL with the mobile phase.

Reference solution (f) Dilute 1.0 mL of reference solution (a) to 20.0 mL with the mobile phase. Dilute 0.5 mL of this solution to 50.0 mL with the mobile phase.

Column:
— *size*: l = 0.15 m, Ø = 3.9 mm;
— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *water R, acetonitrile R* (35:65 *V/V*).

Flow rate 1.4 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µL of test solution (a) and reference solutions (c), (e) and (f).

Run time 5 times the retention time of pentaerythrityl tetranitrate.

Relative retention With reference to pentaerythrityl tetranitrate (retention time = about 2.4 min): impurity B = about 0.7; impurity C = about 3.0.

System suitability Reference solution (e):
— *resolution*: minimum 3.0 between the peaks due to glyceryl trinitrate and pentaerythrityl tetranitrate.

Limits:
— *impurities C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent);
— *unspecified impurities*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (f) (0.10 per cent);
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.6 per cent);
— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (f) (0.05 per cent).

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (b).

Calculate the percentage content of $C_5H_8N_4O_{12}$ from the declared content of *diluted pentaerythrityl tetranitrate CRS*.

STORAGE

Protected from light and heat.

LABELLING

The label states:

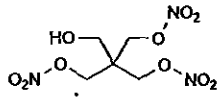
— the percentage content of pentaerythrityl tetranitrate;
— the diluent used.

IMPURITIES

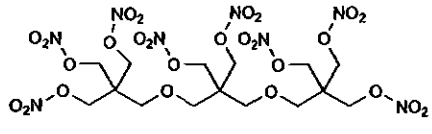
Specified impurities *A, C, D.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B.*

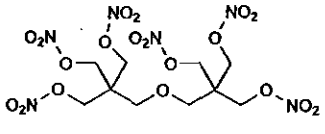
A. NO_3^-: inorganic nitrates,



B. 2,2-bis(hydroxymethyl)propane-1,3-diol trinitrate (pentaerythritol trinitrate),



C. 2,2-bis[[3-hydroxy-2,2-bis(hydroxymethyl) propoxy]methyl]propane-1,3-diol octanitrate (tripentaerythrityl octanitrate),

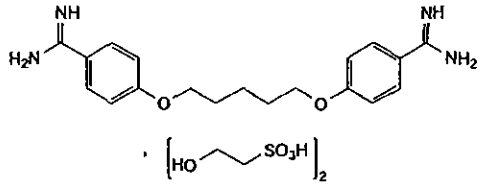


D. 2,2′-(oxybismethylene)bis[2-(hydroxymethyl)propane-1,3-diol] hexanitrate (dipentaerythrityl hexanitrate).

Ph Eur

Pentamidine Isetionate

(Pentamidine Diisetionate, Ph. Eur. monograph 1137)



$C_{23}H_{36}N_4O_{10}S_2$ 592.7 *140-64-7*

Action and use

Antiprotozoal.

Preparation

Pentamidine Injection

Ph Eur

DEFINITION

4,4′-[Pentane-1,5-diylbis(oxy)]dibenzimidamide bis(2-hydroxyethanesulfonate).

Content

98.5 per cent to 101.5 per cent (dried substance).

PRODUCTION

It is considered that alkyl 2-hydroxyethanesulfonate esters are potential impurities in pentamidine diisetionate.

The manufacturing process should be developed taking into consideration the principles of quality risk management, together with considerations of the quality of starting materials, process capability and validation including, where necessary, demonstration that alkyl 2-hydroxyethanesulfonate esters are not detectable in the final product.

CHARACTERS

Appearance

White or almost white powder or colourless crystals, hygroscopic.

Solubility

Freely soluble in water, sparingly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *pentamidine diisetionate CRS.*

B. Dissolve about 40 mg in 5 mL of *water R* and add dropwise with shaking 1 mL of a 10 g/L solution of *sodium chloride R*. Allow to stand for 5 min. The mixture remains clear.

C. Treat 0.15 g by the oxygen-flask method (*2.5.10*). Use 10 mL of *dilute hydrogen peroxide solution R* to absorb the combustion products. The solution gives reaction (a) of sulfates (*2.3.1*).

TESTS

Appearance of solution

The solution is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than intensity 6 of the range of reference solutions of the most appropriate colour (*2.2.2, Method II*).

Dissolve 2.0 g in *water R* and dilute to 20 mL with the same solvent.

pH (*2.2.3*)

4.5 to 6.5.

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dilute 2.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) To 0.1 g in a conical flask, add 40 mL of *water R* and glass beads. Adjust to pH 10.5 with *dilute sodium hydroxide solution R* and boil under reflux for 20 min. Cool and dilute to 50 mL with *water R*. Dilute 1 mL of the solution to 50 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 65 volumes of *methanol R* and 35 volumes of a 30 g/L solution of *ammonium acetate R* previously adjusted to pH 7.5 with *triethylamine R*.

Flow rate 1 mL/min.

Detection Spectrophotometer at 265 nm.

Injection 10 µL.

Run time 3.5 times the retention time of pentamidine.

System suitability Reference solution (b):
— the chromatogram obtained shows 2 principal peaks;
— *resolution*: minimum 2.0 between the 2 principal peaks.

Limits:
— *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);
— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Loss on drying (*2.2.32*)
Maximum 4.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

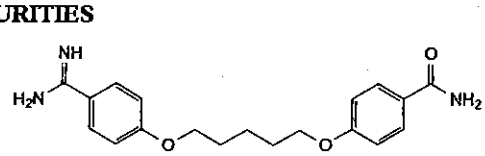
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.250 g in 50 mL of *dimethylformamide R*. Add 0.25 mL of *thymol blue solution R*. Titrate with 0.1 M tetrabutylammonium hydroxide, under a current of *nitrogen R*, until the colour of the indicator changes to blue. Carry out a blank titration.

1 mL of 0.1 M tetrabutylammonium hydroxide is equivalent to 29.63 mg of $C_{23}H_{36}N_4O_{10}S_2$.

STORAGE
In an airtight container.

IMPURITIES

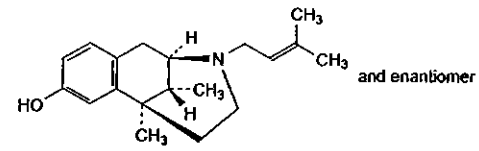


A. 4-[[5-(4-amidinophenoxy)pentyl]oxy]benzenecarboxamide.

Ph Eur

Pentazocine

(Ph. Eur. monograph 1462)



and enantiomer

$C_{19}H_{27}NO$ 285.4 *359-83-1*

Action and use
Opioid receptor agonist; analgesic.

Preparation
Pentazocine Injection

Ph Eur

DEFINITION
Pentazocine contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (2*RS*,6*RS*,11*RS*)-6,11-dimethyl-3-(3-methylbut-2-enyl)-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocin-8-ol, calculated with reference to the dried substance.

CHARACTERS
A white or almost white powder, practically insoluble in water, freely soluble in methylene chloride and soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION
Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the *Ph. Eur. reference spectrum for pentazocine (form A)*.

TESTS

Absorbance (*2.2.25*)
Dissolve 0.100 g in a mixture of 20 mL of *water R* and 1 mL of *1 M hydrochloric acid*, and dilute to 100.0 mL with *water R*. To 10.0 mL add 1 mL of *1 M hydrochloric acid* and dilute to 100.0 mL with *water R*. The absorbance at the absorption maximum at 278 nm is 0.67 to 0.71, calculated with reference to the dried substance.

Related substances
Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel F_{254} plate R*.

Test solution Dissolve 0.20 g of the substance to be examined in *methylene chloride R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dilute 1 mL of the test solution to 100 mL with *methylene chloride R*.

Reference solution (b) Dilute 5 mL of reference solution (a) to 10 mL with *methylene chloride R*.

Reference solution (c) Dilute 5 mL of reference solution (a) to 20 mL with *methylene chloride R*.

Apply to the plate 10 µL of each solution. Develop over a path corresponding to two thirds of the plate height using a mixture of 3 volumes of *isopropylamine R*, 3 volumes of *methanol R* and 94 volumes of *methylene chloride R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Heat the plate at 100-105 °C for 15 min, allow to cool, expose to iodine vapour and re-examine under ultraviolet light at 254 nm. By each method of visualisation: any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot obtained

with reference solution (a) (1 per cent); not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent) and not more than 4 such spots are more intense than the spot in the chromatogram obtained with reference solution (c) (0.25 per cent).

Loss on drying (*2.2.32*)
Not more than 0.5 per cent, determined on 1.000 g by drying at 60 °C at a pressure not exceeding 0.7 kPa for 4 h.

Sulfated ash (*2.4.14*)
Not more than 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.200 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

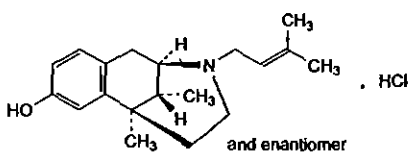
1 mL of *0.1 M perchloric acid* is equivalent to 28.54 mg of $C_{19}H_{27}NO$.

STORAGE
Store protected from light.

Ph Eur

Pentazocine Hydrochloride

(Ph. Eur. monograph 1463)



$C_{19}H_{28}ClNO$ 321.9 *64024-15-3*

Action and use
Opioid receptor agonist; analgesic.

Preparations
Pentazocine Capsules
Pentazocine Tablets

Ph Eur

DEFINITION
Pentazocine hydrochloride contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (2*RS*,6*RS*,11*RS*)-6,11-dimethyl-3-(3-methylbut-2-enyl)-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocin-8-ol hydrochloride, calculated with reference to the dried substance.

CHARACTERS
A white or almost white powder, sparingly soluble in water, soluble in ethanol (96 per cent) and sparingly soluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION
A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the *Ph. Eur. reference spectrum of pentazocine hydrochloride*.

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

pH (*2.2.3*)
Dissolve 0.1 g in 10 mL of *carbon dioxide-free water R*. The pH of the solution is 4.0 to 6.0.

Absorbance (*2.2.25*)
Dissolve 0.100 g in a mixture of 20 mL of *water R* and 1 mL of *1 M hydrochloric acid*, and dilute to 100.0 mL with *water R*. To 10.0 mL add 1 mL of *1 M hydrochloric acid* and dilute to 100.0 mL with *water R*. The absorbance at the absorption maximum at 278 nm is 0.59 to 0.63, calculated with reference to the dried substance.

Related substances
Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel F_{254} plate R*.

Test solution Dissolve 0.20 g in 3 mL of *methanol R* and dilute to 10 mL with *methylene chloride R*.

Reference solution (a) Dilute 1 mL of the test solution to 100 mL with *methylene chloride R*.

Reference solution (b) Dilute 5 mL of reference solution (a) to 10 mL with *methylene chloride R*.

Reference solution (c) Dilute 5 mL of reference solution (a) to 20 mL with *methylene chloride R*.

Apply to the plate 10 µL of each solution. Develop over a path corresponding to two-thirds of the plate height using a mixture of 3 volumes of *isopropylamine R*, 3 volumes of *methanol R* and 94 volumes of *methylene chloride R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Heat the plate at 100-105 °C for 15 min, allow to cool, expose to iodine vapour and re-examine under ultraviolet light at 254 nm. By each method of visualisation: any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot obtained with reference solution (a) (1 per cent); not more than 1 such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent); and not more than 4 such spots are more intense than the spot in the chromatogram obtained with reference solution (c) (0.25 per cent).

Loss on drying (*2.2.32*)
Not more than 0.5 per cent, determined on 1.000 g by drying at 60 °C at a pressure not exceeding 0.7 kPa for 4 h.

Sulfated ash (*2.4.14*)
Not more than 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.250 g in 50 mL of *ethanol (96 per cent) R*. Add 5 mL of *0.01 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflection.

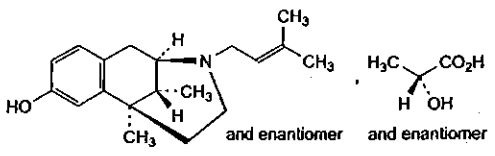
1 mL of *0.1 M sodium hydroxide* is equivalent to 32.19 mg of $C_{19}H_{28}ClNO$.

STORAGE
Store protected from light.

Ph Eur

Pentazocine Lactate

(Ph. Eur. monograph 2000)



$C_{22}H_{33}NO_4$ 375.5 *17146-95-1*

Action and use
Opioid receptor agonist; analgesic.

Preparation
Pentazocine Injection

Ph Eur

DEFINITION
(2*RS*,6*RS*,11*RS*)-6,11-Dimethyl-3-(3-methylbut-2-enyl)-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocin-8-ol (2*RS*)-2-hydroxypropanoate.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white powder.

Solubility
Sparingly soluble in water, freely soluble in methanol, slightly soluble in methylene chloride.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *Ph. Eur. reference spectrum of pentazocine lactate.*

TESTS
pH (*2.2.3*)
5.5 to 6.5.
Dissolve 0.1 g in 10 mL of *carbon dioxide-free water R.*

Absorbance (*2.2.25*)
0.50 to 0.54, determined at the absorption maximum at 278 nm.
Dissolve 0.10 g in 10.0 mL of *1 M hydrochloric acid* and dilute to 100.0 mL with *water R.* Dilute 10.0 mL of the solution to 100.0 mL with *water R.*

Related substances
Thin-layer chromatography (*2.2.27*).
Test solution Dissolve 0.20 g of the substance to be examined in *methylene chloride R* and dilute to 10 mL with the same solvent.
Reference solution (a) Dissolve 100 mg of the substance to be examined in *acetic anhydride R* and dilute to 5 mL with the same solvent. Heat at 80 °C for 10 min. Dilute 1 mL of the solution to 10 mL with *methanol R.* Mix 1 mL of this solution with 1 mL of the test solution.
Reference solution (b) Dilute 1 mL of the test solution to 100 mL with *methylene chloride R.* Dilute 2 mL of this solution to 10 mL with *methylene chloride R.*
Plate *TLC silica gel F_{254} plate R.*
Mobile phase *isopropylamine R, methanol R, methylene chloride R* (3:3:94 *V/V/V*).
Application 10 µL.
Development Over 2/3 of the plate.
Drying In air.
Detection Examine in ultraviolet light at 254 nm. Heat at 100-105 °C for 15 min. Allow to cool. Expose to iodine vapour and re-examine in ultraviolet light at 254 nm.
System suitability Reference solution (a):
— the chromatogram shows 2 clearly separated spots.
Limits By each method of detection:
— *any impurity*: any spots, apart from the principal spot, are not more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

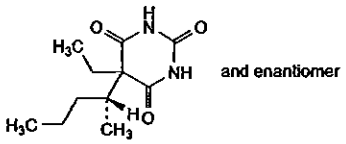
ASSAY
Dissolve 0.300 g in 30 mL of *anhydrous acetic acid R* and add 30 mL of *dioxan R.* Titrate with *0.1 M perchloric acid,* determining the end-point potentiometrically (*2.2.20*).
1 mL of *0.1 M perchloric acid* is equivalent to 37.55 mg of $C_{22}H_{33}NO_4$.

STORAGE
Protected from light.

Ph Eur

Pentobarbital

(Ph. Eur. monograph 0200)



$C_{11}H_{18}N_2O_3$ 226.3 *76-74-4*

Action and use
Barbiturate.

Ph Eur

DEFINITION
5-Ethyl-5-[(2*RS*)-pentan-2-yl]-1,3-diazinane-2,4,6-trione.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder or colourless crystals.

Solubility
Very slightly soluble in water, freely soluble in anhydrous ethanol, practically insoluble in heptane. It forms water-soluble compounds with alkali hydroxides and carbonates and with ammonia.
It shows polymorphism (*5.9*).

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *pentobarbital CRS.*
If the spectra obtained in the solid state show differences, weigh 10 mg of the substance to be examined and 10 mg of

the reference substance separately in glass vials; heat in an oven at 140 °C for 1 h, then allow to stand at room temperature for 3 h. Record new spectra.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution B_9 (*2.2.2, Method II*).

Dissolve 1.0 g in a mixture of 4 mL of *dilute sodium hydroxide solution R* and 6 mL of *water R*.

Acidity

Boil 1.0 g with 50 mL of *water R* for 2 min, allow to cool and filter. To 10 mL of the filtrate add 0.15 mL of *methyl red solution R*. The solution is orange-yellow. Not more than 0.1 mL of *0.1 M sodium hydroxide* is required to produce a pure yellow colour.

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 mL with the mobile phase.

Test solution (b) Dilute 5.0 mL of test solution (a) to 50.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of test solution (b) to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve 2.5 mg of *pentobarbital impurity E CRS* in the mobile phase and dilute to 25 mL with the mobile phase. Dilute 1 mL of the solution to 100 mL with the mobile phase. Dissolve 5 mg of the substance to be examined in 5 mL of this solution.

Reference solution (c) Dissolve 25.0 mg of *pentobarbital CRS* in the mobile phase and dilute to 25.0 mL with the mobile phase. Dilute 5.0 mL of the solution to 50.0 mL with the mobile phase.

Column:
- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: end-capped extra-dense bonded octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 35 volumes of *acetonitrile R1* and 65 volumes of a 1.36 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.5 with *dilute phosphoric acid R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 214 nm.

Injection 10 µL of test solution (a) and reference solutions (a) and (b).

Run time 2.5 times the retention time of pentobarbital.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity E.

Relative retention With reference to pentobarbital (retention time = about 10 min): impurity E = about 0.92.

System suitability Reference solution (b):
- *resolution*: minimum 2.0 between the peaks due to impurity E and pentobarbital.

Calculation of percentage contents:
- for each impurity, use the concentration of pentobarbital in reference solution (a).

Limits:
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.1 per cent;
- *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

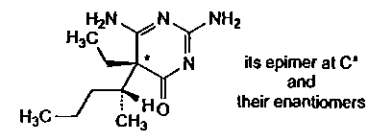
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (c).

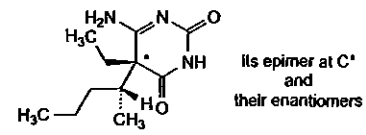
Calculate the percentage content of $C_{11}H_{18}N_2O_3$ taking into account the assigned content of *pentobarbital CRS*.

IMPURITIES

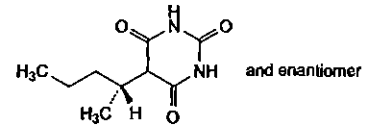
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, E, F.*



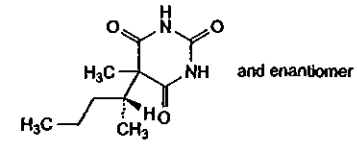
A. mixture of (5*R*)-2,6-diamino-5-ethyl-5-[(2*RS*)-pentan-2-yl]pyrimidin-4(5*H*)-one and (5*S*)-2,6-diamino-5-ethyl-5-[(2*RS*)-pentan-2-yl]pyrimidin-4(5*H*)-one,



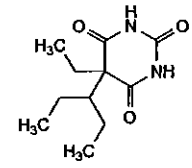
B. mixture of (5*R*)-6-amino-5-ethyl-5-[(2*RS*)-pentan-2-yl] pyrimidine-2,4(3*H*,5*H*)-dione and (5*S*)-6-amino-5-ethyl-5-[(2*RS*)-pentan-2-yl]pyrimidine-2,4(3*H*,5*H*)-dione,



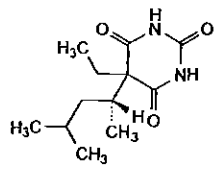
C. 5-[(2*RS*)-pentan-2-yl]-1,3-diazinane-2,4,6-trione,



D. 5-methyl-5-[(2*RS*)-pentan-2-yl]-1,3-diazinane-2,4,6-trione,



E. 5-ethyl-5-(pentan-3-yl)-1,3-diazinane-2,4,6-trione,

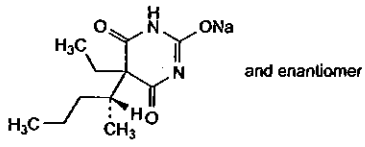


F. 5-ethyl-5-[(2*RS*)-4-methylpentan-2-yl]-1,3-diazinane-2,4,6-trione.

Ph Eur

Pentobarbital Sodium

(Ph. Eur. monograph 0419)



$C_{11}H_{17}N_2NaO_3$ 248.3 *57-33-0*

Action and use
Barbiturate.

Preparation
Pentobarbital Tablets

Ph Eur

DEFINITION
Sodium 5-ethyl-4,6-dioxo-5-[(2*RS*)-pentan-2-yl]-1,4,5,6-tetrahydropyrimidin-2-olate.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, hygroscopic, crystalline powder.

Solubility
Very soluble in water, freely soluble in ethanol (96 per cent), practically insoluble in heptane.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *pentobarbital sodium CRS.*

If the spectra obtained in the solid state show differences, dissolve 10 mg of the substance to be examined and 10 mg of the reference substance separately in 0.2 mL of *methanol R*. Evaporate the solvent by heating in an oven at 80 °C for 2 h. Record new spectra using the residues.

B. Ignite 1 g. The residue gives reaction (a) of sodium (*2.3.1*).

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution B_9 (*2.2.2, Method II*).

Dissolve 1.0 g in 10 mL of *ethanol (50 per cent V/V) R*. Examine immediately after preparation.

pH (*2.2.3*)
9.6 to 11.0, measured immediately after preparation.

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 27.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 mL with the mobile phase.

Test solution (b) Dilute 5.0 mL of test solution (a) to 50.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of test solution (b) to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve 2.5 mg of *pentobarbital impurity E CRS* in the mobile phase and dilute to 25 mL with the mobile phase. Dilute 1 mL of the solution to 100 mL with the mobile phase. Dissolve 5 mg of the substance to be examined in 5 mL of this solution.

Reference solution (c) Dissolve 25.0 mg of *pentobarbital CRS* in the mobile phase and dilute to 25.0 mL with the mobile phase. Dilute 5.0 mL of the solution to 50.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped extra-dense bonded octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 35 volumes of *acetonitrile R1* and 65 volumes of a 1.36 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.5 with *dilute phosphoric acid R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 214 nm.

Injection 10 µL of test solution (a) and reference solutions (a) and (b).

Run time 2.5 times the retention time of pentobarbital.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity E.

Relative retention With reference to pentobarbital (retention time = about 10 min): impurity E = about 0.92.

System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the peaks due to impurity E and pentobarbital.

Calculation of percentage contents:
— for each impurity, use the concentration of pentobarbital sodium in reference solution (a).

Limits:
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.1 per cent;
— *reporting threshold*: 0.05 per cent.

Free pentobarbital
Maximum 3.5 per cent.

Dissolve 2.00 g in 75 mL of *dimethylformamide R*, heating gently if necessary. Titrate with *0.1 M sodium methoxide* until the colour changes from olive-green to blue, using 0.25 mL of a 10 g/L solution of *thymol blue R* in *dimethylformamide R* as indicator. Carry out a blank titration.

1 mL of *0.1 M sodium methoxide* is equivalent to 22.63 mg of pentobarbital.

Loss on drying (*2.2.32*)
Maximum 3.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (c).

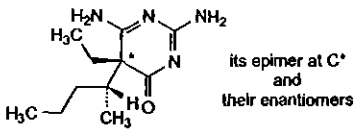
Calculate the percentage content of $C_{11}H_{17}N_2NaO_3$ taking into account the assigned content of *pentobarbital CRS* and a conversion factor of 1.097.

STORAGE

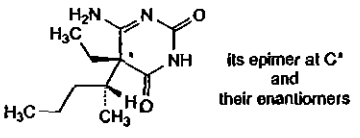
In an airtight container.

IMPURITIES

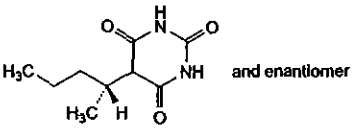
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, E, F.*



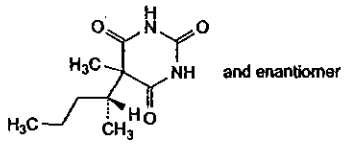
A. mixture of (5*R*)-2,6-diamino-5-ethyl-5-[(2*RS*)-pentan-2-yl]pyrimidin-4(5*H*)-one and (5*S*)-2,6-diamino-5-ethyl-5-[(2*RS*)-pentan-2-yl]pyrimidin-4(5*H*)-one,



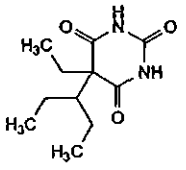
B. mixture of (5*R*)-6-amino-5-ethyl-5-[(2*RS*)-pentan-2-yl] pyrimidine-2,4(3*H*,5*H*)-dione and (5*S*)-6-amino-5-ethyl-5-[(2*RS*)-pentan-2-yl]pyrimidine-2,4(3*H*,5*H*)-dione,



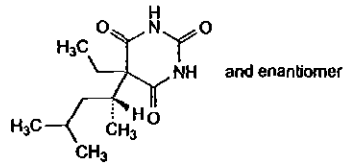
C. 5-[(2*RS*)-pentan-2-yl]-1,3-diazinane-2,4,6-trione,



D. 5-methyl-5-[(2*RS*)-pentan-2-yl]-1,3-diazinane-2,4,6-trione,



E. 5-ethyl-5-(pentan-3-yl)-1,3-diazinane-2,4,6-trione,

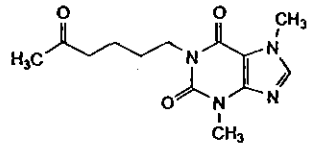


F. 5-ethyl-5-[(2*RS*)-4-methylpentan-2-yl]-1,3-diazinane-2,4,6-trione.

Ph Eur

Pentoxifylline

(Ph. Eur. monograph 0851)



$C_{13}H_{18}N_4O_3$ 278.3 *6493-05-6*

Action and use

Vasodilator.

Ph Eur

DEFINITION

3,7-Dimethyl-1-(5-oxohexyl)-3,7-dihydro-1*H*-purine-2,6-dione.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Soluble in water, freely soluble in methylene chloride, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: A, B.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 103 °C to 107 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *pentoxifylline CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 20 mg of *pentoxifylline CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *methanol R, ethyl acetate R* (15:85 *V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. It gives the reaction of xanthines (*2.3.1*).

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50 mL with the same solvent.

Appearance of solution

A 40 per cent *V/V* solution of solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

Acidity

To 8 mL of solution S add 12 mL of *carbon dioxide-free water R* and 0.05 mL of *bromothymol blue solution R1*. The solution is green or yellow. Not more than 0.2 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to blue.

Related substances

Liquid chromatography (*2.2.29*).

Solution A 5.44 g/L solution of *potassium dihydrogen phosphate R*.

Solvent mixture *methanol R*, solution A (50:50 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

Reference solution (a) Dilute 2.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 5.0 mL of this solution to 100.0 mL with the solvent mixture.

Reference solution (b) Dilute 10.0 mL of reference solution (a) to 50.0 mL with the solvent mixture.

Reference solution (c) Dissolve 2 mg of *caffeine R* (impurity F) and 2 mg of *theophylline R* (impurity C) in the solvent mixture, add 1 mL of the test solution and dilute to 10 mL with the solvent mixture.

Reference solution (d) Dissolve 5.0 mg of *caffeine R* (impurity F), 5.0 mg of *theobromine R* (impurity A) and 5.0 mg of *theophylline R* (impurity C) in the solvent mixture and dilute to 100.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 25.0 mL with the solvent mixture.

Column:
- *size*: *l* = 0.25 m, Ø = 4.0 mm;
- *stationary phase*: *base-deactivated octylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 30 °C.

Mobile phase:
- *mobile phase A*: *methanol R*, solution A (30:70 *V/V*);
- *mobile phase B*: solution A, *methanol R* (30:70 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 6	85	15
6 - 13	85 → 10	15 → 90
13 - 30	10	90
30 - 35	10 → 85	90 → 15
35 - 45	85	15

Flow rate 1 mL/min.

Detection Spectrophotometer at 272 nm.

Injection 10 µL.

Relative retention With reference to pentoxifylline (retention time = about 12 min): impurity A = about 0.3; impurity C = about 0.4; impurity F = about 0.5; impurity J = about 1.6.

System suitability Reference solution (c):
- *resolution*: minimum 4.0 between the peaks due to impurities C and F.

Limits:
- *impurities A, C, F*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.1 per cent);
- *impurity J*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b) (0.02 per cent).

Chlorides (*2.4.4*)

Maximum 100 ppm.

Place 20 mL of solution S in a separating funnel and shake with 2 quantities, each of 20 mL, of *2-methylpropanol R*. Dilute 10 mL of the aqueous layer to 15 mL with *water R*.

Sulfates (*2.4.13*)

Maximum 200 ppm, determined on 15 mL of solution S.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 5 mL of *anhydrous acetic acid R*. Add 20 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 27.83 mg of $C_{13}H_{18}N_4O_3$.

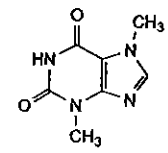
STORAGE

Protected from light.

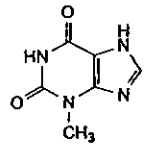
IMPURITIES

Specified impurities *A, C, F, J.*

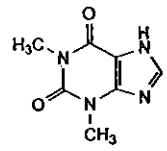
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, D, E, G, H, I, K.*



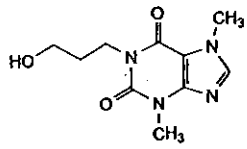
A. theobromine,



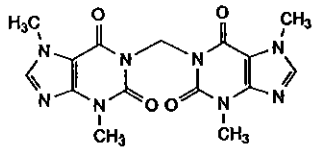
B. 3-methyl-3,7-dihydro-1*H*-purine-2,6-dione,



C. theophylline,

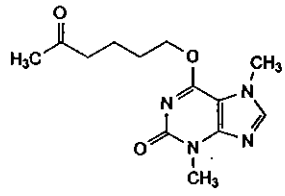


D. 1-(3-hydroxypropyl)-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione,

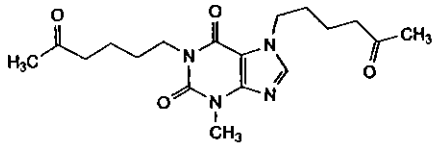


E. 1,1′-methylenebis(3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione),

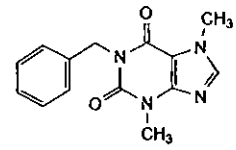
F. caffeine,



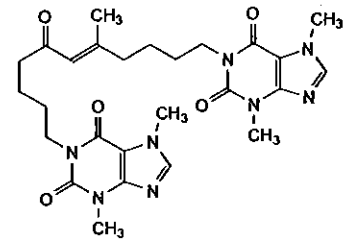
G. 3,7-dimethyl-6-(5-oxohexyloxy)-3,7-dihydro-2*H*-purin-2-one,



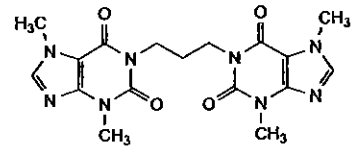
H. 3-methyl-1,7-bis(5-oxohexyl)-3,7-dihydro-1*H*-purine-2,6-dione,



I. 1-benzyl-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione,



J. 1-[(5*E*)-11-(3,7-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-1-yl)-5-methyl-7-oxoundec-5-enyl]-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione,

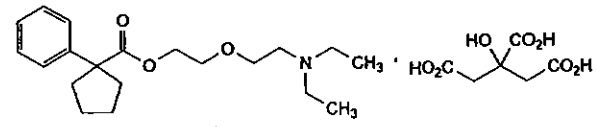


K. 1,1′-(propane-1,3-diyl)bis(3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione).

Ph Eur

Pentoxyverine Citrate

(Pentoxyverine Hydrogen Citrate, Ph. Eur. monograph 1621)



$C_{26}H_{39}NO_{10}$ 525.6 *23142-01-0*

Action and use
Cough suppressant.

Ph Eur

DEFINITION
2-[2-(Diethylamino)ethoxy]ethyl 1-phenylcyclopentanecarboxylate dihydrogen 2-hydroxypropane-1,2,3-tricarboxylate.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water, very soluble in glacial acetic acid, freely soluble in methanol, soluble in alcohol and in methylene chloride.

mp
About 93 °C.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of pentoxyverine hydrogen citrate.*

B. Dissolve 0.25 g in 5 mL of *water R*. The solution gives the reaction of citrates (*2.3.1*).

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

pH (*2.2.3*)

3.3 to 3.7 for solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 mL with the mobile phase.

Reference solution Introduce 5.0 mg of *pentoxyverine impurity A CRS* and 5.0 mg of *pentoxyverine impurity B CRS* in a conical flask, add 5.0 mL of the test solution and dilute to 100.0 mL with the mobile phase. Dilute 3.0 mL of the solution to 50.0 mL with the mobile phase.

Column:
- *size*: *l* = 0.15 m, Ø = 3.9 mm,
- *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm) with a pore size of 10 nm and a carbon loading of 12 per cent,
- *temperature*: 50 °C.

Mobile phase Mix 35 volumes of *acetonitrile R* and 65 volumes of a 1.5 g/L solution of *sodium heptanesulfonate R* adjusted to pH 3.0 with *dilute sulfuric acid R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 205 nm.

Injection 20 µL.

Run time 3 times the retention time of pentoxyverine.

Relative retention With reference to pentoxyverine (retention time = about 6 min): impurity B = about 0.8; impurity A = about 1.5.

System suitability Reference solution:
- *resolution*: minimum of 5.0 between the peaks due to pentoxyverine and to impurity A,
- *signal-to-noise ratio*: minimum of 100 for the peak due to pentoxyverine,
- *symmetry factor*: maximum of 2.0 for the peak due to pentoxyverine.

Limits:
- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.3 per cent),
- *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.3 per cent),
- *any other impurity*: not more than one-third of the area of the peak due to pentoxyverine in the chromatogram obtained with the reference solution (0.1 per cent),
- *total of any other impurity*: not more than the area of the peak due to pentoxyverine in the chromatogram obtained with the reference solution (0.3 per cent),
- *disregard limit*: 0.1 times the area of the peak due to pentoxyverine in the chromatogram obtained with the reference solution (0.03 per cent); disregard any peak with a retention time less than or equal to 2.5 min.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 4 h.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

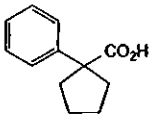
Dissolve 0.400 g in 70 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 52.56 mg of $C_{26}H_{39}NO_{10}$.

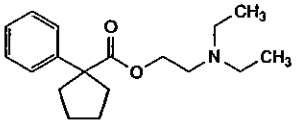
STORAGE

Protected from light.

IMPURITIES



A. 1-phenylcyclopentanecarboxylic acid,



B. 2-(diethylamino)ethyl 1-phenylcyclopentanecarboxylate (caramiphen).

Ph Eur

Pepsin

(Pepsin Powder, Ph. Eur. monograph 0682)

9001-75-6

Action and use

Proteolytic enzyme.

Ph Eur

DEFINITION

Powder prepared from the gastric mucosa of pigs, cattle or sheep. It contains gastric proteinases, active in acid medium (pH 1 to 5).

Activity Not less than 0.5 Ph. Eur. U./mg (dried substance).

PRODUCTION

The animals from which pepsin powder is derived must fulfil the requirements for the health of animals suitable for human consumption.

CHARACTERS

Appearance

White or slightly yellow, crystalline or amorphous powder, hygroscopic.

Table 0682.-1

	Tubes								
	S_1	S_{1b}	S_2	S_{2b}	S_3	S_{3b}	T	T_b	B
Dilute hydrochloric acid R2 (mL)	0.5	0.5	0.25	0.25			0.25	0.25	1.0
Reference solution (mL)	0.5	0.5	0.75	0.75	1.0	1.0			
Test solution (mL)							0.75	0.75	
Trichloroacetic acid solution R (mL)		10.0		10.0		10.0		10.0	10.0
Mix		+		+		+		+	+
Water bath at 25 °C	+	+	+	+	+	+	+	+	+
Haemoglobin solution R (mL)		5.0		5.0		5.0		5.0	5.0
Mix		+		+		+		+	+
Haemoglobin solution R (mL)	5.0		5.0		5.0		5.0		
Mix	+		+		+		+		
Water bath at 25 °C, 10 min	+	+	+	+	+	+	+	+	+
Trichloroacetic acid solution R (mL)	10.0		10.0		10.0		10.0		
Mix	+		+		+		+		
Filter	+	+	+	+	+	+	+	+	+

Solubility

Soluble in water, practically insoluble in ethanol (96 per cent).

The solution in water may be slightly opalescent with a weak acidic reaction.

IDENTIFICATION

In a mortar, pound 30 mg of *fibrin blue R*. Suspend in 20 mL of *dilute hydrochloric acid R2*. Filter the suspension on a filter paper and wash with *dilute hydrochloric acid R2* until a colourless filtrate is obtained. Perforate the filter paper and wash the *fibrin blue R* through it into a conical flask using 20 mL of *dilute hydrochloric acid R2*. Shake before use. Dissolve a quantity of the substance to be examined, equivalent to not less than 20 Ph. Eur. U., in 2 mL of *dilute hydrochloric acid R2* and adjust to pH 1.6 ± 0.1. Add 1 mL of this solution to a test-tube containing 4 mL of the fibrin blue suspension, mix and place in a water-bath at 25 °C with gentle shaking. Prepare a blank solution at the same time and in the same manner using 1 mL of *water R*. After 15 min of incubation the blank solution is colourless and the test solution is blue.

TESTS

Loss on drying (*2.2.32*)

Maximum 5.0 per cent, determined on 0.500 g by drying at 60 °C over *diphosphorus pentoxide R* at a pressure not exceeding 670 Pa for 4 h.

Microbial contamination

TAMC: acceptance criterion 10^4 CFU/g (*2.6.12*).

TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

Absence of *Escherichia coli* (*2.6.13*).

Absence of *Salmonella* (*2.6.13*).

ASSAY

The activity of pepsin powder is determined by comparing the quantity of peptides, non-precipitable by *trichloroacetic acid solution R* and assayed using the *phosphomolybdotungstic reagent R*, which are released per minute from a substrate of *haemoglobin solution R*, with the quantity of such peptides released by *pepsin powder BRP* from the same substrate in the same conditions.

For the test solution and the reference solution, prepare the solution and carry out the dilution at 0 °C to 4 °C.

Avoid shaking and foaming during preparation of the test and reference solutions.

Test solution Immediately before use, prepare a solution of the substance to be examined expected to contain 0.5 Ph. Eur. U./mL in *dilute hydrochloric acid R2*; before dilution to volume, adjust to pH 1.6 ± 0.1, if necessary, using *1 M hydrochloric acid*.

Reference solution Less than 15 min before use, prepare a solution of *pepsin powder BRP* containing 0.5 Ph. Eur. U./mL in *dilute hydrochloric acid R2*; before dilution to volume, adjust to pH 1.6 ± 0.1, if necessary, using *1 M hydrochloric acid*.

Designate tubes in duplicate T, T_b, S_1, S_{1b}, S_2, S_{2b}, S_3, S_{3b}; designate a tube B.

Add *dilute hydrochloric acid R2* to the tubes as follows:

B: 1.0 mL

S_1 and S_{1b}: 0.5 mL

S_2, S_{2b} and T and T_b: 0.25 mL

Add the reference solution to the tubes as follows:

S_1 and S_{1b}: 0.5 mL

S_2 and S_{2b}: 0.75 mL

S_3 and S_{3b}: 1.0 mL

Add 0.75 mL of the test solution to tubes T and T_b.

Add 10.0 mL of *trichloroacetic acid solution R* to tubes S_{1b}, S_{2b}, S_{3b}, T_b and B. Mix by shaking.

Place the tubes and *haemoglobin solution R* in a water bath at 25 ± 0.1 °C. When temperature equilibrium is reached, add 5.0 mL of *haemoglobin solution R* to tubes B, S_{1b}, S_{2b}, S_{3b} and T_b. Mix.

At time zero add 5.0 mL of *haemoglobin solution R* successively and at intervals of 30 s to tubes S_1, S_2, S_3 and T.

Mix immediately after each addition.

Exactly 10 min after adding the *haemoglobin solution R*, stop the reaction by adding, at intervals of 30 s, 10.0 mL of *trichloroacetic acid solution R* to tubes S_1, S_2, S_3 and T (the use of a fast-flowing or blow-out pipette is recommended) and mix.

Filter the contents of each tube (samples and blanks) twice through the same suitable filter paper previously washed with a 50 g/L solution of *trichloroacetic acid R*, then with *water R* and dried. Discard the first 5 mL of filtrate. Place 3.0 mL of each filtrate separately in a tube containing 20 mL of *water R*. Mix.

A suitable filter paper complies with the following test Filter 5 mL of a 50 g/L solution of *trichloroacetic acid R* through a 7 cm disc of white filter paper: the absorbance (*2.2.25*) of the filtrate, measured at 275 nm using unfiltered *trichloroacetic acid R* solution as the compensation liquid, is less than 0.04.

Add to each tube 1.0 mL of *sodium hydroxide solution R* and 1.0 mL of *phosphomolybdotungstic reagent R*, beginning with the blanks and then the samples of each set, in a known order.

A schematic presentation of the above operations is shown in Table 0682.-1.

After 15 min measure the absorbance (*2.2.25*) of solutions S_1, S_2, S_3, S_{1b}, S_{2b}, S_{3b} and T at 540 nm using the filtrate obtained from tube B as the compensation liquid. Correct the average absorbance values for the filtrates obtained from tubes S_1, S_2 and S_3 by subtracting the average values obtained for the filtrates from tubes S_{1b}, S_{2b}, S_{3b} respectively.

Draw a calibration curve of the corrected values against volume of reference solution used. Determine the activity of the substance to be examined using the corrected absorbance for the test solution ($T - T_b$) together with the calibration curve and taking into account the dilution factors.

STORAGE

Store in an airtight container, protected from light, at a temperature of 2 °C to 8 °C.

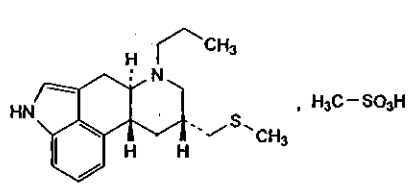
LABELLING

The label states the activity in European Pharmacopoeia Units per milligram.

Ph Eur

Pergolide Mesilate

(Ph. Eur. monograph 1555)



$C_{20}H_{30}N_2O_3S_2$ 410.6 *66104-23-2*

Action and use

Dopamine receptor agonist; treatment of Parkinson's disease.

Ph Eur

DEFINITION

(6a*R*,9*R*,10a*R*)-9-[(Methylsulfanyl)methyl]-7-propyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline monomethanesulfonate.

Content

97.5 per cent to 102.0 per cent (dried substance).

PRODUCTION

It is considered that alkyl methanesulfonate esters are genotoxic and are potential impurities in pergolide mesilate. The manufacturing process should be developed taking into consideration the principles of quality risk management, together with considerations of the quality of starting materials, process capability and validation. The general methods *2.5.37. Methyl, ethyl and isopropyl methanesulfonate in methanesulfonic acid*, *2.5.38. Methyl, ethyl and isopropyl methanesulfonate in active substances* and *2.5.39. Methanesulfonyl chloride in methanesulfonic acid* are available to assist manufacturers.

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, sparingly soluble in methanol, slightly soluble in ethanol (96 per cent) and in methylene chloride, very slightly soluble in acetone.

IDENTIFICATION

A. Specific optical rotation (*2.2.7*): −23 to −17 (dried substance).

Dissolve 0.25 g in *dimethylformamide R* and dilute to 25.0 mL with the same solvent.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *pergolide mesilate CRS*.

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 30.0 mg of the substance to be examined in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *methanol R*. Dilute 1.0 mL of this solution to 10.0 mL with *methanol R*.

Reference solution (b) Dissolve 10 mg of *4,4'-dimethoxybenzophenone R* in *methanol R* and dilute to 10 mL with the same solvent. To 1 mL of the solution add 2 mL of the test solution and dilute to 100 mL with *methanol R*. Dilute 1 mL of this solution to 10 mL with *methanol R*.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase:

- *mobile phase A*: mix 5.0 mL of *morpholine for chromatography R* with 995 mL of *water R* and adjust to pH 7.0 with *phosphoric acid R*; use within 24 h;
- *mobile phase B*: *acetonitrile R*, *methanol R*, *tetrahydrofuran R* (1:1:1 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 35	70 → 0	30 → 100
35 - 40	0 → 70	100 → 30
40 - 50	70	30

Flow rate 1 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µL.

System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the peaks due to 4,4′-dimethoxybenzophenone (1st peak) and pergolide (2nd peak).

Limits:
— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 105 °C for 1 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Liquid chromatography (*2.2.29*).

Solution A Dissolve 5.0 mg of *DL-methionine R* in 500 mL of *0.01 M hydrochloric acid*. Add 500 mL of *methanol R* and mix.

Test solution Dissolve 65.0 mg of the substance to be examined in solution A and dilute to 100.0 mL with solution A. Dilute 10.0 mL of this solution to 100.0 mL with solution A.

Reference solution Dissolve 65.0 mg of *pergolide mesilate CRS* in solution A and dilute to 100.0 mL with solution A. Dilute 10.0 mL of this solution to 100.0 mL with solution A.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated octylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 40 °C.

Mobile phase Mix 1 volume of *acetonitrile R*, 1 volume of *methanol R* and 2 volumes of a mixture prepared as follows: dissolve 2.0 g of *sodium octanesulfonate R* in *water R*, add 1.0 mL of *anhydrous acetic acid R* and dilute to 1000 mL with *water R*.

Flow rate 1 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µL.

Retention time Pergolide = about 9 min.

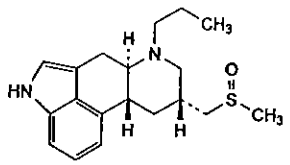
System suitability Reference solution:
— *symmetry factor*: maximum 1.5 for the peak due to pergolide.

Calculate the percentage content of $C_{20}H_{30}N_2O_3S_2$ from the assigned content of *pergolide mesilate CRS*.

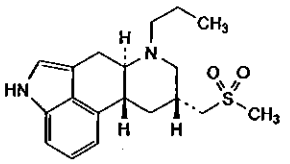
STORAGE
Protected from light.

IMPURITIES
Specified impurities *A*.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B*.



A. (6a*R*,9*R*,10a*R*)-9-[(methylsulfinyl)methyl]-7-propyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline (pergolide sulfoxide),

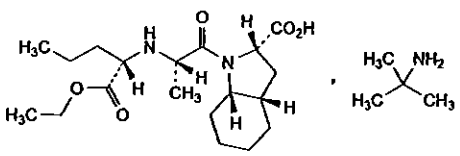


B. (6a*R*,9*R*,10a*R*)-9-[(methylsulfonyl)methyl]-7-propyl-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline (pergolide sulfone).

Ph Eur

Perindopril Erbumine

(Perindopril tert-Butylamine, Ph. Eur. monograph 2019)



$C_{23}H_{43}N_3O_5$ 441.6 *107133-36-8*

Action and use
Angiotensin converting enzyme inhibitor.

Preparation
Perindopril Erbumine Tablets

Ph Eur

DEFINITION
2-Methylpropan-2-amine (2*S*,3a*S*,7a*S*)-1-{(2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)butyl]amino]propanoyl}octahydro-1*H*-indole-2-carboxylate.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white, slightly hygroscopic, crystalline powder.

Solubility
Freely soluble in water and in ethanol (96 per cent), soluble or sparingly soluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION
A. Specific optical rotation (*2.2.7*): −69 to −66 (anhydrous substance).

Dissolve 0.250 g in *ethanol (96 per cent) R* and dilute to 25.0 mL with the same solvent.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *perindopril tert-butylamine CRS*.

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *methylene chloride R*, evaporate to dryness and record new spectra using the residues.

C. Examine the chromatograms obtained in the test for impurity A.

Results In the chromatogram obtained with the test solution a spot is observed with the same R_F as the spot with the higher R_F in the chromatogram obtained with reference solution (c) (*tert*-butylamine).

TESTS

Impurity A

Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.20 g of the substance to be examined in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dissolve 5 mg of *perindopril impurity A CRS* in *methanol R* and dilute to 25.0 mL with the same solvent.

Reference solution (b) Dilute 5.0 mL of reference solution (a) to 20.0 mL with *methanol R*.

Reference solution (c) To 5 mL of reference solution (a) add 5 mL of a 20 g/L solution of *1,1-dimethylethylamine R* in *methanol R*.

Plate *TLC silica gel plate R*.

Mobile phase *glacial acetic acid R, toluene R, methanol R* (1:40:60 *V/V/V*).

Application 10 µL of the test solution and reference solutions (b) and (c).

Development Over 2/3 of the plate.

Drying In a current of warm air.

Detection Expose to iodine vapour for at least 20 h.

System suitability Reference solution (c):
— the chromatogram shows 2 clearly separated spots.

Limit:
— *impurity A*: any spot due to impurity A is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent).

Stereochemical purity

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20 mg of the substance to be examined in *ethanol (96 per cent) R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *ethanol (96 per cent) R*. Dilute 1.0 mL of this solution to 10.0 mL with *ethanol (96 per cent) R*.

Reference solution (b) Dissolve 10 mg of *perindopril for stereochemical purity CRS* (containing impurity I) in *ethanol (96 per cent) R* and dilute to 5 mL with the same solvent.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 50 °C for the column and the tubing preceding the column (the method has been developed with a temperature of 50 °C for at least 30 cm of the tubing preceding the column).

Mobile phase Mix, in the following order, 21.7 volumes of *acetonitrile R1*, 0.3 volumes of *pentanol R*, and 78 volumes of a 1.50 g/L solution of *sodium heptanesulfonate R* previously adjusted to pH 2.0 with a mixture of equal volumes of *perchloric acid R* and *water for chromatography R*.

Flow rate 0.8 mL/min.

Detection Spectrophotometer at 215 nm.

Equilibration Minimum 4 h.

Injection 10 µL.

Identification of impurities Use the chromatogram supplied with *perindopril for stereochemical purity CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity I.

Run time 1.5 times the retention time of perindopril.

Relative retention With reference to perindopril (retention time = about 100 min): impurity I = about 0.9.

System suitability:
— the chromatogram obtained with reference solution (b) is similar to the chromatogram supplied with *perindopril for stereochemical purity CRS*;
— *signal-to-noise ratio*: minimum 3 for the principal peak in the chromatogram obtained with reference solution (a);
— *peak-to-valley ratio*: minimum 3, where H_p = height above the baseline of the peak due to impurity I and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to perindopril in the chromatogram obtained with reference solution (b).

Limits:
— *impurity I*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *disregard limit*: disregard any peak with a relative retention with reference to perindopril of less than 0.6 or more than 1.4.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use or maintain them at a temperature below 10 °C.*

Test solution Dissolve 60 mg of the substance to be examined in mobile phase A and dilute to 20.0 mL with mobile phase A.

Reference solution (a) Dissolve 3 mg of *perindopril for peak identification CRS* (containing impurities B, E, F, H and K) in 1 mL of mobile phase A.

Reference solution (b) Dilute 1.0 mL of the test solution to 200.0 mL with mobile phase A.

Reference solution (c) Dilute 1.0 mL of reference solution (b) to 10.0 mL with mobile phase A.

Column:
— *size*: *l* = 0.15 m, Ø = 4 mm;
— *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 60 °C for the column and the tubing preceding the column.

Mobile phase:
— *mobile phase A*: *water for chromatography R* adjusted to pH 2.5 with a mixture of equal volumes of *perchloric acid R*; and *water for chromatography R*;

— *mobile phase B*: 0.03 per cent *V/V* solution of *perchloric acid R* in *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	95	5
5 - 60	95 → 40	5 → 60

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram supplied with *perindopril for peak identification CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities B, E, F, H and K.

Relative retention With reference to perindopril (retention time = about 25 min): impurity B = about 0.68; impurity K = about 0.72; impurity E = about 1.2; impurity F = about 1.6; impurity H = about 1.8 (impurity H may be eluted as 1 or 2 peaks).

System suitability Reference solution (a):
— *peak-to-valley ratio*: minimum 3, where H_p = height above the baseline of the peak due to impurity B and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity K.

Limits:
— *impurity E*: not more than 0.8 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent);
— *impurity B*: not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
— *impurities F, H*: for each impurity, not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
— *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Water (*2.5.12*)
Maximum 1.0 per cent, determined on 0.50 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.160 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 22.08 mg of $C_{23}H_{43}N_3O_5$.

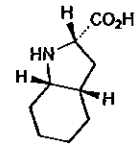
STORAGE

In an airtight container.

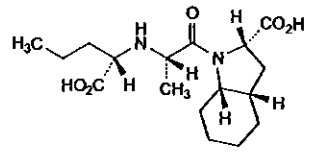
IMPURITIES

Specified impurities *A, B, E, F, H, I.*

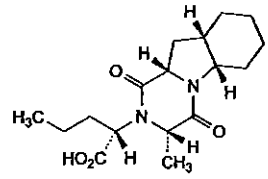
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C, D, G, J, K, L, M, N, O, P, Q, R, S, T, U, V, W, X, Y, Z, AA, BB, CC.*



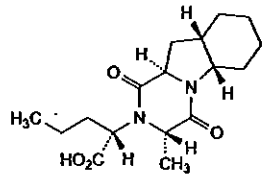
A. (2*S*,3a*S*,7a*S*)-octahydro-1*H*-indole-2-carboxylic acid,



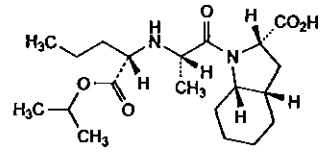
B. (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[[(1*S*)-1-carboxybutyl]amino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid (perindoprilat),



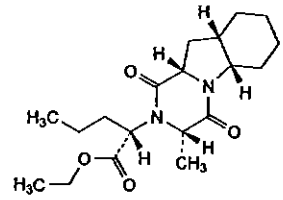
C. (2*S*)-2-[(3*S*,5a*S*,9a*S*,10a*S*)-3-methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl]pentanoic acid,



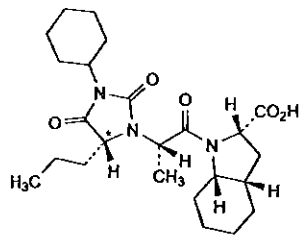
D. (2*S*)-2-[(3*S*,5a*S*,9a*S*,10a*R*)-3-methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl]pentanoic acid,



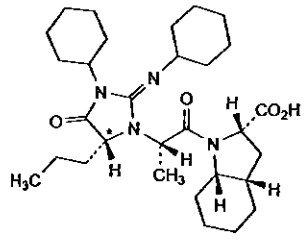
E. (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[[(1*S*)-1-[(1-methylethoxy)carbonyl]butyl]amino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid,



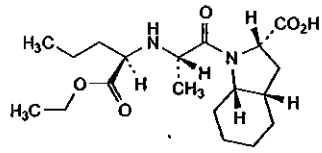
F. ethyl (2*S*)-2-[(3*S*,5a*S*,9a*S*,10a*S*)-3-methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl]pentanoate,



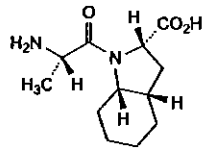
G. (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[(5*RS*)-3-cyclohexyl-2,4-dioxo-5-propylimidazolidin-1-yl]propanoyl]octahydro-1*H*-indole-2-carboxylic acid,



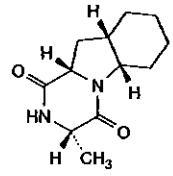
H. (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[(5*RS*)-3-cyclohexyl-2-(cyclohexylimino)-4-oxo-5-propylimidazolidin-1-yl] propanoyl]octahydro-1*H*-indole-2-carboxylic acid,



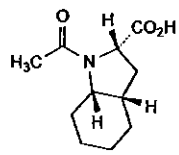
I. (2*RS*,3a*RS*,7a*RS*)-1-[(2*RS*)-2-[[(1*SR*)-1-(ethoxycarbonyl)butyl]amino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid ((±)-1″-*epi*-perindopril),



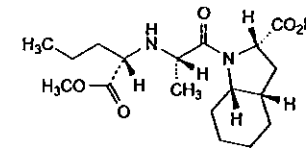
J. (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-aminopropanoyl]octahydro-1*H*-indole-2-carboxylic acid,



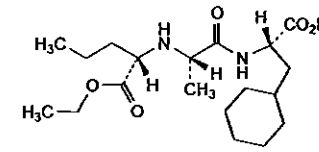
K. (3*S*,5a*S*,9a*S*,10a*S*)-3-methyldecahydropyrazino[1,2-*a*] indole-1,4-dione,



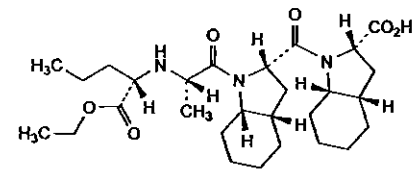
L. (2*S*,3a*S*,7a*S*)-1-acetyloctahydro-1*H*-indole-2-carboxylic acid,



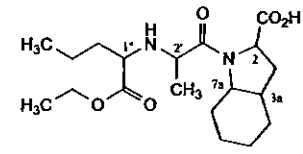
M. (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[[(1*S*)-1-(methoxycarbonyl)butyl] amino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid,



N. (2*S*)-3-cyclohexyl-2-[[(2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)butyl]amino]propanoyl]amino]propanoic acid,



O. (2*S*,3a*S*,7a*S*)-1-[[(2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)butyl]amino]propanoyl]octahydro-1*H*-indol-2-yl]carbonyl]octahydro-1*H*-indole-2-carboxylic acid,



1-[2-[[1-(ethoxycarbonyl)butyl]amino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid,

P. (2*RS*,3a*RS*,7a*RS*)-, (2′*SR*)-, (1″*RS*)-: (±)-2′-*epi*-perindopril,

Q. (2*RS*,3a*RS*,7a*SR*)-, (2′*RS*)-, (1″*RS*)-: (±)-7a-*epi*-perindopril,

R. (2*RS*,3a*SR*,7a*RS*)-, (2′*RS*)-, (1″*RS*)-: (±)-3a-*epi*-perindopril,

S. (2*SR*,3a*RS*,7a*RS*)-, (2′*RS*)-, (1″*RS*)-: (±)-2-*epi*-perindopril,

T. (2*RS*,3a*RS*,7a*RS*)-, (2′*SR*)-, (1″*SR*)-: (±)-1″,2′-di-*epi*-perindopril,

U. (2*RS*,3a*RS*,7a*SR*)-, (2′*RS*)-, (1″*SR*)-: (±)-1″,7a-di-*epi*-perindopril,

V. (2*SR*,3a*SR*,7a*RS*)-, (2′*RS*)-, (1″*RS*)-: (±)-2,3a-di-*epi*-perindopril,

W. (2*SR*,3a*RS*,7a*RS*)-, (2′*RS*)-, (1″*SR*)-: (±)-1″,2-di-*epi*-perindopril,

X. (2*SR*,3a*RS*,7a*SR*)-, (2′*RS*)-, (1″*RS*)-: (±)-2,7a-di-*epi*-perindopril,

Y. (2*SR*,3a*RS*,7a*RS*)-, (2′*SR*)-, (1″*RS*)-: (±)-2,2′-di-*epi*-perindopril,

Z. (2*RS*,3a*SR*,7a*RS*)-, (2′*RS*)-, (1″*SR*)-: (±)-1″,3a-di-*epi*-perindopril,

AA. (2*RS*,3a*SR*,7a*SR*)-, (2′*RS*)-, (1″*RS*)-: (±)-3a,7a-di-*epi*-perindopril,

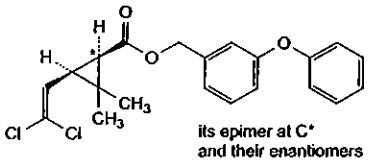
BB. (2*RS*,3a*SR*,7a*RS*)-, (2′*SR*)-, (1″*RS*)-: (±)-2′,3a-di-*epi*-perindopril,

CC. (2*RS*,3a*RS*,7a*SR*)-, (2′*SR*)-, (1″*RS*)-: (±)-2′,7a-di-*epi*-perindopril.

Ph Eur

Permethrin (25:75)

(Ph. Eur. monograph 1762)



$C_{21}H_{20}Cl_2O_3$ 391.3 *52645-53-1*

Undefined *cis*/*trans* ratio

Action and use
Insecticide.

Ph Eur

DEFINITION

Mixture of the *cis* and *trans* isomers of (3-phenoxyphenyl)methyl (1*RS*,2*RS*)-2-(2,2-dichloroethenyl)-3,3-dimethylcyclopropane-1-carboxylate.

Content
- *total*: 98.0 per cent to 102.0 per cent (anhydrous substance);
- *cis isomer*: 23.0 per cent to 27.0 per cent (anhydrous substance);
- *trans isomer*: 73.0 per cent to 77.0 per cent (anhydrous substance).

CHARACTERS

Appearance
Colourless or slightly brownish viscous liquid, semi-solid or crystalline solid.

Solubility
Practically insoluble in water, very soluble in heptane, freely soluble in anhydrous ethanol, sparingly soluble in ethylene glycol.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *permethrin (25:75) CRS*.

B. Examine the chromatograms obtained in the assay.

Results The 2 principal peaks in the chromatogram obtained with the test solution are similar in retention time and in *cis*/*trans* ratio to the 2 principal peaks in the chromatogram obtained with the reference solution (*cis*/*trans* ratio = about 0.3).

TESTS

Related substances
Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution Warm the substance to be examined at 70-85 °C for 20 min. Dissolve 1.0 g of this substance in *heptane R* and dilute to 20.0 mL with the same solvent.

Reference solution (a) Dissolve 50 mg of *permethrin for system suitability CRS* (containing impurities B, C and G) in *heptane R* and dilute to 1.0 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *heptane R*. Dilute 1.0 mL of this solution to 20.0 mL with *heptane R*.

Column:
- *material*: fused silica;
- *size*: *l* = 15 m, Ø = 0.53 mm;
- *stationary phase*: *methylpolysiloxane R* (film thickness 1.5 µm).

Carrier gas *nitrogen for chromatography R*.

Flow rate 12.0 mL/min.

Split ratio 1:10.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 2	45
	2 - 26.5	45 → 290
	26.5 - 40.5	290
Injection port		250
Detector		290

Detection Flame ionisation.

Injection 1 µL.

Identification of impurities Use the chromatogram supplied with *permethrin for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities B, C and G.

Relative retention With reference to permethrin (retention time = about 24 min): impurity B = about 0.5; impurity C = about 0.7; impurity G = about 0.9.

System suitability Reference solution (a):
- *resolution*: minimum 2.0 between the peaks due to impurity G and permethrin.

Limits:
- *correction factor*: for the calculation of content, multiply the peak area of impurity B by 1.5;
- *impurities B, C*: for each impurity, maximum 0.15 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.5 per cent;
- *reporting threshold*: 0.05 per cent (reference solution (b)).

Water (*2.5.32*)
Maximum 0.25 per cent, determined on 0.100 g using the evaporation technique:
- *temperature*: 110 °C;
- *heating time*: 3 min.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution Warm the substance to be examined at 70-85 °C for 20 min. Dissolve 0.100 g of this substance in the mobile phase using sonication and dilute to 50.0 mL with the mobile phase.

Reference solution Warm *permethrin (25:75) CRS* at 70-85 °C for 20 min. Dissolve 0.100 g of this substance in the mobile phase using sonication and dilute to 50.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *silica gel for chromatography R* (5 µm);
— *temperature*: 30 °C.

Mobile phase *dioxan R, heptane R* (1.1:98.9 *V/V*).

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µL.

Run time Twice the retention time of *cis*-permethrin.

Retention time *cis*-permethrin = about 6 min.

Elution order *cis*-permethrin, *trans*-permethrin.

System suitability Reference solution:
— *resolution*: minimum 2.0 between the peaks due to *cis*-permethrin and *trans*-permethrin.

Calculation of percentage contents:
— calculate the percentage content of *cis*-permethrin and *trans*-permethrin by normalisation;
— calculate the percentage content of $C_{21}H_{20}Cl_2O_3$ taking into account the assigned content of *permethrin (25:75) CRS*.

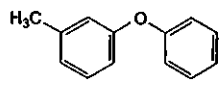
STORAGE

Protected from light.

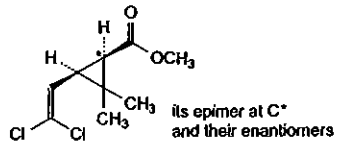
IMPURITIES

Specified impurities *B, C.*

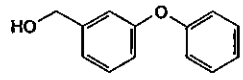
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, D, E, F, G, H.*



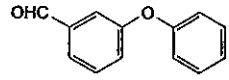
A. 1-methyl-3-phenoxybenzene,



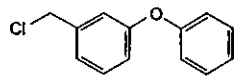
B. methyl (1*RS*,2*RS*)-2-(2,2-dichloroethenyl)-3,3-dimethylcyclopropane-1-carboxylate,



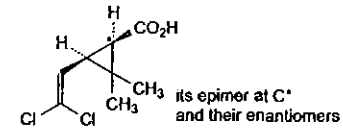
C. (3-phenoxyphenyl)methanol,



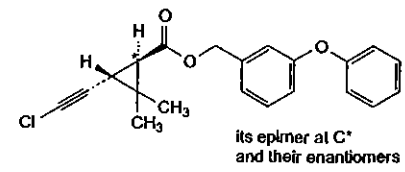
D. 3-phenoxybenzaldehyde,



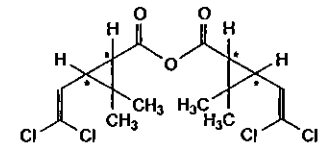
E. 1-(chloromethyl)-3-phenoxybenzene,



F. (1*RS*,2*RS*)-2-(2,2-dichloroethenyl)-3,3-dimethylcyclopropane-1-carboxylic acid,



G. 3-(phenoxyphenyl)methyl (1*RS*,2*RS*)-2-(chloroethynyl)-3,3-dimethylcyclopropane-1-carboxylate,

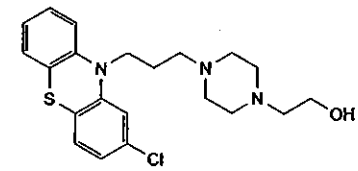


H. 2-(2,2-dichloroethenyl)-3,3-dimethylcyclopropane-1-carboxylic anhydride.

Ph Eur

Perphenazine

(Ph. Eur. monograph 0629)



$C_{21}H_{26}ClN_3OS$ 404.0 *58-39-9*

Action and use

Dopamine receptor antagonist; neuroleptic.

Ph Eur

DEFINITION

2-[4-[3-(2-Chloro-10*H*-phenothiazin-10-yl)propyl]piperazin-1-yl]ethanol.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish-white, crystalline powder.

Solubility

Practically insoluble in water, freely soluble in methylene chloride, soluble in ethanol (96 per cent). It dissolves in dilute solutions of hydrochloric acid.

IDENTIFICATION

A. Melting point (*2.2.14*): 96 °C to 100 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *perphenazine CRS.*

TESTS

Appearance of solution
The solution is clear (*2.2.1*).

Dissolve 0.20 g in 10 mL of *methanol R*.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use. Carry out the test protected from light.*

Test solution Dissolve 20 mg of the substance to be examined in mobile phase A and dilute to 10.0 mL with mobile phase A.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

Reference solution (b) Dissolve 2 mg of *perphenazine for system suitability CRS* (containing impurities A and B) in 1.0 mL of mobile phase A.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: spherical *base-deactivated octylsilyl silica gel for chromatography R* (4 µm);
— *temperature*: 30 °C.

Mobile phase:
— *mobile phase A*: mix 35 volumes of *acetonitrile R* and 65 volumes of a 7 g/L solution of *sodium dihydrogen phosphate R*;
— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	100	0
5 - 10	100 → 80	0 → 20
10 - 33	80 → 30	20 → 70
33 - 48	30 → 100	70 → 0

Flow rate 1.3 mL/min.

Detection Spectrophotometer at 245 nm.

Injection 10 µL.

Identification of impurities Use the chromatogram supplied with *perphenazine for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and B.

Relative retention With reference to perphenazine (retention time = about 12 min): impurity A = about 0.3; impurity B = about 0.8.

System suitability Reference solution (b):
— *resolution*: minimum 4.0 between the peaks due to impurity B and perphenazine.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity A by 0.6;
— *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *impurity B*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 65 °C for 4 h.

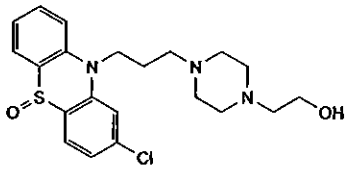
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.150 g in 25 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (*2.2.20*).

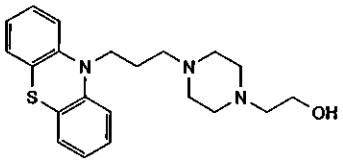
1 mL of *0.1 M perchloric acid* is equivalent to 20.20 mg of $C_{21}H_{26}ClN_3OS$.

STORAGE
Protected from light.

IMPURITIES
Specified impurities *A, B.*



A. 2-[4-[3-(2-chloro-5-oxido-10*H*-phenothiazin-10-yl)propyl] piperazin-1-yl]ethanol,

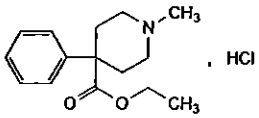


B. 2-[4-[3-(10*H*-phenothiazin-10-yl)propyl]piperazin-1-yl] ethanol.

Ph Eur

Pethidine Hydrochloride

(Ph. Eur. monograph 0420)



$C_{15}H_{22}ClNO_2$ 283.8 *50-13-5*

Action and use
Opioid receptor agonist; analgesic.

Preparations
Pethidine Injection
Pethidine Tablets

Ph Eur

DEFINITION
Ethyl 1-methyl-4-phenylpiperidine-4-carboxylate hydrochloride.

Content
99.0 per cent to 101.0 per cent (dried substance).

PRODUCTION

If intended for use in the manufacture of parenteral preparations, the manufacturing process is validated to show that the content of impurity B is not more than 0.1 ppm.

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very soluble in water, freely soluble in alcohol.

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 187 °C to 190 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of pethidine hydrochloride.*

C. Dissolve 0.1 g in 10 mL of *ethanol R* and add 10 mL of *picric acid solution R*. A crystalline precipitate is formed which, when washed with *water R* and dried at 100-105 °C, melts (*2.2.14*) at 186 °C to 193 °C. Mix equal quantities of the precipitate and the substance to be examined and determine the melting point of the mixture. The melting point is at least 20 °C lower than that of the precipitate.

D. To 5 mL of solution S (see Tests) add 5 mL of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 10 mL of solution S add 0.2 mL of *methyl red solution R* and 0.2 mL of *0.01 M sodium hydroxide*. The solution is yellow. Add 0.3 mL of *0.01 M hydrochloric acid*. The solution is red.

Impurity B

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 0.100 g of the substance to be examined in a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R* and dilute to 25.0 mL with the same mixture of solvents.

Test solution (b) Dissolve 0.125 g of the substance to be examined in a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R* and dilute to 10.0 mL with the same mixture of solvents.

Reference solution (a) Dilute 0.5 mL of test solution (a) to 100.0 mL with a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R*.

Reference solution (b) Dissolve 10.0 mg of *pethidine impurity A CRS* in a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R* and dilute to 100.0 mL with the same mixture of solvents.

Reference solution (c) Dissolve 12.5 mg of *1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine R* in a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R* and dilute to 10.0 mL with the same mixture of solvents. Dilute 1.0 mL of the solution to 100.0 mL with a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R*.

Reference solution (d) Dilute 5.0 mL of reference solution (b) and 1.0 mL of reference solution (c) to 100.0 mL with a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R*.

Column:

— *size*: l = 0.25 m, Ø = 4.0 mm,

— *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 340 m^2/g, a pore size of 10 nm and a carbon loading of 19 per cent.

Mobile phase:

— *mobile phase A*: mix equal volumes of a 42.0 g/L solution of *sodium perchlorate R* and of a 11.6 g/L solution of *phosphoric acid R*, adjust to pH 2.0 with *triethylamine R*,

— *mobile phase B*: *acetonitrile R*,

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	80 → 75	20 → 25
15 - 31	75 → 55	25 → 45
31 - 40	55	45

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 50 µL; inject test solution (b) and reference solution (d).

Relative retention With reference to pethidine (retention time = about 24 min): impurity B = about 0.66; impurity A = about 0.68.

System suitability Reference solution (d):

— *signal-to-noise ratio*: minimum 10 for the first peak,

— *peak-to-valley ratio*: minimum 4, where H_p = height above the baseline of the peak due to impurity B, and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity A.

Limit:

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (10 ppm) if intended for non-parenteral administration.

Related substances

Liquid chromatography (*2.2.29*) as described in the test for impurity B with the following modifications.

Injection 20 µL; inject test solution (a) and reference solution (a).

Limits:

— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent),

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.220 g in 50 mL of *alcohol R*. Add 5.0 mL of *0.01 M hydrochloric acid*. Titrate with *0.1 M sodium hydroxide*

determining the end-point potentiometrically (*2.2.20*). Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 28.38 mg of $C_{15}H_{22}ClNO_2$.

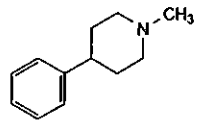
STORAGE

In an airtight container, protected from light.

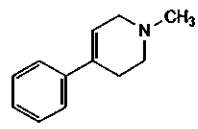
LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

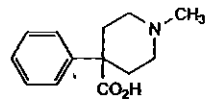
IMPURITIES



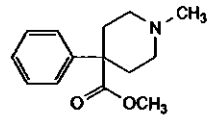
A. 1-methyl-4-phenylpiperidine (MPP),



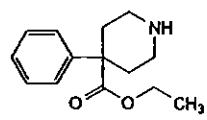
B. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP),



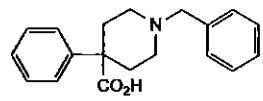
C. 1-methyl-4-phenylpiperidine-4-carboxylic acid,



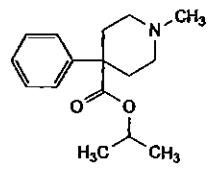
D. methyl 1-methyl-4-phenylpiperidine-4-carboxylate,



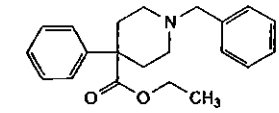
E. ethyl 4-phenylpiperidine-4-carboxylate,



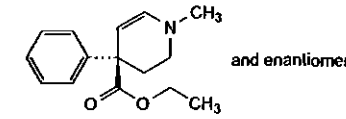
F. 1-benzyl-4-phenylpiperidine-4-carboxylic acid,



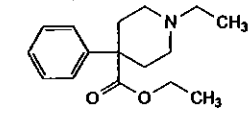
G. 1-methylethyl 1-methyl-4-phenylpiperidine-4-carboxylate,



H. ethyl 1-benzyl-4-phenylpiperidine-4-carboxylate,



I. ethyl (4*RS*)-1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine-4-carboxylate,

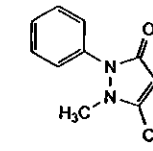


J. ethyl 1-ethyl-4-phenylpiperidine-4-carboxylate.

Ph Eur

Phenazone

(Ph. Eur. monograph 0421)



$C_{11}H_{12}N_2O$ 188.2 *60-80-0*

Action and use

Analgesic; used to test hepatic drug-metabolizing activity.

Ph Eur

DEFINITION

1,5-Dimethyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Very soluble in water, in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

First identification: A, B.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 109 °C to 113 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison phenazone CRS.

C. To 1 mL of solution S (see Tests) add 4 mL of *water R* and 0.25 mL of *dilute sulfuric acid R*. Add 1 mL of *sodium nitrite solution R*; a green colour develops.

D. To 1 mL of solution S add 4 mL of *water R* and 0.5 mL of *ferric chloride solution R2*. A red colour develops which is discharged on the addition of *dilute sulfuric acid R*.

TESTS

Solution S
Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity
To 10 mL of solution S add 0.1 mL of *phenolphthalein solution R*; the solution is colourless. Add 0.2 mL of *0.01 M sodium hydroxide*; the solution is red. Add 0.25 mL of *methyl red solution R* and 0.4 mL of *0.01 M hydrochloric acid*; the solution is red or yellowish-red.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 5 mg of *phenazone impurity A CRS* in the mobile phase, add 10 mL of the test solution and dilute to 20.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution (c) Dissolve 5.0 mg of *phenazone impurity A CRS* in the mobile phase and dilute to 20.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:
— *size*: l = 0.15 m, Ø = 6.0 mm;
— *stationary phase*: spherical *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 6.8 g of *potassium dihydrogen phosphate R* in *water R* and dilute to 1000 mL with the same solvent. Add 2 mL of *triethylamine R* and adjust to pH 7.0 with *sodium hydroxide solution R*. Add 430 mL of *methanol R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µL.

Run time 3 times the retention time of phenazone.

Relative retention With reference to phenazone (retention time = about 13 min): impurity A = about 0.8.

System suitability Reference solution (b):
— *resolution*: minimum 3.0 between the peaks due to impurity A and phenazone.

Limits:
— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.05 per cent);
— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent);
— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *disregard limit*: 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.03 per cent).

Chlorides (*2.4.4*)
Maximum 100 ppm.
Dilute 10 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 100 ppm.
Dissolve 1.5 g in *distilled water R* and dilute to 15 mL with the same solvent.

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 6 h.

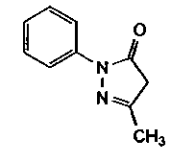
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.150 g in 20 mL of *water R*. Add 2 g of *sodium acetate R*, 1 mL of *dilute acetic acid R* and 25.0 mL of *0.05 M iodine*. Allow to stand protected from light for 30 min. Add 25 mL of *methylene chloride R* and shake until the precipitate dissolves. Titrate with *0.1 M sodium thiosulfate*, using 1 mL of *starch solution R*, added towards the end of the titration, as indicator. Carry out a blank titration.

1 mL of *0.05 M iodine* is equivalent to 9.41 mg of $C_{11}H_{12}N_2O$.

STORAGE
Protected from light.

IMPURITIES
Specified impurities *A*.

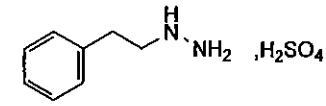


A. 5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one.

Ph Eur

Phenelzine Sulfate

Phenelzine Sulphate



234.3 *156-51-4*

Action and use
Monoamine oxidase inhibitor; antidepressant.

Preparation
Phenelzine Tablets

DEFINITION
Phenelzine Sulfate is phenethylhydrazine hydrogen sulfate. It contains not less than 98.0% and not more than 102.0% of $C_8H_{12}N_2,H_2SO_4$, calculated with reference to the dried substance.

CHARACTERISTICS
A white powder or pearly platelets.

Freely soluble in *water*; practically insoluble in *ethanol (96%)* and in *ether*.

IDENTIFICATION

A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.1% w/v solution in 0.05M *sulfuric acid* exhibits three well-defined maxima, at 252, 258 and 263 nm. The *absorbances* at the maxima are about 0.62, about 0.77 and about 0.58, respectively.

B. Dissolve 0.1 g in 5 mL of *water*, make alkaline with 5M *sodium hydroxide* and add 1 mL of *cupri-tartaric solution* R1. A red precipitate is produced.

C. Yields reaction A characteristic of *sulfates*, Appendix VI.

TESTS

Melting point
164° to 168°, Appendix V A.

Loss on drying
When dried over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 24 hours, loses not more than 1.0% of its weight. Use 1 g.

Sulfated ash
Not more than 0.1%, Appendix IX A.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) 0.026% w/v of the substance to be examined in the mobile phase.

(2) 0.026% w/v *phenelzine sulfate BPCRS in the mobile phase.*

CHROMATOGRAPHIC CONDITIONS

(a) A stainless steel column (15 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 210 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

40 volumes of *methanol R2* and 60 volumes of a solution of 0.216% w/v *sodium octanesulfonate* and 0.68% w/v *potassium dihydrogen phosphate* previously adjusted to pH 3.0 with *orthophosphoric acid.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the principal peak is between 0.8 and 2.0.

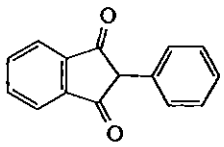
DETERMINATION OF CONTENT

Calculate the content of $C_8H_{12}N_2,H_2SO_4$ in the substance being examined using the declared content of $C_8H_{12}N_2$, H_2SO_4 in *phenelzine sulfate BPCRS.*

STORAGE

Phenelzine Sulfate should be protected from light.

Phenindione



$C_{15}H_{10}O_2$ 222.2 *83-12-5*

Action and use
Oral anticoagulant (indanedione).

Preparation
Phenindione Tablets

DEFINITION

Phenindione is 2-phenylindane-1,3-dione. It contains not less than 98.0% and not more than 102.0% of $C_{15}H_{10}O_2$, calculated with reference to the dried substance.

CHARACTERISTICS

Soft, white or creamy white crystals.

Very slightly soluble in *water*; slightly soluble in *ethanol (96%)* and in *ether*. Solutions are yellow to red.

IDENTIFICATION

The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of phenindione *(RS 268).*

TESTS

Melting point
148° to 151°, Appendix V A.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) 0.25% w/v of the substance being examined in *methanol.*

(2) Dilute 1 volume of solution (1) to 200 volumes with *methanol.*

(3) 0.0005% w/v each of *phenindione BPCRS, phenylacetic acid* (impurity 3), *benzalphthalide* (impurity 4) and *phthalic acid* (impurity 5) in *methanol.*

(4) Dilute 1 volume of solution (2) to 10 volumes of *methanol.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3.5 µm) (X-bridge shield C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 30°.

(e) Use a chilled auto-sampler temperature of 4°.

(f) Use a detection wavelength of 220 nm.

(g) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 10 volumes *acetonitrile*, 10 volumes of a 1.36% w/v *dipotassium hydrogen phosphate* solution previously adjusted to pH 3.0 with *orthophosphoric acid*, and 80 volumes of *water.*

Mobile phase B 10 volumes *water* and 90 volumes *acetonitrile.*

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-0.5	80	20	isocratic
0.5-10	80→50	20→50	linear gradient
10-13	50	50	isocratic
13-21	50→30	50→70	linear gradient
21-22	30→80	70→20	linear gradient
22-25	80	20	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to phenindione (retention time about 7 minutes) are: impurity 5, about 0.2; impurity 3, about 0.4; impurity 1, about 0.6; impurity 4, about 1.8 and impurity 2, about 2.4.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3) the *resolution* between the peaks due to impurity 5 and impurity 3 is at least 4.6.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to impurity 1 or 2 is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3% of each);

the area of any other *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

Loss on drying

When dried at 105° for 2 hours, loses not more than 1.0% of its weight. Use 1 g.

Sulfated ash

Not more than 0.1%, Appendix IX A.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

Solution A 2% v/v *glacial acetic acid* in *acetonitrile*.

(1) Mix with the aid of ultrasound 25 mg of Phenindione in 20 mL of 0.01M *sodium hydroxide* and add 50 mL of solution A. Dilute to 100 mL with solution A.

(2) 25 mg of *phenindione BPCRS* in 20 mL of 0.01M *sodium hydroxide* and 30 mL of solution A. Make up to 100 mL with solution A.

(3) 5 mg each of *phenindione BPCRS* and *phenylacetic acid* (impurity 3) in 5 mL of 0.01M *sodium hydroxide* and 5 mL of solution A. Make up to 20 mL with solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use an autosampler temperature of 4°.

(f) Use a detection wavelength of 250 nm.

(g) Inject 10 µL of each solution.

MOBILE PHASE

40 volumes *acetonitrile* and 60 volumes of 0.68 % w/v *potassium dihydrogen phosphate* previously adjusted to pH 3.5 with *orthophosphoric acid*.

SYSTEM SUITABILITY

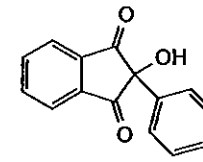
The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity 3 and phenindione is at least 6.0.

DETERMINATION OF CONTENT

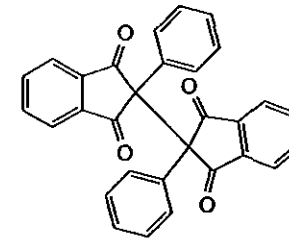
Calculate the content of $C_{15}H_{10}O_2$ using the declared content of $C_{15}H_{10}O_2$ in *phenindione BPCRS*.

IMPURITIES

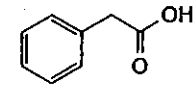
The impurities limited by the requirements of this monograph include:



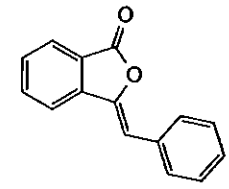
1. 2-hydroxy-2-phenyl-1*H*-indene-1,3(2*H*)-dione



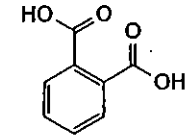
2. 2′-diphenyl-1*H*,1′*H*-[2,2′-bi-indene]-1,1′,3,3′(2*H*,2′*H*)-tetrone



3. phenylacetic acid



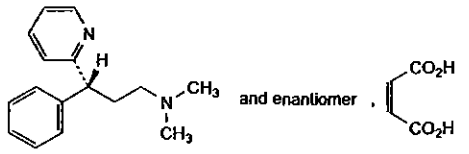
4. 3-benzylidene-2-benzofuran-1(3*H*)-one (benzalphthalide)



5. phthalic acid

Pheniramine Maleate

(Ph. Eur. monograph 1357)



$C_{20}H_{24}N_2O_4$ 356.4 *132-20-7*

Action and use
Histamine H_1 receptor antagonist; antihistamine.

Ph Eur

DEFINITION

(3*RS*)-*N*,*N*-Dimethyl-3-phenyl-3-(pyridin-2-yl)propan-1-amine (*Z*)-butenedioate.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Very soluble in water, freely soluble in ethanol (96 per cent), in methanol and in methylene chloride.

IDENTIFICATION

First identification: C.

Second identification: A, B, D.

A. Melting point (*2.2.14*): 106 °C to 109 °C.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 40.0 mg in *0.1 M hydrochloric acid* and dilute to 100.0 mL with the same acid. Dilute 5.0 mL of the solution to 50.0 mL with *0.1 M hydrochloric acid.*

Spectral range 220-320 nm.

Absorption maximum At 265 nm.

Shoulder At 261 nm.

Specific absorbance at the absorption maximum 200 to 220.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *pheniramine maleate CRS.*

D. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.10 g of the substance to be examined in *methanol R* and dilute to 5.0 mL with the same solvent.

Reference solution (a) Dissolve 65 mg of *maleic acid R* in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 0.10 g of *pheniramine maleate CRS* in *methanol R* and dilute to 5.0 mL with the same solvent.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *water R, anhydrous formic acid R, methanol R, di-isopropyl ether R* (3:7:20:70 *V/V/V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Detection Examine in ultraviolet light at 254 nm.

Results The chromatogram obtained with the test solution shows 2 clearly separated spots; the upper spot is similar in position and size to the spot in the chromatogram obtained with reference solution (a); the lower spot is similar in position and size to the lower spot in the chromatogram obtained with reference solution (b).

TESTS

Solution S
Dissolve 2.0 g in *water R* and dilute to 20.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

pH (*2.2.3*)
4.5 to 5.5.

Dissolve 0.20 g in 20 mL of *carbon dioxide-free water R.*

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R*, mobile phase A (10:90 *V/V*).

Test solution Dissolve 20.0 mg of the substance to be examined in the solvent mixture and dilute to 20.0 mL with the solvent mixture.

Reference solution (a) Dissolve 10.0 mg of *pheniramine impurity A CRS* and 10 mg of *4-benzylpyridine R* (impurity B) in 10.0 mL of the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dilute 1.0 mL of reference solution (a) to 50.0 mL with the solvent mixture.

Reference solution (d) Dilute 1.0 mL of the test solution to 10.0 mL with reference solution (a). Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.30 m, Ø = 3.9 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (10 µm).

Mobile phase:
— *mobile phase A*: dissolve 5.056 g of *sodium heptanesulfonate R* in 900 mL of *water R*, adjust to pH 2.5 with *dilute phosphoric acid R* and dilute to 1000 mL with *water R*;
— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	90	10
2 - 37	90 → 62	10 → 38

Flow rate 1 mL/min.

Detection Spectrophotometer at 264 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A and B.

Relative retention With reference to pheniramine (retention time = about 31 min): maleic acid = about 0.1; impurity A = about 0.9; impurity B = about 0.97.

System suitability Reference solution (d):
— *resolution*: minimum 1.5 between the peaks due to impurity B and pheniramine.

Limits:
— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

— *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: maximum 1.0 per cent;
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard the peak due to maleic acid.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 3 h.

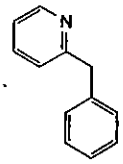
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.130 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

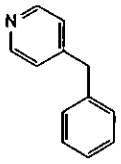
1 mL of *0.1 M perchloric acid* is equivalent to 17.82 mg of $C_{20}H_{24}N_2O_4$.

STORAGE
Protected from light.

IMPURITIES
Specified impurities *A, B.*



A. 2-benzylpyridine,

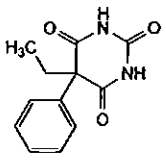


B. 4-benzylpyridine.

Ph Eur

Phenobarbital

(Ph. Eur. monograph 0201)



$C_{12}H_{12}N_2O_3$ 232.2 *50-06-6*

Action and use
Barbiturate.

Preparations
Phenobarbital Elixir
Phenobarbital Tablets

Ph Eur

DEFINITION
5-Ethyl-5-phenylpyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder or colourless crystals.

Solubility
Very slightly soluble in water, freely soluble in ethanol (96 per cent).

It forms water-soluble compounds with alkali hydroxides, carbonates and ammonia.

IDENTIFICATION
First identification: A, B.

Second identification: A, C, D.

A. Determine the melting point (*2.2.14*) of the substance to be examined. Mix equal parts of the substance to be examined and *phenobarbital CRS* and determine the melting point of the mixture. The difference between the melting points (which are about 176 °C) is not greater than 2 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *phenobarbital CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *ethanol (96 per cent) R* and dilute to 10.0 mL with the same solvent.

Reference solution Dissolve 10 mg of *phenobarbital CRS* in *ethanol (96 per cent) R* and dilute to 10.0 mL with the same solvent.

Plate *TLC silica gel GF_{254} plate R.*

Mobile phase *concentrated ammonia R, ethanol (96 per cent) R, methylene chloride R* (5:15:80 *V/V/V*); use the lower layer.

Application 10 µL.

Development Over 2/3 of the plate.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. It gives the reaction of non-nitrogen substituted barbiturates (*2.3.1*).

TESTS
Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Dissolve 1.0 g in a mixture of 4 mL of *dilute sodium hydroxide solution R* and 6 mL of *water R*.

Acidity
Boil 1.0 g with 50 mL of *water R* for 2 min, allow to cool and filter. To 10 mL of the filtrate add 0.15 mL of *methyl red solution R*. The solution is orange-yellow. Not more than 0.1 mL of *0.1 M sodium hydroxide* is required to produce a pure yellow colour.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.125 g of the substance to be examined in 5.0 mL of *methanol R* and dilute to 25.0 mL with the mobile phase.

Reference solution (a) Mix 1.0 mL of the test solution and 20.0 mL of *methanol R* and dilute to 100.0 mL with the mobile phase. Mix 1.0 mL of this solution with 2.0 mL of *methanol R* and dilute to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *phenobarbital impurity A CRS* and 5.0 mg of *phenobarbital impurity B CRS* in 2.0 mL of *methanol R* and dilute to 10.0 mL with the mobile phase. Mix 1.0 mL of this solution with 20.0 mL of *methanol R* and dilute to 100.0 mL with the mobile phase.

Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 6.60 g of *sodium acetate R* in 900 mL of *water R*, add 3 mL of *glacial acetic acid R*, adjust to pH 4.5 with *glacial acetic acid R* and dilute to 1000 mL with *water R*. Mix 60 volumes of this solution with 40 volumes of *methanol R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL.

Run time 2.1 times the retention time of phenobarbital.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and B.

Relative retention With reference to phenobarbital (retention time = about 14 min): impurity A = about 0.2; impurity B = about 0.3.

System suitability Reference solution (b):
— *resolution*: minimum 1.5 between the peaks due to impurities A and B.

Limits:
— *impurity A*: not more than 1.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
— *impurity B*: not more than 1.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying *(2.2.32)*
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

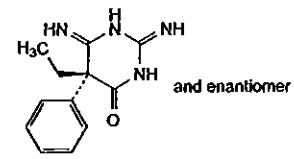
Sulfated ash *(2.4.14)*
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.200 g in 40 mL of *ethanol (96 per cent) R* and add 20 mL of *water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically *(2.2.20)*.

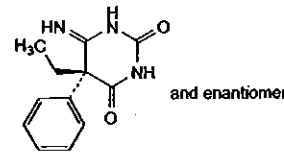
1 mL of *0.1 M sodium hydroxide* is equivalent to 23.22 mg of $C_{12}H_{12}N_2O_3$.

IMPURITIES
Specified impurities *A, B.*

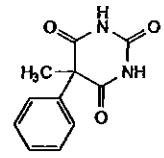
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) C.



A. (5*RS*)-5-ethyl-2,6-diimino-5-phenyltetrahydropyrimidin-4(1*H*)-one,



B. (5*RS*)-5-ethyl-6-imino-5-phenyldihydropyrimidine-2,4(1*H*,3*H*)-dione,

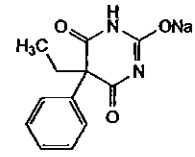


C. 5-methyl-5-phenylpyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione.

Ph Eur

Phenobarbital Sodium

(Ph. Eur. monograph 0630)



$C_{12}H_{11}N_2NaO_3$ 254.2 *57-30-7*

Action and use
Barbiturate.

Preparations
Phenobarbital Injection
Paediatric Phenobarbital Oral Solution
Phenobarbital Sodium Tablets

Ph Eur

DEFINITION
Sodium derivative of 5-ethyl-5-phenylpyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder, hygroscopic.

Solubility
Freely soluble in carbon dioxide-free water (a small fraction may be insoluble), soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification: A, B, E.

Second identification: A, C, D, E.

A. Acidify 10 mL of solution S (see Tests) with *dilute hydrochloric acid R* and shake with 20 mL of *ether R*. Separate the ether layer, wash with 10 mL of *water R*, dry over *anhydrous sodium sulfate R* and filter. Evaporate the filtrate to dryness and dry the residue at 100-105 °C (test residue). Determine the melting point (*2.2.14*) of the test residue. Mix equal parts of the test residue and *phenobarbital CRS* and determine the melting point of the mixture. The difference between the melting points (which are about 176 °C) is not greater than 2 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Use the test residue obtained in identification test A.

Comparison *phenobarbital CRS.*

If the spectra obtained in the solid state show differences, dissolve the test residue and the reference substance separately in *anhydrous ethanol R*, evaporate to dryness and record new spectra using the residues.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *ethanol (50 per cent V/V) R* and dilute to 10.0 mL with the same solvent.

Reference solution Dissolve 9 mg of *phenobarbital CRS* in *ethanol (50 per cent V/V) R* and dilute to 10.0 mL with the same solvent.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *concentrated ammonia R, ethanol (96 per cent) R, methylene chloride R* (5:15:80 *V/V/V*); use the lower layer.

Application 10 µL.

Development Over 2/3 of the plate.

Detection Examine immediately in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. It gives the reaction of non-nitrogen substituted barbiturates (*2.3.1*).

E. It gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S
Dissolve 5.0 g in *ethanol (50 per cent V/V) R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

pH (*2.2.3*)
Maximum 10.2.

Dissolve 5.0 g as completely as possible in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 55.0 mg of the substance to be examined in 2.0 mL of *methanol R* and dilute to 10.0 mL with the mobile phase.

Test solution (b) Dissolve 25.0 mg of the substance to be examined in 10.0 mL of *methanol R* and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Mix 1.0 mL of test solution (b) and 20.0 mL of *methanol R* and dilute to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *phenobarbital impurity A CRS* and 5.0 mg of *phenobarbital impurity B CRS* in 2.0 mL of *methanol R* and dilute to 10.0 mL with the mobile phase. Mix 1.0 mL of the solution and 20.0 mL of *methanol R* and dilute to 100.0 mL with the mobile phase.

Reference solution (c) Dissolve 25.0 mg of *phenobarbital CRS* in 10.0 mL of *methanol R* and dilute to 50.0 mL with the mobile phase.

Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 6.6 g of *sodium acetate R* in 900 mL of *water R*, add 3 mL of *glacial acetic acid R*, adjust to pH 4.5 with *glacial acetic acid R* and dilute to 1000 mL with *water R*. Mix 60 volumes of this solution and 40 volumes of *methanol R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL of test solution (a) and reference solutions (a) and (b).

Run time 2.5 times the retention time of phenobarbital.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and B.

Relative retention With reference to phenobarbital (retention time = about 13 min): impurity A = about 0.29; impurity B = about 0.32.

System suitability Reference solution (b):
— *resolution*: minimum 1.5 between the peaks due to impurities A and B.

Calculation of percentage contents:
— for impurity A, use the concentration of impurity A in reference solution (b);
— for impurity B, use the concentration of impurity B in reference solution (b);
— for impurities other than A and B, use the concentration of phenobarbital sodium in reference solution (a).

Limits:
— *impurities A, B*: for each impurity, maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.2 per cent;
— *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)
Maximum 7.0 per cent, determined on 0.500 g by drying in an oven at 150 °C for 4 h.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection 10 µL of test solution (b) and reference solution (c).

Run time 1.5 times the retention time of phenobarbital.

Calculate the percentage content of $C_{12}H_{11}N_2NaO_3$ taking into account the assigned content of *phenobarbital CRS* and a conversion factor of 1.095.

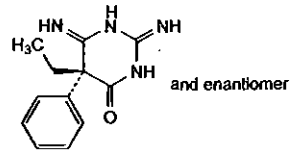
STORAGE

In an airtight container.

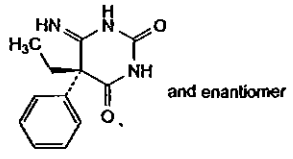
IMPURITIES

Specified impurities *A, B.*

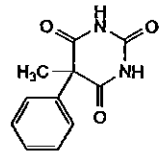
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C, E.*



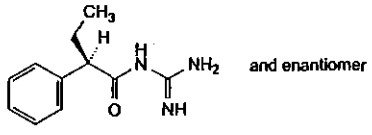
A. (5*RS*)-5-ethyl-2,6-diimino-5-phenyltetrahydropyrimidin-4(1*H*)-one,



B. (5*RS*)-5-ethyl-6-imino-5-phenyldihydropyrimidine-2,4(1*H*,3*H*)-dione,



C. 5-methyl-5-phenylpyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione,

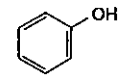


E. (2*RS*)-*N*-carbamimidoyl-2-phenylbutanamide.

Ph Eur

Phenol

(Ph. Eur. monograph 0631)



C_6H_6O 94.1 *108-95-2*

Action and use

Antiseptic; antimicrobial preservative; antipruritic.

Preparations

Aqueous Phenol Injection

Oily Phenol Injection

Phenol and Glycerol Injection

Ph Eur

DEFINITION

Content

99.0 per cent to 100.5 per cent.

CHARACTERS

Appearance

Colourless or faintly pink or faintly yellowish, crystals or crystalline masses, deliquescent.

Solubility

Soluble in water, very soluble in ethanol (96 per cent), in glycerol and in methylene chloride.

IDENTIFICATION

A. Dissolve 0.5 g in 2 mL of *concentrated ammonia R*. The substance dissolves completely. Dilute to about 100 mL with *water R*. To 2 mL of this solution add 0.05 mL of *strong sodium hypochlorite solution R*. A blue colour develops and becomes progressively more intense.

B. To 1 mL of solution S (see Tests) add 10 mL of *water R* and 0.1 mL of *ferric chloride solution R1*. A violet colour is produced which disappears on addition of 5 mL of *2-propanol R*.

C. To 1 mL of solution S add 10 mL of *water R* and 1 mL of *bromine water R*. A white precipitate is formed.

TESTS

Solution S

Dissolve 1.0 g in *water R* and dilute to 15 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_6 (*2.2.2, Method II*).

Acidity

To 2 mL of solution S add 0.05 mL of *methyl orange solution R*. The solution is yellow.

Freezing point (*2.2.18*)

Minimum 39.5 °C.

Residue on evaporation

Maximum 0.05 per cent.

Evaporate 5.000 g to dryness on a water-bath and dry at 100-105 °C for 1 h. The residue weighs a maximum of 2.5 mg.

ASSAY

Dissolve 2.000 g in *water R* and dilute to 1000.0 mL with the same solvent. Transfer 25.0 mL of the solution to a ground-glass-stoppered flask and add 50.0 mL of *0.0167 M bromide-bromate* and 5 mL of *hydrochloric acid R*, close the

flask, allow to stand with occasional swirling for 30 min. Then allow to stand for 15 min. Add 5 mL of a 200 g/L solution of *potassium iodide R*, shake and titrate with *0.1 M sodium thiosulfate* until a faint yellow colour remains. Add 0.5 mL of *starch solution R* and 10 mL of *chloroform R* and continue the titration with vigorous shaking. Carry out a blank titration.

1 mL of *0.0167 M bromide-bromate* is equivalent to 1.569 mg of C_6H_6O.

STORAGE

In an airtight container, protected from light.

Ph Eur

Liquefied Phenol

Action and use

Antiseptic; antimicrobial preservative; antipruritic.

When Phenol is to be mixed with collodion, fixed oils or paraffins, melted Phenol should be used and not Liquefied Phenol.

DEFINITION

Liquefied Phenol contains not less than 77.0% and not more than 81.5% w/w of Phenol in Purified Water. It is prepared by adding Purified Water to melted Phenol.

CHARACTERISTICS

A colourless or faintly coloured liquid; caustic.

Soluble in *water*; miscible with *ethanol (96%)*, with *ether* and with *glycerol*.

IDENTIFICATION

A. Dissolve 0.6 g in 2 mL of 13.5M *ammonia* and dilute to 100 mL with *water*. To 2 mL of the resulting solution add 0.05 mL of *sodium hypochlorite solution*. A blue colour is produced which becomes progressively more intense.

B. Dilute 1 mL of a 15% w/v solution to 10 mL and add 0.1 mL of *iron(III) chloride solution R1*. A violet colour is produced which is discharged on the addition of *propan-2-ol*.

C. To 1 mL of a 15% w/v solution add 10 mL of *water* and 1 mL of *bromine water*. A white or yellowish white precipitate is produced.

TESTS

Acidity

To 2 mL of a 15% w/v solution add 0.05 mL of *methyl orange solution*. The solution is yellow.

Clarity and colour of solution

A solution of 1.0 mL in 14 mL of *water*, at 20°, is *clear*, Appendix IV A, and not more intensely coloured than *reference solution* R_7 or B_7, Appendix IV B, Method II.

Weight per mL

1.055 to 1.060 g, Appendix V G.

Non-volatile matter

When heated on a water bath and dried at 105°, leaves not more than 0.05% w/v of residue.

ASSAY

Dissolve 2.5 g in sufficient *water* to produce 1000 mL, transfer 25 mL to a 500 mL glass-stoppered flask and add 50 mL of *0.05M bromine VS* and 5 mL of *hydrochloric acid*, stopper, swirl occasionally over a period of 30 minutes and allow to stand for 15 minutes. Add 5 mL of a 20% w/v solution of *potassium iodide* taking care to avoid loss of bromine, shake thoroughly and titrate with 0.1M *sodium thiosulfate VS* until only a faint yellow colour remains. Add 0.1 mL of *starch mucilage* and 10 mL of *chloroform* and complete the titration with vigorous shaking. Repeat the operation without the material being examined.

The difference between the titrations represents the amount of bromine required. Each mL of *0.05M bromine VS* is equivalent to 1.569 mg of C_6H_6O.

STORAGE

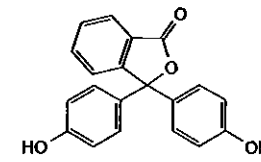
Liquefied Phenol should be protected from light. Liquefied Phenol may congeal or deposit crystals if stored at a temperature below 4°. It should be completely melted before use.

LABELLING

The label states 'contains 80% phenol'.

Phenolphthalein

(Ph. Eur. monograph 1584)



$C_{20}H_{14}O_4$ 318.3 *77-09-8*

Action and use

Stimulant laxative.

Ph Eur

DEFINITION

Phenolphthalein contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of 3,3-bis(4-hydroxyphenyl)isobenzofuran-1(3*H*)-one, calculated with reference to the dried substance.

CHARACTERS

A white or almost white powder, practically insoluble in water, soluble in alcohol.

It melts at about 260 °C.

IDENTIFICATION

A. Dissolve 25.0 mg in *alcohol R* and dilute to 100.0 mL with the same solvent (solution A). To 2.0 mL of solution A add 5.0 mL of *1 M hydrochloric acid*and dilute to 50.0 mL with *alcohol R* (solution A_1). To 10.0 mL of solution A add 5.0 mL of *1 M hydrochloric acid* and dilute to 50.0 mL with *alcohol R* (solution A_2). To 2.0 mL of solution A add 5.0 mL of *1 M sodium hydroxide* and dilute to 50.0 mL with *alcohol R* (solution B). Examined between 220 nm and 250 nm (*2.2.25*), solution A_1 shows an absorption maximum at 229 nm. The specific absorbance at the maximum at 229 nm is 922 to 1018. Examined between 250 nm and 300 nm, solution A_2 shows an absorption maximum at 276 nm. The specific absorbance at the maximum at 276 nm is 142 to 158. Examined between 230 nm and 270 nm, solution B shows an absorption maximum at 249 nm. The specific absorbance at the maximum at 249 nm is 744 to 822.

B. Dissolve about 10 mg in *alcohol R*. Add 1 mL of *dilute sodium hydroxide solution R*. The solution is red. Add 5 mL of *dilute sulfuric acid R*. The colour disappears.

TESTS

Solution S

To 2.0 g add 40 mL of *distilled water R* and heat to boiling. Cool and filter.

Appearance of solution

Dissolve 0.20 g in 5 mL of *alcohol R*. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

Acidity or alkalinity

To 10 mL of solution S add 0.15 mL of *bromothymol blue solution R1*. Add 0.05 mL of *0.01 M hydrochloric acid*, the solution is yellow. Add 0.10 mL of *0.01 M sodium hydroxide*, the solution is blue.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel F_{254} plate R*.

Test solution Dissolve 0.5 g of the substance to be examined in *alcohol R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dilute 1 mL of the test solution to 10 mL with *alcohol R*. Dilute 5 mL of this solution to 100 mL with *alcohol R*.

Reference solution (b) Dissolve 25 mg of *fluorene R* in *alcohol R*, add 0.5 mL of the test solution and dilute to 10 mL with *alcohol R*.

Apply to the plate 5 µL of the test solution and 5 µL of each of the reference solutions. Develop over a path corresponding to two-thirds of the plate height using a mixture of 50 volumes of *acetone R* and 50 volumes of *methylene chloride R*. Allow the plate to dry in air. Examine in ultraviolet light at 254 nm and re-examine after exposure to ammonia vapour. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

Chlorides (*2.4.4*)

Dilute 10 mL of solution S to 15 mL with *water R*. The solution complies with the limit test for chlorides (100 ppm).

Sulfates (*2.4.13*)

15 mL of solution S complies with the limit test for sulfates (200 ppm).

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 5 mL of *dimethylformamide R*. Add 5 mL of *sodium carbonate solution R*, 10 mL of *sodium hydrogen carbonate solution R*, 35 mL of *water R* and 50.0 mL of *0.05 M iodine*. Add 10 mL of *methylene chloride R* and 20 mL of *dilute sulfuric acid R*. Titrate the excess of iodine with *0.1 M sodium thiosulfate*, using 0.3 mL of *starch solution R* added towards the end of the titration, as indicator. Carry out a blank titration.

1 mL of *0.05 M iodine* is equivalent to 3.979 mg of $C_{20}H_{14}O_4$.

STORAGE

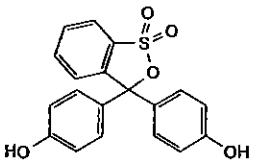
Store protected from light.

Ph Eur

Phenolsulfonphthalein

Phenolsulphonphthalein

(Ph. Eur. monograph 0242)



$C_{19}H_{14}O_5S$ 354.4 *143-74-8*

Action and use

Measurement of renal function; marker in drug absorption studies.

Ph Eur

DEFINITION

Phenolsulfonphthalein (phenol red) contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of 3,3-bis(4-hydroxyphenyl)-3*H*-2,1-benzoxathiole 1,1-dioxide, calculated with reference to the dried substance.

CHARACTERS

A bright-red to dark-red, crystalline powder, very slightly soluble in water, slightly soluble in alcohol.

IDENTIFICATION

A. Dissolve 10 mg in a 10 g/L solution of *sodium carbonate R* and dilute to 200.0 mL with the sodium carbonate solution. Dilute 5.0 mL of the solution to 100.0 mL with a 10 g/L solution of *sodium carbonate R*. Examined between 400 nm and 630 nm (*2.2.25*), the solution shows an absorption maximum at 558 nm. The specific absorbance at the maximum is 1900 to 2100.

B. Dissolve about 10 mg in 1 mL of *dilute sodium hydroxide solution R* and add 9 mL of *water R*. The solution is deep red. To 5 mL of the solution add a slight excess of *dilute sulfuric acid R*. The colour becomes orange.

C. To 5 mL of the solution prepared for identification test B add 1 mL of *0.0167 M bromide-bromate* and 1 mL of *dilute hydrochloric acid R*, shake and allow to stand for 15 min. Make alkaline with *dilute sodium hydroxide solution R*. An intense violet-blue colour is produced.

TESTS

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel GF_{254} R* as the coating substance.

Test solution Dissolve 0.1 g of the substance to be examined in *0.1 M sodium hydroxide* and dilute to 5 mL with the same solvent.

Reference solution Dilute 0.5 mL of the test solution to 100 mL with *0.1 M sodium hydroxide*.

Apply separately to the plate 10 µL of each solution. Develop over a path of 15 cm using a mixture of 25 volumes of *glacial acetic acid R*, 25 volumes of *water R* and 100 volumes of *tert-pentyl alcohol R*. Allow the plate to dry in air until the solvent has evaporated and expose the plate to the vapour from *concentrated ammonia R*. Examine in ultraviolet light at 254 nm. Not more than one spot, apart from the principal spot, appears in the chromatogram obtained with the test solution and this spot is not more intense than the spot in

the chromatogram obtained with the reference solution (0.5 per cent).

Insoluble matter
To 1.0 g of the finely powdered substance to be examined add 12 mL of *sodium hydrogen carbonate solution R*. Allow to stand for 1 h, shaking frequently. Dilute to 100 mL with *water R* and allow to stand for 15 h. Centrifuge at 2000 *g* to 3000 *g*, for 30 min, decant the supernatant and wash the residue with 25 mL of a 10 g/L solution of *sodium hydrogen carbonate R* and then 25 mL of *water R*. Dry at 100 °C to 105 °C. The residue weighs not more than 5 mg (0.5 per cent).

Loss on drying *(2.2.32)*
Not more than 1.0 per cent, determined on 1.00 g of the powdered substance to be examined by drying in an oven at 105 °C.

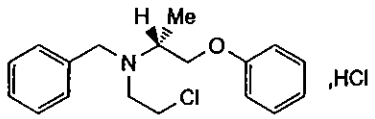
Sulfated ash *(2.4.14)*
Not more than 0.2 per cent, determined on 0.5 g.

ASSAY
Dissolve 0.900 g in 15 mL of *1 M sodium hydroxide* and dilute to 250.0 mL with *water R*. To 10.0 mL of the solution in a glass-stoppered flask add 25 mL of *glacial acetic acid R*, 20.0 mL of *0.0167 M potassium bromate*, 5 mL of a 100 g/L solution of *potassium bromide R* and 5 mL of *hydrochloric acid R*. Allow to stand protected from light for 15 min, add 10 mL of a 100 g/L solution of *potassium iodide R* and titrate immediately with *0.1 M sodium thiosulfate*, using 0.1 mL of *starch solution R* as indicator.

1 mL of *0.0167 M potassium bromate* is equivalent to 4.43 mg of $C_{19}H_{14}O_5S$.

Ph Eur

Phenoxybenzamine Hydrochloride



and enantiomer

$C_{18}H_{22}ClNO,HCl$ 340.3 *63-92-3*

Action and use
Alpha-adrenoceptor antagonist.

Preparation
Phenoxybenzamine Capsules

DEFINITION
Phenoxybenzamine Hydrochloride is (*RS*)-benzyl(2-chloroethyl)1-methyl-2-phenoxyethylamine hydrochloride. It contains not less than 98.5% and not more than 101.0% of $C_{18}H_{22}ClNO,HCl$, calculated with reference to the dried substance.

CHARACTERISTICS
A white or almost white, crystalline powder.

Sparingly soluble in *water*; freely soluble in *ethanol (96%)*.

IDENTIFICATION
A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of phenoxybenzamine hydrochloride *(RS 271)*.

B. Dissolve 0.5 g in 50 mL of *ethanol-free chloroform* and extract with three 20-mL quantities of 0.01M *hydrochloric acid*. Filter the chloroform layer through absorbent cotton and dilute 5 mL of the filtrate to 250 mL with *ethanol-free chloroform*. The *light absorption* of the resulting solution, Appendix II B, in the range 250 to 350 nm exhibits two maxima, at 272 nm and 279 nm. The *absorbances* at the maxima are about 1.1 and about 0.90, respectively.

C. Yields the reactions characteristic of *chlorides*, Appendix VI.

TESTS
Melting point
137.5° to 140°, Appendix V A.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *acetonitrile*.

(1) 0.08% w/v of the substance being examined.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute 1 volume of solution (2) to 10 volumes.

(4) To 10 volumes of solution (1) add 0.5 volume of 0.1M *sodium hydroxide*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5µm) (HiChrom Kromasil is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1 mL per minute.

(d) Use a column temperature of 25°.

(e) Use a detection wavelength of 268 nm.

(f) Inject 10 µL of each solution.

(g) Allow the chromatography to proceed for three times the retention time of phenoxybenzamine.

MOBILE PHASE

45 volumes of a 0.22% w/v solution of *anhydrous sodium dihydrogen orthophosphate*, previously adjusted to pH 3.0 with *orthophosphoric acid* and 55 volumes of *acetonitrile*.

When the chromatograms are recorded under the prescribed conditions, the relative retention with reference to phenoxybenzamine (retention time = about 11 minutes) is: phenoxybenzamine tertiary amine, about 0.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to phenoxybenzamine and the secondary peak with a relative retention of about 1.3 is at least 4.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to phenoxybenzamine tertiary amine is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0 %);

the area of any other *secondary peak* is not greater than 4 times the area of the principal peak in the chromatogram obtained with solution (3) (0.4%);

the sum of the areas of all the *secondary peaks* is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

Loss on drying
When dried over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 24 hours, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash
Not more than 0.1%, Appendix IX A.

ASSAY
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *acetonitrile*.

(1) 0.02% w/v of the substance being examined.

(2) 0.02% w/v of *phenoxybenzamine hydrochloride BPCRS*.

(3) To 10 mL of solution (1) add 0.5 mL of 0.1M *sodium hydroxide*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to phenoxybenzamine and the secondary peak with a relative retention of about 1.3 is at least 4.0.

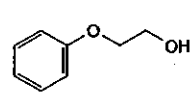
DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{22}ClNO,HCl$ using the declared content of $C_{18}H_{22}ClNO,HCl$ in *phenoxybenzamine hydrochloride BPCRS*.

IMPURITIES
The impurities limited by the requirements of this monograph include phenoxybenzamine tertiary amine.

Phenoxyethanol

(Ph. Eur. monograph 0781)



$C_8H_{10}O_2$ 138.2 *122-99-6*

Action and use
Antimicrobial preservative; also used topically in treatment of bacterial infections.

Ph Eur

DEFINITION
2-Phenoxyethanol.

Content
99.0 per cent *m/m* to 100.5 per cent *m/m*.

CHARACTERS

Appearance
Colourless, slightly viscous liquid.

Solubility
Slightly soluble in water, in arachis oil and in olive oil, miscible with acetone, with ethanol (96 per cent) and with glycerol.

IDENTIFICATION
First identification: C.

Second identification: A, B, D.

A. Refractive index (*2.2.6*): 1.537 to 1.539.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 80.0 mg in *water R* and dilute to 100.0 mL with the same solvent. Dilute 10.0 mL of the solution to 100.0 mL with *water R*.

Spectral range 240-350 nm.

Absorption maxima At 269 nm and 275 nm.

Specific absorbances at the absorption maxima:
— at 269 nm: 95 to 105;
— at 275 nm: 75 to 85.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *phenoxyethanol CRS*.

D. Shake 2 mL with a mixture of 4 g of *potassium permanganate R*, 5.4 g of *sodium carbonate R* and 75 mL of *water R* for 30 min. Add 25 g of *sodium chloride R* and stir continuously for 60 min, filter and acidify with *hydrochloric acid R* to about pH 1.7. The melting point of the precipitate, after recrystallisation from *water R*, is 96 °C to 99 °C (*2.2.14*).

TESTS

Relative density (*2.2.5*)
1.105 to 1.110.

Related substances
Gas chromatography (*2.2.28*).

Test solution Dilute 5.0 g of the substance to be examined to 20.0 mL with *anhydrous ethanol R*.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *anhydrous ethanol R*.

Reference solution (b) Dissolve 25.0 mg of *phenol R* (impurity A) in *anhydrous ethanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (c) Mix 1.0 mL of reference solution (a) and 1.0 mL of reference solution (b) and dilute to 10.0 mL with *anhydrous ethanol R*.

Reference solution (d) Dilute 1.0 mL of reference solution (a) to 10.0 mL with *anhydrous ethanol R*.

Column:
— *material*: fused silica;
— *size*: *l* = 30 m, Ø = 0.25 mm;
— *stationary phase*: *macrogol 20 000 R* (0.25 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.0 mL/min.

Split ratio 1:100.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 13	90 → 220
	13 - 23	220
Injection port		250
Detector		270

Detection Flame ionisation.

Injection 1 µL of the test solution and reference solutions (c) and (d).

Elution order Impurity A, phenoxyethanol.

Retention time Phenoxyethanol = about 13 min.

System suitability Reference solution (c):
— *resolution*: minimum 15 between the peaks due to impurity A and phenoxyethanol.

Calculation of percentage contents:
— for each impurity, use the concentration of phenoxyethanol in reference solution (d).

Limits:
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.3 per cent;
— *reporting threshold*: 0.05 per cent.

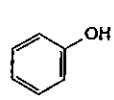
ASSAY

To 2.000 g in an acetylation flask fitted with an air condenser, add 10.0 mL of freshly prepared *acetic anhydride solution R1* and heat with frequent shaking in a water-bath for 45 min. Cool and carefully add 10 mL of *water R*. Heat for a further 2 min. Cool, add 10 mL of *butanol R*, shake vigorously and titrate the excess of acetic acid with *1 M sodium hydroxide* using 0.2 mL of *phenolphthalein solution R* as indicator. Repeat the procedure without the substance to be examined. The difference between the volumes used in the titrations represents the amount of acetic anhydride required for the acetylation of the substance to be examined.

1 mL of *1 M sodium hydroxide* is equivalent to 0.1382 g of $C_8H_{10}O_2$.

IMPURITIES

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) A.

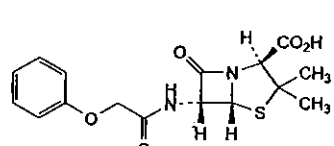


A. phenol.

Ph Eur

Phenoxymethylpenicillin

(Ph. Eur. monograph 0148)



$C_{16}H_{18}N_2O_5S$ 350.4 *87-08-1*

Action and use
Penicillin antibacterial.

Ph Eur

DEFINITION

(2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-[(2-phenoxyacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

Substance produced by the growth of certain strains of *Penicillium notatum* or related microorganisms.

Content
95.0 per cent to 102.0 per cent for the sum of the percentage contents of phenoxymethylpenicillin and 4-hydroxyphenoxymethylpenicillin (anhydrous substance).

CHARACTERS

Appearance
White or almost white, slightly hygroscopic, crystalline powder.

Solubility
Very slightly soluble in water, soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: B.

Second identification: A, C, D.

A. pH (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *phenoxymethylpenicillin CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 25 mg of the substance to be examined in 5 mL of *acetone R.*

Reference solution (a) Dissolve 25 mg of *phenoxymethylpenicillin CRS* in 5 mL of *acetone R.*

Reference solution (b) Dissolve 25 mg of *benzylpenicillin potassium CRS* and 25 mg of *phenoxymethylpenicillin potassium CRS* in 5 mL of *water R.*

Plate *TLC silanised silica gel plate R.*

Mobile phase Mix 30 volumes of *acetone R* and 70 volumes of a 154 g/L solution of *ammonium acetate R* adjusted to pH 5.0 with *glacial acetic acid R.*

Application 1 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Expose to iodine vapour until the spots appear and examine in daylight.

System suitability Reference solution (b):
— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Place about 2 mg in a test-tube about 150 mm long and 15 mm in diameter. Moisten with 0.05 mL of *water R* and add 2 mL of *sulfuric acid-formaldehyde reagent R.* Mix the contents of the tube by swirling; the solution is reddish-brown. Place the test-tube on a water-bath for 1 min; a dark reddish-brown colour develops.

TESTS

pH (*2.2.3*)
2.4 to 4.0.

Suspend 50 mg in 10 mL of *carbon dioxide-free water R.*

Related substances
Liquid chromatography (*2.2.29*).

Solution A To 250 mL of *0.2 M potassium dihydrogen phosphate R* add 500 mL of *water R*, adjust to pH 6.5 with an 8.4 g/L solution of *sodium hydroxide R* and dilute to 1000 mL with *water R.*

Test solution (a) Dissolve 50.0 mg of the substance to be examined in solution A and dilute to 50.0 mL with solution A.

Test solution (b) *Prepare immediately before use.* Dissolve 80.0 mg of the substance to be examined in solution A and dilute to 20.0 mL with solution A.

Reference solution (a) Dissolve 55.0 mg of *phenoxymethylpenicillin potassium CRS* in solution A and dilute to 50.0 mL with solution A.

Reference solution (b) Dissolve 9 mg of *phenoxymethylpenicillin for system suitability CRS* (containing impurities B, D, E and F) in 2 mL of solution A.

Reference solution (c) Dilute 1.0 mL of test solution (b) to 100.0 mL with solution A.

Reference solution (d) Dilute 1.0 mL of reference solution (c) to 20.0 mL with solution A.

Column:
- *size*: l = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm);
- *temperature*: 50 °C.

Mobile phase:
- *mobile phase A*: *phosphate buffer solution pH 3.4 R, methanol R, water for chromatography R* (10:30:60 *V/V/V*);
- *mobile phase B*: *phosphate buffer solution pH 3.4 R, methanol R, water for chromatography R* (5:60:35 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	85	15
2 - 5	85 → 70	15 → 30
5 - 17	70 → 0	30 → 100
17 - 22	0	100

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL of test solution (b) and reference solutions (b), (c) and (d).

Identification of impurities Use the chromatogram supplied with *phenoxymethylpenicillin for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B, D, E and F.

Relative retention With reference to phenoxymethylpenicillin (retention time = about 11 min): impurity B = about 0.29; impurity D = about 0.38; impurity E = about 0.55 and 0.61; impurity F = about 0.88 and 0.95.

System suitability:
- *resolution*: minimum 3.0 between the peaks due to the epimers of impurity F in the chromatogram obtained with reference solution (b);
- *signal-to-noise ratio*: minimum 20 for the principal peak in the chromatogram obtained with reference solution (d).

Calculation of percentage contents:
- *correction factors*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.5; impurity E = 1.3;
- for each impurity, use the concentration of phenoxymethylpenicillin in reference solution (c).

Limits:
- *impurity E (sum of isomers), impurity F (sum of epimers)*: for each impurity, maximum 1.0 per cent;
- *impurity B*: maximum 0.2 per cent;
- *any other impurity*: for each impurity, maximum 0.15 per cent;
- *sum of impurities other than D*: maximum 3.0 per cent;
- *reporting threshold*: 0.05 per cent.

Impurity D (4-hydroxyphenoxymethylpenicillin)
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (c).

Calculation of percentage content:
- *correction factor*: multiply the peak area of impurity D by 1.7;
- use the concentration of phenoxymethylpenicillin in reference solution (c).

Limit Maximum 1.0 per cent (anhydrous substance).

Water
(*2.5.12*): maximum 0.5 per cent, determined on 1.00 g.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (a) and reference solution (a).

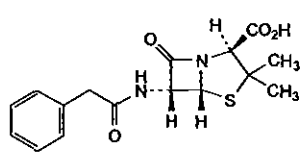
Calculate the percentage content of $C_{16}H_{18}N_2O_5S$ taking into account the assigned content of *phenoxymethylpenicillin potassium CRS* and a conversion factor of 0.902.

Calculate the sum of the percentage contents of phenoxymethylpenicillin and of 4-hydroxyphenoxymethylpenicillin.

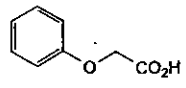
STORAGE
In an airtight container.

IMPURITIES
Specified impurities *B, D, E, F.*

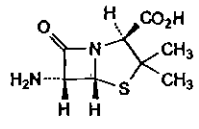
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, C.*



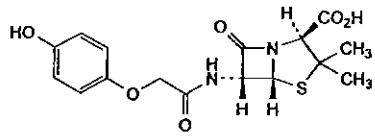
A. (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[(2-phenylacetyl) amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (benzylpenicillin),



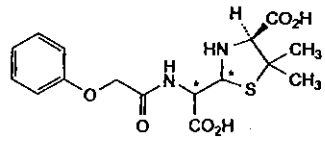
B. phenoxyacetic acid,



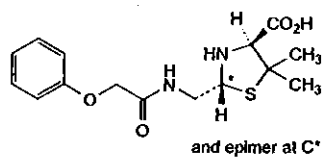
C. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),



D. (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[[2-(4-hydroxyphenoxy) acetyl]amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (4-hydroxyphenoxymethylpenicillin),



E. (4*S*)-2-[carboxy[(2-phenoxyacetyl)amino]methyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid (penicilloic acids of phenoxymethylpenicillin),



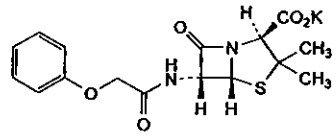
and epimer at C*

F. (2*RS*,4*S*)-5,5-dimethyl-2-[[(2-phenoxyacetyl) amino]methyl]-1,3-thiazolidine-4-carboxylic acid (penilloic acids of phenoxymethylpenicillin).

Ph Eur

Phenoxymethylpenicillin Potassium

(Ph. Eur. monograph 0149)



$C_{16}H_{17}KN_2O_5S$ 388.5 *132-98-9*

Action and use
Penicillin antibacterial.

Preparations
Phenoxymethylpenicillin Oral Solution
Phenoxymethylpenicillin Tablets

Ph Eur

DEFINITION

Potassium (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[(2-phenoxyacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate.

Substance produced by the growth of certain strains of *Penicillium notatum* or related microorganisms.

Content
95.0 per cent to 102.0 per cent for the sum of the percentage contents of phenoxymethylpenicillin potassium and 4-hydroxyphenoxymethylpenicillin potassium (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

First identification: A, D.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *phenoxymethylpenicillin potassium CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 25 mg of the substance to be examined in 5 mL of *water R.*

Reference solution (a) Dissolve 25 mg of *phenoxymethylpenicillin potassium CRS* in 5 mL of *water R.*

Reference solution (b) Dissolve 25 mg of *benzylpenicillin potassium CRS* and 25 mg of *phenoxymethylpenicillin potassium CRS* in 5 mL of *water R.*

Plate *TLC silanised silica gel plate R.*

Mobile phase Mix 30 volumes of *acetone R* and 70 volumes of a 154 g/L solution of *ammonium acetate R* adjusted to pH 5.0 with *glacial acetic acid R.*

Application 1 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Expose to iodine vapour until the spots appear and examine in daylight.

System suitability Reference solution (b):
— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Place about 2 mg in a test-tube about 150 mm long and 15 mm in diameter. Moisten with 0.05 mL of *water R* and add 2 mL of *sulfuric acid-formaldehyde reagent R.* Mix the contents of the tube by swirling; the solution is reddish-brown. Place the test-tube on a water-bath for 1 min; a dark reddish-brown colour develops.

D. It gives reaction (a) of potassium (*2.3.1*).

TESTS

pH (*2.2.3*)
5.5 to 7.5.

Dissolve 50 mg in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Solution A To 250 mL of *0.2 M potassium dihydrogen phosphate R* add 500 mL of *water R,* adjust to pH 6.5 with an 8.4 g/L solution of *sodium hydroxide R* and dilute to 1000 mL with *water R.*

Test solution (a) Dissolve 50.0 mg of the substance to be examined in solution A and dilute to 50.0 mL with solution A.

Test solution (b) *Prepare immediately before use.* Dissolve 80.0 mg of the substance to be examined in solution A and dilute to 20.0 mL with solution A.

Reference solution (a) Dissolve 50.0 mg of *phenoxymethylpenicillin potassium CRS* in solution A and dilute to 50.0 mL with solution A.

Reference solution (b) Dissolve 8 mg of *phenoxymethylpenicillin for system suitability CRS* (containing impurities B, D, E and F) in 2 mL of solution A.

Reference solution (c) Dilute 1.0 mL of test solution (b) to 100.0 mL with solution A.

Reference solution (d) Dilute 1.0 mL of reference solution (c) to 20.0 mL with solution A.

Column:
- *size: l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (3 µm);
- *temperature:* 50 °C.

Mobile phase:
- *mobile phase A: phosphate buffer solution pH 3.4 R, methanol R, water for chromatography R* (10:30:60 *V/V/V*);
- *mobile phase B: phosphate buffer solution pH 3.4 R, methanol R, water for chromatography R* (5:60:35 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	85	15
2 - 5	85 → 70	15 → 30
5 - 17	70 → 0	30 → 100
17 - 22	0	100

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL of test solution (b) and reference solutions (b), (c) and (d).

Identification of impurities Use the chromatogram supplied with *phenoxymethylpenicillin for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B, D, E and F.

Relative retention With reference to phenoxymethylpenicillin (retention time = about 11 min): impurity B = about 0.29; impurity D = about 0.38; impurity E = about 0.55 and 0.61; impurity F = about 0.88 and 0.95.

System suitability:
- *resolution*: minimum 3.0 between the peaks due to the epimers of impurity F in the chromatogram obtained with reference solution (b);
- *signal-to-noise ratio*: minimum 20 for the principal peak in the chromatogram obtained with reference solution (d).

Calculation of percentage contents:
- *correction factors*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.6; impurity E = 1.3;
- for each impurity, use the concentration of phenoxymethylpenicillin potassium in reference solution (c).

Limits:
- *impurity E (sum of isomers), impurity F (sum of epimers)*: for each impurity, maximum 1.0 per cent;
- *impurity B*: maximum 0.3 per cent;
- *any other impurity*: for each impurity, maximum 0.15 per cent;
- *sum of impurities other than D*: maximum 3.0 per cent;
- *reporting threshold*: 0.05 per cent.

Impurity D (4-hydroxyphenoxymethylpenicillin)
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (c).

Calculation of percentage content:
- *correction factor*: multiply the peak area of impurity D by 1.7;
- use the concentration of phenoxymethylpenicillin potassium in reference solution (c).

Limit Maximum 4.0 per cent (anhydrous substance).

Water
(*2.5.12*): maximum 1.0 per cent, determined on 1.00 g.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

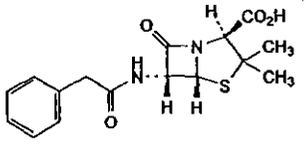
Injection Test solution (a) and reference solution (a).

Calculate the percentage content of $C_{16}H_{17}KN_2O_5S$ taking into account the assigned content of *phenoxymethylpenicillin potassium CRS*.

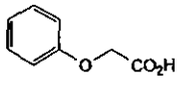
Calculate the sum of the percentage contents of phenoxymethylpenicillin potassium and of 4-hydroxyphenoxymethylpenicillin potassium.

IMPURITIES
Specified impurities *B, D, E, F.*

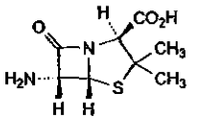
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, C.*



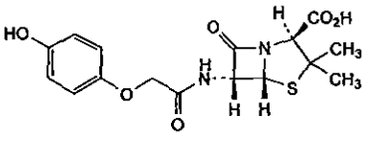
A. (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[(2-phenylacetyl) amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (benzylpenicillin),



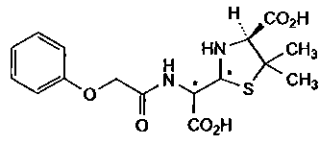
B. phenoxyacetic acid,



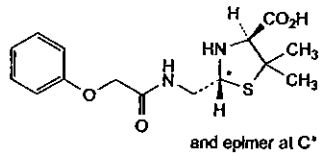
C. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),



D. (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[[2-(4-hydroxyphenoxy) acetyl]amino]-4-thia- 1-azabicyclo[3.2.0]heptane-2-carboxylic acid (4-hydroxyphenoxymethylpenicillin),



E. (4*S*)-2-[carboxy[(2-phenoxyacetyl)amino]methyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid (penicilloic acids of phenoxymethylpenicillin),



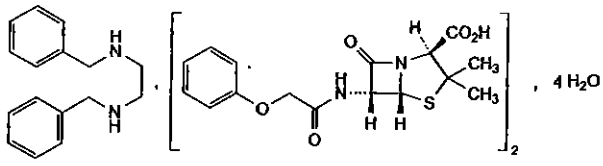
and epimer at C*

F. (2*RS*,4*S*)-5,5-dimethyl-2-[[(2-phenoxyacetyl)amino]methyl]-1,3-thiazolidine-4-carboxylic acid (penilloic acids of phenoxymethylpenicillin).

Ph Eur

Phenoxymethylpenicillin (Benzathine) Tetrahydrate

(Ph. Eur. monograph 2636)



$C_{48}H_{56}N_6O_{10}S_2,4H_2O$ 1013 *2113728-41-7*

Action and use
Penicillin antibacterial.

Ph Eur

DEFINITION

N^1,N^2-Dibenzylethane-1,2-diamine bis[(2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-(2-phenoxyacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate] tetrahydrate.

Salt of a substance produced by the growth of certain strains of *Penicillium notatum* or related micro-organisms.

Content
— 94.0 per cent to 102.0 per cent for the sum of benzathine phenoxymethylpenicillin and benzathine 4-hydroxyphenoxymethylpenicillin (anhydrous substance) with correction for dispersing or suspending agents;
— *benzathine*: 23.0 per cent to 26.0 per cent (anhydrous substance).

Dispersing or suspending agents (e.g. lecithin) may be added.

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Very slightly soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *benzathine phenoxymethylpenicillin CRS.*

TESTS

pH (*2.2.3*)
4.0 to 6.5.

Suspend 0.600 g in 20 mL of *carbon dioxide-free water R.*

Related substances
Liquid chromatography (*2.2.29*).

Solution A To 250 mL of *0.2 M potassium dihydrogen phosphate R* add 500 mL of *water R*, adjust to pH 6.5 with an 8.4 g/L solution of *sodium hydroxide R* and dilute to 1000 mL with *water R*.

Test solution (a) Dissolve 40.0 mg of the substance to be examined in 10 mL of *methanol R* and dilute to 100.0 mL with solution A.

Test solution (b) *Prepare immediately before use.* Dissolve 80.0 mg of the substance to be examined in 10 mL of *methanol R* and dilute to 20.0 mL with solution A.

Reference solution (a) Dissolve 40.0 mg of *benzathine phenoxymethylpenicillin CRS* in 10 mL of *methanol R* and dilute to 100.0 mL with solution A.

Reference solution (b) Dissolve 8 mg of *benzathine phenoxymethylpenicillin for system suitability CRS* (containing impurities B, D, E, F, G, H and I) in 1 mL of *methanol R* and dilute to 2 mL with solution A.

Reference solution (c) Dilute 1.0 mL of reference solution (a) to 10.0 mL with a mixture of equal volumes of *methanol R* and solution A.

Reference solution (d) Dilute 1.0 mL of reference solution (c) to 25.0 mL with a mixture of equal volumes of *methanol R* and solution A.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm);
— *temperature*: 50 °C.

Mobile phase:
— *mobile phase A*: *phosphate buffer solution pH 3.4 R, methanol R, water for chromatography R* (10:30:60 *V/V/V*);
— *mobile phase B*: *phosphate buffer solution pH 3.4 R, water for chromatography R, methanol R* (2.5:27.5:70 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	90	10
2 - 5	90 → 75	10 → 25
5 - 17	75 → 0	25 → 100
17 - 24	0	100

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL of test solution (b) and reference solutions (b), (c) and (d).

Identification of impurities Use the chromatogram supplied with *benzathine phenoxymethylpenicillin for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B, D, E, F, G, H and I.

Relative retention With reference to phenoxymethylpenicillin (retention time = about 10 min): benzathine = about 0.2; impurity B = about 0.3; impurity D = about 0.4; impurity E = about 0.60 and 0.64; impurity F = about 0.91 and 0.97; impurity H = about 1.36; impurity G = about 1.39; impurity I = about 2.4.

System suitability:
- — *resolution*: minimum 3.0 between the peaks due to the epimers of impurity F in the chromatogram obtained with reference solution (b);
- — *signal-to-noise ratio*: minimum 10 for the principal peak in the chromatogram obtained with reference solution (d).

Calculation of percentage contents:
- — *correction factors*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.5; impurity D = 1.7; impurity E = 1.3;
- — for each impurity, use the concentration of phenoxymethylpenicillin in reference solution (c).

Limits:
- — *impurity G*: maximum 2.0 per cent;
- — *impurity E (sum of isomers), impurity F (sum of epimers)*: for each impurity, maximum 1.0 per cent;
- — *impurities D, H, I*: for each impurity, maximum 0.5 per cent;
- — *impurity B*: maximum 0.2 per cent;
- — *any other impurity*: for each impurity, maximum 0.15 per cent;
- — *sum of impurities other than D*: maximum 4.0 per cent;
- — *reporting threshold*: 0.05 per cent; disregard the peak due to benzathine.

Water (*2.5.12, Method B*)
5.0 per cent to 8.0 per cent, determined on 0.200 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (a) and reference solution (a).

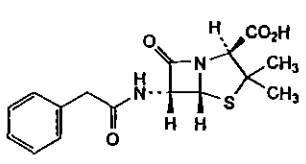
Calculate the percentage contents of benzathine ($C_{16}H_{20}N_2$) and benzathine phenoxymethylpenicillin ($C_{48}H_{56}N_6O_{10}S_2$) taking into account the assigned content of *benzathine phenoxymethylpenicillin CRS*.

Calculate the sum of the percentage contents of benzathine phenoxymethylpenicillin and of benzathine 4-hydroxyphenoxymethylpenicillin.

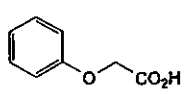
IMPURITIES

Specified impurities *B, D, E, F, G, H, I.*

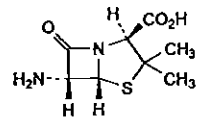
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, C.*



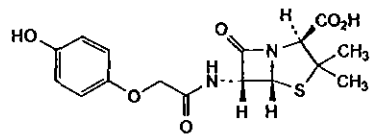
A. (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (benzylpenicillin),



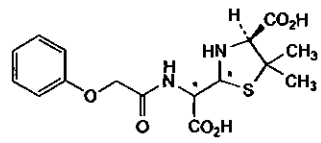
B. phenoxyacetic acid,



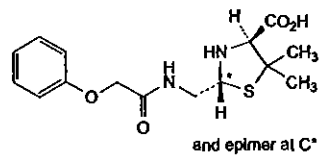
C. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),



D. (2*S*,5*R*,6*R*)-6-[2-(4-hydroxyphenoxy)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (4-hydroxyphenoxymethylpenicillin),

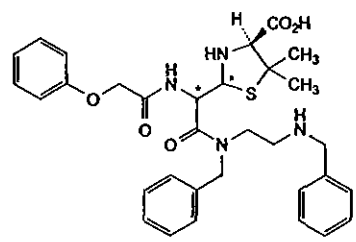


E. (2*Ξ*,4*S*)-2-[(*Ξ*)-carboxy(2-phenoxyacetamido)methyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid (penicilloic acids of phenoxymethylpenicillin),



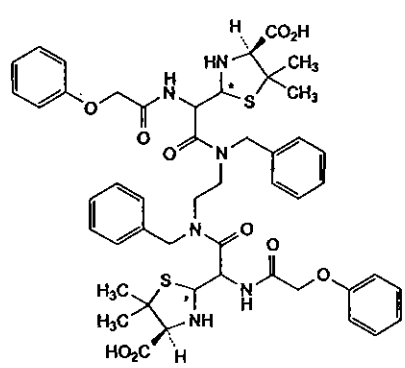
and epimer at C*

F. (2*RS*,4*S*)-5,5-dimethyl-2-[(2-phenoxyacetamido)methyl]-1,3-thiazolidine-4-carboxylic acid (penilloic acids of phenoxymethylpenicillin),



G. (2*Ξ*,4*S*)-2-[(1*Ξ*)-2-[benzyl[2-(benzylamino)ethyl]amino]-2-oxo-1-(2-phenoxyacetamido)ethyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid,

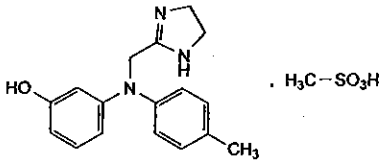
H. unknown structure,



I. (2*Ξ*,2′*Ξ*,4*S*,4′*S*)-2,2′-[ethane-1,2-diylbis[(benzylazanediyl)[2-oxo-1-(2-phenoxyacetamido)ethane-2,1-diyl]]]bis(5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid).

Phentolamine Mesilate

(Ph. Eur. monograph 1138)



$C_{18}H_{23}N_3O_4S$ 377.5 *65-28-1*

Action and use
Alpha-adrenoceptor antagonist.

Preparation
Phentolamine Injection

Ph Eur

DEFINITION

3-[[(4,5-Dihydro-1*H*-imidazol-2-yl)methyl](4-methylphenyl)amino]phenol methanesulfonate.

Content
98.0 per cent to 101.0 per cent (dried substance).

PRODUCTION

It is considered that alkyl methanesulfonate esters are genotoxic and are potential impurities in phentolamine mesilate. The manufacturing process should be developed taking into consideration the principles of quality risk management, together with considerations of the quality of starting materials, process capability and validation.
The general methods *2.5.37. Methyl, ethyl and isopropyl methanesulfonate in methanesulfonic acid, 2.5.38. Methyl, ethyl and isopropyl methanesulfonate in active substances* and *2.5.39. Methanesulfonyl chloride in methanesulfonic acid* are available to assist manufacturers.

CHARACTERS

Appearance
White or almost white, slightly hygroscopic, crystalline powder.

Solubility
Freely soluble in water and in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

First identification: C, E.

Second identification: A, B, D, E.

A. Melting point (*2.2.14*): 178 °C to 182 °C.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 60.0 mg in *water R* and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of the solution to 100.0 mL with *water R*.

Spectral range 230-350 nm.

Absorption maximum At 278 nm.

Specific absorbance at the absorption maximum 220 to 245.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *phentolamine mesilate CRS*.

D. Dissolve 0.5 g in a mixture of 5 mL of *ethanol (96 per cent) R* and 5 mL of a 10 g/L solution of *hydrochloric acid R* and add 0.5 mL of a 5 g/L solution of *ammonium vanadate R*. A light green precipitate is produced.

E. Mix 50 mg with 0.2 g of *sodium hydroxide R*, heat to fusion and continue heating for a few seconds. Allow to cool and add 0.5 mL of warm *water R*. Acidify with *dilute hydrochloric acid R* and heat. Sulfur dioxide is evolved, which turns moistened *starch iodate paper R* blue.

TESTS

Acidity
Dissolve 0.1 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent. Add 0.1 mL of *methyl red solution R*. If the solution is red, not more than 0.05 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator to yellow.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 25 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dilute 5.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 2.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution (b) Dissolve 5 mg of *phentolamine for system suitability CRS* (containing impurities A and C) in the mobile phase and dilute to 10 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *phenylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 30 °C.

Mobile phase Mix 33 volumes of *acetonitrile for chromatography R* and 67 volumes of a 0.5 g/L solution of *ammonium acetate R* previously adjusted to pH 5.9 with *dilute acetic acid R*.

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 10 µL.

Run time 1.5 times the retention time of phentolamine.

Identification of impurities Use the chromatogram supplied with *phentolamine for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and C.

Relative retention With reference to phentolamine (retention time = about 15 min): impurity A = about 0.7; impurity C = about 1.2.

System suitability Reference solution (b):
— *resolution*: minimum 3.0 between the peaks due to phentolamine and impurity C.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity A by 1.7;
— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

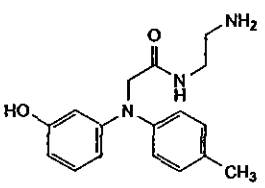
ASSAY
Dissolve 0.300 g in 100 mL of *2-propanol R1*. Titrate under a stream of nitrogen with *0.1 M tetrabutylammonium hydroxide in 2-propanol*. Determine the end-point potentiometrically (*2.2.20*), using a glass indicator electrode and a suitable reference electrode. Carry out a blank titration.

1 mL of *0.1 M tetrabutylammonium hydroxide in 2-propanol* is equivalent to 37.75 mg of $C_{18}H_{23}N_3O_4S$.

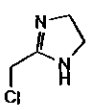
STORAGE
In an airtight container, protected from light.

IMPURITIES
Specified impurities *A.*

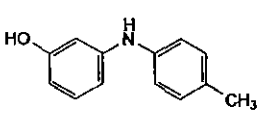
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C.*



A. *N*-(2-aminoethyl)-2-[(3-hydroxyphenyl)(4-methylphenyl) amino]acetamide,



B. 2-(chloromethyl)-4,5-dihydro-1*H*-imidazole,

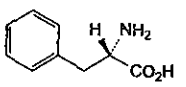


C. 3-[(4-methylphenyl)amino]phenol.

Ph Eur

Phenylalanine

(Ph. Eur. monograph 0782)



$C_9H_{11}NO_2$ 165.2 *63-91-2*

Action and use
Amino acid.

Ph Eur

DEFINITION
(2*S*)-2-Amino-3-phenylpropanoic acid.

Product of fermentation or of protein hydrolysis.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder, or shiny, white flakes.

Solubility
Sparingly soluble in water, very slightly soluble in ethanol (96 per cent). It dissolves in dilute mineral acids and in dilute solutions of alkali hydroxides.

IDENTIFICATION
First identification: A, B.

Second identification: A, C, D.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *phenylalanine CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in a mixture of equal volumes of *glacial acetic acid R* and *water R* and dilute to 50 mL with the same mixture of solvents.

Reference solution Dissolve 10 mg of *phenylalanine CRS* in a mixture of equal volumes of *glacial acetic acid R* and *water R* and dilute to 50 mL with the same mixture of solvents.

Plate *TLC silica gel plate R.*

Mobile phase *glacial acetic acid R, water R, butanol R* (20:20:60 *V/V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *ninhydrin solution R* and heat at 105 °C for 15 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. To about 10 mg add 0.5 g of *potassium nitrate R* and 2 mL of *sulfuric acid R*. Heat on a water-bath for 20 min. Allow to cool. Add 5 mL of a 50 g/L solution of *hydroxylamine hydrochloride R* and allow to stand in iced water for 10 min. Add 9 mL of *strong sodium hydroxide solution R*. A violet-red or violet-brown colour develops.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Dissolve 0.5 g in a 103 g/L solution of *hydrochloric acid R* and dilute to 10 mL with the same solution.

Specific optical rotation (*2.2.7*)

−35.5 to −33.0 (dried substance).

Dissolve 0.50 g in *water R* and dilute to 25.0 mL with the same solvent.

Ninhydrin-positive substances

Amino acid analysis (*2.2.56*). For analysis, use Method 1.

The concentrations of the test solution and the reference solutions may be adapted according to the sensitivity of the equipment used. The concentrations of all solutions are adjusted so that the system suitability requirements described in general chapter *2.2.46* are fulfilled, keeping the ratios of concentrations between all solutions as described.

Solution A *dilute hydrochloric acid R1* or a sample preparation buffer suitable for the apparatus used.

Test solution Dissolve 30.0 mg of the substance to be examined in solution A and dilute to 50.0 mL with solution A.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with solution A. Dilute 2.0 mL of this solution to 10.0 mL with solution A.

Reference solution (b) Dissolve 30.0 mg of *proline R* in solution A and dilute to 100.0 mL with solution A. Dilute 1.0 mL of the solution to 250.0 mL with solution A.

Reference solution (c) Dilute 6.0 mL of *ammonium standard solution (100 ppm NH_4) R* to 50.0 mL with solution A. Dilute 1.0 mL of this solution to 100.0 mL with solution A.

Reference solution (d) Dissolve 30 mg of *isoleucine R* and 30 mg of *leucine R* in solution A and dilute to 50.0 mL with solution A. Dilute 1.0 mL of the solution to 200.0 mL with solution A.

Blank solution Solution A.

Inject suitable, equal amounts of the test, blank and reference solutions into the amino acid analyser. Run a program suitable for the determination of physiological amino acids.

System suitability Reference solution (d):

— *resolution*: minimum 1.5 between the peaks due to isoleucine and leucine.

Calculation of percentage contents:

— for any ninhydrin-positive substance detected at 570 nm, use the concentration of phenylalanine in reference solution (a);

— for any ninhydrin-positive substance detected at 440 nm, use the concentration of proline in reference solution (b); if a peak is above the reporting threshold at both wavelengths, use the result obtained at 570 nm for quantification.

Limits:

— *any ninhydrin-positive substance*: for each impurity, maximum 0.2 per cent;

— *total*: maximum 0.5 per cent;

— *reporting threshold*: 0.05 per cent.

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Chlorides (*2.4.4*)

Maximum 200 ppm.

Dissolve 0.25 g in 3 mL of *dilute nitric acid R* and dilute to 15 mL with *water R*. The solution, without further addition of nitric acid, complies with the test.

Sulfates (*2.4.13*)

Maximum 300 ppm.

Dissolve 0.5 g in a mixture of 5 volumes of *dilute hydrochloric acid R* and 25 volumes of *distilled water R* and dilute to 15 mL with the same mixture of solvents.

Ammonium

Amino acid analysis (*2.2.56*) as described in the test for ninhydrin-positive substances with the following modifications.

Injection Test solution, reference solution (c) and blank solution.

Limit:

— *ammonium at 570 nm*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.02 per cent), taking into account the peak due to ammonium in the chromatogram obtained with the blank solution.

Iron (*2.4.9*)

Maximum 10 ppm.

In a separating funnel, dissolve 1.0 g in 10 mL of *dilute hydrochloric acid R*. Shake with 3 quantities, each of 10 mL, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 mL of *water R* and shake for 3 min. Use the aqueous layer.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 3 mL of *anhydrous formic acid R*. Add 30 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

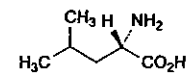
1 mL of *0.1 M perchloric acid* is equivalent to 16.52 mg of $C_9H_{11}NO_2$.

STORAGE

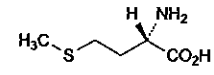
Protected from light.

IMPURITIES

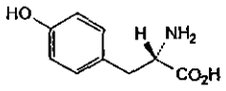
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D.*



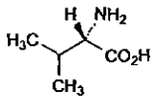
A. (2*S*)-2-amino-4-methylpentanoic acid (leucine),



B. (2*S*)-2-amino-4-(methylsulfanyl)butanoic acid (methionine),



C. (2*S*)-2-amino-3-(4-hydroxyphenyl)propanoic acid (tyrosine),

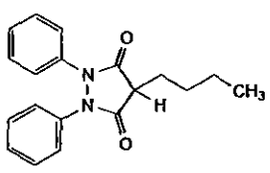


D. (2*S*)-2-amino-3-methylbutanoic acid (valine).

Ph Eur

Phenylbutazone

(Ph. Eur. monograph 0422)



$C_{19}H_{20}N_2O_2$ 308.4 *50-33-9*

Action and use
Cyclo-oxygenase inhibitor; pyrazolone analgesic.

Ph Eur

DEFINITION
4-Butyl-1,2-diphenylpyrazolidine-3,5-dione.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, sparingly soluble in alcohol. It dissolves in alkaline solutions.

IDENTIFICATION
First identification: A, C.
Second identification: A, B, D.

A. Melting point (*2.2.14*): 104 °C to 107 °C.

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 25 mg of the substance to be examined in a mixture of equal volumes of *ethanol R* and *methylene chloride R* and dilute to 25 mL with the same mixture of solvents.

Reference solution Dissolve 25 mg of *phenylbutazone CRS* in a mixture of equal volumes of *ethanol R* and *methylene chloride R* and dilute to 25 mL with the same mixture of solvents.

Plate *TLC silica gel GF_{254} plate R.*

Mobile phase *acetone R, methylene chloride R* (20:80 *V/V*).

Application 5 µL.

Development Over a path of 10 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *phenylbutazone CRS.*

D. To 0.1 g add 1 mL of *glacial acetic acid R* and 2 mL of *hydrochloric acid R* and heat the mixture under a reflux condenser for 30 min. Cool, add 10 mL of *water R* and filter. To the filtrate add 3 mL of a 7 g/L solution of *sodium nitrite R*. A yellow colour is produced. To 1 mL of the solution add a solution of 10 mg of *β-naphthol R* in 5 mL of *sodium carbonate solution R*. A brownish-red to violet-red precipitate is formed.

TESTS

Solution S
Dissolve 1.0 g with shaking in 20 mL of *dilute sodium hydroxide solution R* and maintain the solution at 25 °C for 3 h.

Appearance of solution
Solution S is clear (*2.2.1*).

Acidity or alkalinity
Heat to boiling 1.0 g in 50 mL of *water R*, cool with shaking in a closed flask and filter. To 25 mL of the filtrate add 0.5 mL of *phenolphthalein solution R*. The solution is colourless. Not more than 0.5 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator. Add 0.6 mL of *0.01 M hydrochloric acid* and 0.1 mL of *methyl red solution R*; the solution is red or orange.

Absorbance (*2.2.25*)
Maximum 0.20 for solution S at 420 nm in a 4 cm cell.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 100.0 mg of the substance to be examined in *acetonitrile R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *acetonitrile R*. Dilute 1.0 mL to 10.0 mL with *acetonitrile R*.

Reference solution (b) Dissolve 5 mg of *phenylbutazone impurity B CRS* and 5 mg of *1,2-diphenylhydrazine R* in *acetonitrile R*, add 0.5 mL of the test solution and dilute to 50 mL with *acetonitrile R*. Dilute 2.5 mL to 10 mL with *acetonitrile R*.

Reference solution (c) Dissolve 1.0 mg of *benzidine R* in *acetonitrile R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL to 100.0 mL with *acetonitrile R*. Dilute 5.0 mL to 10.0 mL with *acetonitrile R*.

Column:
— *size*: *l* = 0.125 m, Ø = 4.0 mm,
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm),
— *temperature*: 30 °C.

Mobile phase:
— *mobile phase A*: dissolve 1.36 g of *sodium acetate R* in *water R*, adjust to pH 5.2 with a 52.5 g/L solution of *citric acid monohydrate R* and dilute to 1000 mL with *water R*,
— *mobile phase B*: *acetonitrile R*,

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	70	30
10 - 20	70 → 40	30 → 60
20 - 35	40	60
35 - 40	40 → 70	60 → 30

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 240 nm.

Injection 20 µL; inject the test solution and reference solutions (a) and (b).

Relative retentions With reference to phenylbutazone (retention time = about 13 min): impurity E = about 0.2; impurity A = about 0.5; impurity B = about 1.2; impurity C = about 1.3; impurity D = about 1.7.

System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the peaks due to phenylbutazone and to impurity B.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity C by 0.55,
— *impurities A, B*: for each impurity, not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent),
— *impurity C*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.20 per cent),
— *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),
— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.025 per cent); disregard any peak due to impurity E.

Impurity E

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Detection Spectrophotometer at 280 nm.

Injection Test solution and reference solution (c).

System suitability Reference solution (c):
— *signal-to-noise ratio*: minimum 10 for the principal peak.

Limit:
— *impurity E*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (5 ppm).

Loss on drying (*2.2.32*)

Maximum 0.2 per cent, determined on 1.000 g by drying *in vacuo* at 80 °C for 4 h.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

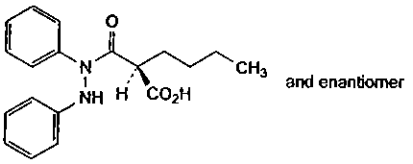
Dissolve 0.250 g in 25 mL of *acetone R* and add 0.5 mL of *bromothymol blue solution R1*. Titrate with *0.1 M sodium hydroxide* until a blue colour is obtained which persists for 15 s. Carry out a blank titration.

1 mL of *0.1 M sodium hydroxide* is equivalent to 30.84 mg of $C_{19}H_{20}N_2O_2$.

STORAGE

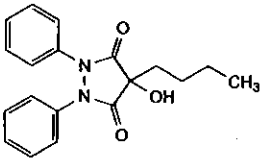
Protected from light.

IMPURITIES



and enantiomer

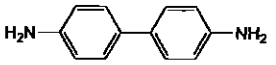
A. (2*RS*)-2-[(1,2-diphenyldiazanyl)carbonyl]hexanoic acid,



B. 4-butyl-4-hydroxy-1,2-diphenylpyrazolidine-3,5-dione,

C. C_6H_5-NH-NH-C_6H_5: 1,2-diphenyldiazane (1,2-diphenylhydrazine),

D. C_6H_5-N=N-C_6H_5: 1,2-diphenyldiazene,

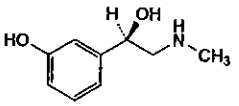


E. biphenyl-4,4′-diamine (benzidine).

Ph Eur

Phenylephrine

(Ph. Eur. monograph 1035)



$C_9H_{13}NO_2$ 167.2 *59-42-7*

Action and use

Alpha-adrenoceptor agonist.

Ph Eur

DEFINITION

(1*R*)-1-(3-Hydroxyphenyl)-2-(methylamino)ethanol.

Content

99.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, sparingly soluble in methanol, slightly soluble in ethanol (96 per cent). It dissolves in dilute mineral acids and in dilute solutions of alkali hydroxides.

mp

About 174 °C.

IDENTIFICATION

First identification: A, B.

Second identification: A, C, D.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *phenylephrine CRS*.

C. Thin-layer chromatography (*2.2.27*).

Solvent mixture A mixture of equal volumes of *methylene chloride R* and methanolic hydrochloric acid (*hydrochloric acid R* diluted 10-fold with *methanol R*).

Test solution Dissolve 0.1 g of the substance to be examined in the solvent mixture and dilute to 5 mL with the solvent mixture.

Reference solution Dissolve 20 mg of *phenylephrine CRS* in the solvent mixture and dilute to 1 mL with the solvent mixture.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *concentrated ammonia R, methanol R, methylene chloride R* (0.5:25:70 *V/V/V*).

Application 10 µL.

Development Over a path of 15 cm.

Drying In a current of cold air.

Detection Examine in ultraviolet light at 254 nm; spray with a 1 g/L solution of *fast red B salt R* in a 50 g/L solution of *sodium carbonate R* and examine in daylight.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve about 10 mg in 1 mL of *1 M hydrochloric acid*, add 0.05 mL of *copper sulfate solution R* and 1 mL of a 200 g/L solution of *sodium hydroxide R*. A violet colour develops. Add 1 mL of *ether R* and shake. The upper layer remains colourless.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

Dissolve 1 g in *1 M hydrochloric acid* and dilute to 10 mL with the same acid.

Specific optical rotation (*2.2.7*)

−53 to −57 (dried substance).

Dissolve 1.250 g in *1 M hydrochloric acid* and dilute to 25.0 mL with the same acid.

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture *dilute hydrochloric acid R*, mobile phase B, mobile phase A (5:200:800 *V/V/V*).

Buffer solution pH 2.8 Dissolve 3.25 g of *sodium octanesulfonate monohydrate R* in 1000 mL of *water for chromatography R* by stirring for 30 min and adjust to pH 2.8 with *dilute phosphoric acid R*.

Test solution Dissolve 41.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dilute 5.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 2.0 mL of this solution to 100.0 mL with the solvent mixture.

Reference solution (b) Dissolve the contents of a vial of *phenylephrine hydrochloride for peak identification CRS* (containing impurities C and E) in 2 mL of the solvent mixture.

Column:
- *size:* l = 0.055 m, Ø = 4.0 mm;
- *stationary phase:* *end-capped octadecylsilyl silica gel for chromatography R1* (3 µm);
- *temperature:* 45 °C.

Mobile phase:
- *mobile phase A:* *acetonitrile R1*, buffer solution pH 2.8 (10:90 *V/V*);
- *mobile phase B:* buffer solution pH 2.8, *acetonitrile R1* (10:90 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	93	7
3 - 13	93 → 70	7 → 30
13 - 14	70 → 93	30 → 7

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 10 µL.

Identification of impurities Identify the peaks due to impurities C and E using the chromatogram obtained with reference solution (b).

Relative retention With reference to phenylephrine (retention time = about 2.8 min): impurity C = about 1.3; impurity E = about 3.6.

System suitability:
- *symmetry factor:* maximum 1.9 for the principal peak in the chromatogram obtained with the test solution;
- *peak-to-valley ratio:* minimum 5.0, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to phenylephrine in the chromatogram obtained with reference solution (b).

Limits:
- *correction factors:* for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity C = 0.5; impurity E = 0.5;
- *impurities C, E:* for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *unspecified impurities:* for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total:* not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *disregard limit:* 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 60 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 16.72 mg of $C_9H_{13}NO_2$.

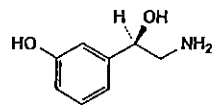
STORAGE

In an airtight container, protected from light.

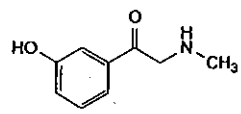
IMPURITIES

Specified impurities *C, E.*

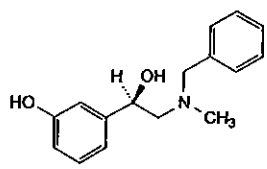
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) A, D.



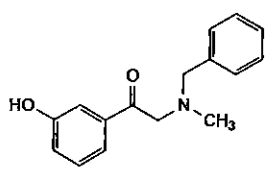
A. (1*R*)-2-amino-1-(3-hydroxyphenyl)ethanol (norphenylephrine),



C. 1-(3-hydroxyphenyl)-2-(methylamino)ethanone (phenylephrone),



D. (1*R*)-2-(benzylmethylamino)-1-(3-hydroxyphenyl)ethanol (benzylphenylephrine),

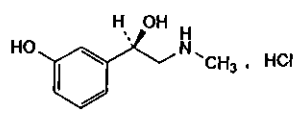


E. 2-(benzylmethylamino)-1-(3-hydroxyphenyl)ethanone (benzylphenylephrone).

Ph Eur

Phenylephrine Hydrochloride

(Ph. Eur. monograph 0632)



$C_9H_{14}ClNO_2$ 203.7 *61-76-7*

Action and use
Alpha-adrenoceptor agonist.

Preparations
Phenylephrine Eye Drops
Phenylephrine Injection
Phenylephrine Intracameral Injection

Ph Eur

DEFINITION
(1*R*)-1-(3-Hydroxyphenyl)-2-(methylamino)ethanol hydrochloride.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water and in ethanol (96 per cent).

mp
About 143 °C.

IDENTIFICATION
First identification: A, C, E.

Second identification: A, B, D, E.

A. Specific optical rotation (see Tests).

B. Melting point (*2.2.14*): 171 °C to 176 °C.

Dissolve 0.3 g in 3 mL of *water R*, add 1 mL of *dilute ammonia R1* and initiate crystallisation by scratching the wall of the tube with a glass rod. Wash the crystals with iced *water R* and dry at 105 °C for 2 h.

C. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *phenylephrine hydrochloride CRS.*

D. Dissolve about 10 mg in 1 mL of *water R* and add 0.05 mL of a 125 g/L solution of *copper sulfate pentahydrate R* and 1 mL of a 200 g/L solution of *sodium hydroxide R*. A violet colour is produced. Add 1 mL of *ether R* and shake; the upper layer remains colourless.

E. It gives reaction (a) of chlorides (*2.3.1*).

TESTS
Solution S
Dissolve 2.00 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity
To 10 mL of solution S add 0.1 mL of *methyl red solution R* and 0.2 mL of *0.01 M sodium hydroxide*. The solution is yellow. Not more than 0.4 mL of *0.01 M hydrochloric acid* is required to change the colour of the indicator to red.

Specific optical rotation (*2.2.7*)
−43 to −47 (dried substance), determined on solution S.

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture Mobile phase B, mobile phase A (20:80 *V/V*).

Buffer solution pH 2.8 Dissolve 3.25 g of *sodium octanesulfonate monohydrate R* in 1000 mL of *water for chromatography R* by stirring for 30 min and adjust to pH 2.8 with *dilute phosphoric acid R*.

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dilute 5.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 2.0 mL of this solution to 100.0 mL with the solvent mixture.

Reference solution (b) Dissolve the contents of a vial of *phenylephrine hydrochloride for peak identification CRS* (containing impurities C and E) in 2 mL of the solvent mixture.

Column:
- *size*: *l* = 0.055 m, Ø = 4.0 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R1* (3 µm);
- *temperature*: 45 °C.

Mobile phase:
- *mobile phase A*: *acetonitrile R1*, buffer solution pH 2.8 (10:90 *V/V*);
- *mobile phase B*: buffer solution pH 2.8, *acetonitrile R1* (10:90 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	93	7
3 - 13	93 → 70	7 → 30
13 - 14	70 → 93	30 → 7

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 10 µL.

Identification of impurities Identify the peaks due to impurities C and E using the chromatogram obtained with reference solution (b).

Relative retention With reference to phenylephrine (retention time = about 2.8 min): impurity C = about 1.3; impurity E = about 3.6.

System suitability:
- *symmetry factor*: maximum 1.9 for the principal peak in the chromatogram obtained with the test solution;
- *peak-to-valley ratio*: minimum 5.0, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to phenylephrine in the chromatogram obtained with reference solution (b).

Limits:
- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity C = 0.5; impurity E = 0.5;
- *impurities C, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Sulfates (*2.4.13*)
Maximum 500 ppm, determined on solution S.

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

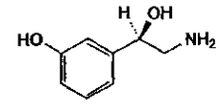
Dissolve 0.150 g in a mixture of 0.5 mL of *0.1 M hydrochloric acid* and 80 mL of *ethanol (96 per cent) R*. Carry out a potentiometric titration (*2.2.20*) using *0.1 M ethanolic sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M ethanolic sodium hydroxide* is equivalent to 20.37 mg of $C_9H_{14}ClNO_2$.

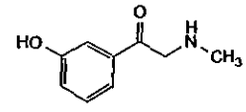
IMPURITIES

Specified impurities *C, E.*

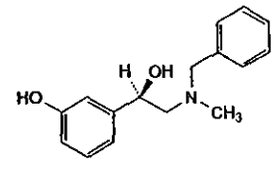
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, D.*



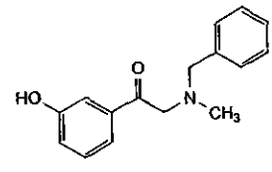
A. (1*R*)-2-amino-1-(3-hydroxyphenyl)ethanol (norphenylephrine),



C. 1-(3-hydroxyphenyl)-2-(methylamino)ethanone (phenylephrone),



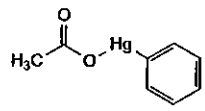
D. (1*R*)-2-(benzylmethylamino)-1-(3-hydroxyphenyl)ethanol (benzylphenylephrine),



E. 2-(benzylmethylamino)-1-(3-hydroxyphenyl)ethanone (benzylphenylephrone).

Phenylmercuric Acetate

(Ph. Eur. monograph 2042)



$C_8H_8HgO_2$ 336.7 *62-38-4*

Action and use
Antiseptic, antimicrobial preservative.

Ph Eur

DEFINITION

Content
98.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance
White or yellowish, crystalline powder or small, colourless crystals.

Solubility
Slightly soluble in water, soluble in acetone and in alcohol.

IDENTIFICATION
First identification: A.
Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *Ph. Eur. reference spectrum of phenylmercuric acetate.*

B. To 5 mL of solution S (see Tests) add 5 mL of *water R* and 0.1 mL of *sodium sulfide solution R*. A white precipitate is formed that darkens slowly on heating.

C. To 10 mL of solution S add 2 mL of *potassium iodide solution R* and shake vigorously. Filter. The filtrate gives reaction (b) of acetates (*2.3.1*).

TESTS

Solution S
Dissolve 0.250 g in 40 mL of *water R* by heating to boiling. Allow to cool and dilute to 50 mL with *water R*. Prepare the solution immediately before use.

Appearance of solution
Solution S is not more opalescent than reference suspension II (*2.2.1*) and is colourless (*2.2.2, Method II*).

Ionised mercury
Maximum 0.2 per cent.

To 2 mL of solution S add 8 mL of *water R*, 2 mL of *potassium iodide solution R* and 3 mL of *dilute hydrochloric acid R*. Filter. The filtrate is not more coloured than the potassium iodide solution used. Wash the precipitate with 3 mL of *water R*. Combine the filtrate and the washings, add 2 mL of *dilute sodium hydroxide solution R* and dilute to 20 mL with *water R*. 12 mL of this solution complies with test A for heavy metals (*2.4.8*). Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Polymercuric benzene compounds
Maximum 1.5 per cent.

Shake 0.2 g with 10 mL of *acetone R*. Filter. Wash the residue twice with 5 mL of *acetone R*. Dry the residue at 105 °C for 1 h. The residue weighs a maximum of 3 mg.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 0.500 g by drying in an oven at 45 °C for 15 h.

ASSAY
Dissolve with heating 0.300 g in 100 mL of *water R*. Cool and add 3 mL of *nitric acid R*. Titrate with *0.1 M ammonium thiocyanate* using 2 mL of *ferric ammonium sulfate solution R2* as indicator, until a persistent reddish-yellow colour is obtained.

1 mL of *0.1 M ammonium thiocyanate* is equivalent to 33.67 mg of phenylmercuric acetate.

STORAGE
Protected from light.

Ph Eur

Phenylmercuric Borate

(Ph. Eur. monograph 0103)

Action and use
Antiseptic; antimicrobial preservative.

Ph Eur

DEFINITION
Compound consisting of equimolecular proportions of phenylmercuric orthoborate and phenylmercuric hydroxide ($C_{12}H_{13}BHg_2O_4$; M_r 633) or of the dehydrated form (metaborate, $C_{12}H_{11}BHg_2O_3$; M_r 615) or of a mixture of the 2 compounds.

Content
— *mercury* (Hg; A_r 200.6): 64.5 per cent to 66.0 per cent (dried substance),
— *borates expressed as H_3BO_3*: 9.8 per cent to 10.3 per cent (dried substance).

CHARACTERS

Appearance
White or slightly yellowish, crystalline powder or colourless, shiny crystals.

Solubility
Slightly soluble in water and in ethanol (96 per cent).

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Preparation Discs.
Comparison *Ph. Eur. reference spectrum of phenylmercuric borate.*

B. To 2 mL of solution S (see Tests) add 8 mL of *water R* and 0.1 mL of *sodium sulfide solution R*. A white precipitate is formed that darkens slowly on heating.

C. Dissolve about 20 mg in 2 mL of *methanol R*.
The solution is clear and colourless. Ignite; the solution burns with a green-edged flame.

TESTS

Solution S
Dissolve 0.25 g by sprinkling it on the surface of 25 mL of boiling *water R*, cool and dilute to 25 mL with *water R*.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Ionised mercury
Maximum 0.01 per cent.

To 10 mL of solution S add 2 mL of *potassium iodide solution R* and 3 mL of *dilute hydrochloric acid R*. Filter. The filtrate is colourless. Wash the precipitate with 3 mL of *water R*. Combine the filtrate and the washings, add 2 mL of *dilute sodium hydroxide solution R* and dilute to 20 mL with *water R*. 12 mL of this solution complies with test A for heavy metals (*2.4.8*). Prepare the reference solution using a mixture of 2.5 mL of *lead standard solution (2 ppm Pb) R* and 7.5 mL of *water R*.

Loss on drying (*2.2.32*)
Maximum 3.5 per cent, determined on 0.50 g by drying in an oven at 45 °C for 15 h ± 30 min.

ASSAY

Mercury
Dissolve 0.300 g in 100 mL of *water R* and add 3 mL of *nitric acid R*. Titrate with *0.1 M ammonium thiocyanate*, using 2 mL of *ferric ammonium sulfate solution R2* as indicator, until a persistent reddish-yellow colour is obtained.

1 mL of *0.1 M ammonium thiocyanate* is equivalent to 20.06 mg of Hg.

Borates
Dissolve 0.600 g with heating in 25 mL of *water R*. Dissolve 10 g of *sorbitol R* in the hot solution and cool. Titrate with *0.1 M sodium hydroxide*, using 0.5 mL of *phenolphthalein solution R* as indicator, until a persistent pink colour is obtained. Carry out a blank titration.

1 mL of *0.1 M sodium hydroxide* is equivalent to 6.18 mg of H_3BO_3.

STORAGE
Protected from light.

Ph Eur

Phenylmercuric Nitrate

(Ph. Eur. monograph 0783)

Action and use
Antiseptic; antimicrobial preservative.

Ph Eur

DEFINITION
Mixture of phenylmercuric nitrate ($C_6H_5HgNO_3$; M_r 339.7) and phenylmercuric hydroxide (C_6H_5HgOH; M_r 294.7).

Content
62.5 per cent to 64.0 per cent of Hg (A_r 200.6) (dried substance).

CHARACTERS

Appearance
White or pale yellow powder.

Solubility
Very slightly soluble in water and in ethanol (96 per cent), slightly soluble in hot water. It dissolves in glycerol and in fatty oils.

IDENTIFICATION
A. To 5 mL of solution S (see Tests) add 8 mL of *water R* and 0.1 mL of *sodium sulfide solution R*. A white precipitate is formed that darkens slowly on heating.

B. To 1 mL of a saturated solution of the substance to be examined add 1 mL of *dilute hydrochloric acid R*. A white, flocculent precipitate is formed.

C. To 5 mL of solution S add 1 mL of *dilute hydrochloric acid R*, 2 mL of *methylene chloride R* and 0.2 mL of *dithizone solution R*. Shake. The lower layer is orange-yellow.

D. About 10 mg gives the reaction of nitrates (*2.3.1*).

TESTS

Solution S
To 0.1 g add 45 mL of *water R* and heat to boiling with shaking. Cool, filter and dilute to 50 mL with *water R*.

Appearance of solution
Solution S is colourless (*2.2.2, Method II*).

Inorganic mercuric compounds
Maximum 0.1 per cent.

To 10 mL of solution S add 2 mL of *potassium iodide solution R* and 3 mL of *dilute hydrochloric acid R*. Filter. The filtrate is colourless. Wash the precipitate with 2 mL of *water R*. Combine the filtrate and washings, add 2 mL of *dilute sodium hydroxide solution R* and dilute to 20 mL with *water R*. 12 mL of the solution complies with test A for heavy metals (*2.4.8*). Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying *in vacuo* for 24 h.

ASSAY
Dissolve 0.150 g in a mixture of 10 mL of *dilute nitric acid R* and 90 mL of *water R*, heating to boiling. Cool to 15-20 °C. Titrate with *0.1 M ammonium thiocyanate* using 2 mL of *ferric ammonium sulfate solution R2* as indicator, until a persistent reddish-yellow colour is obtained. Carry out a blank titration.

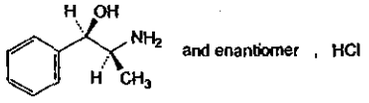
1 mL of *0.1 M ammonium thiocyanate* is equivalent to 20.06 mg of Hg.

STORAGE
Protected from light.

Ph Eur

Phenylpropanolamine Hydrochloride

(Ph. Eur. monograph 0683)



$C_9H_{14}ClNO$ 187.7 *154-41-6*

Action and use
Adrenoceptor agonist.

Ph Eur

DEFINITION
(1*RS*,2*SR*)-2-Amino-1-phenylpropan-1-ol hydrochloride.

Content
99.0 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water and in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 194 °C to 197 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *phenylpropanolamine hydrochloride CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 20 mg of the substance to be examined in *ethanol (96 per cent) R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 10 mg of *phenylpropanolamine hydrochloride CRS* in *ethanol (96 per cent) R* and dilute to 5 mL with the same solvent.

Plate *TLC silica gel G plate R.*

Pretreatment Spray with a 20 g/L solution of *disodium tetraborate R,* using 8 mL for a plate 100 mm x 200 mm and dry in a stream of cold air for 30 min.

Mobile phase *concentrated ammonia R, ethanol (96 per cent) R, butanol R* (6:24:70 *V/V/V*).

Application 10 µL as bands of 10 mm by 3 mm.

Development Over 1/2 of the plate.

Drying In a current of warm air.

Detection Allow to cool, then spray with a 2 g/L solution of *ninhydrin R* in *ethanol (96 per cent) R* and heat at 110 °C for 15 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 1.25 g in *water R* and dilute to 25 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 10 mL of solution S add 0.1 mL of *methyl red solution R* and 0.2 mL of *0.01 M sodium hydroxide.* The solution is yellow. Add 0.4 mL of *0.01 M hydrochloric acid.* The solution is red.

Related substances

Liquid chromatography (*2.2.29*).

Buffer solution Dissolve 3.4 g of *potassium dihydrogen phosphate R* in 500 mL of *water for chromatography R* and adjust to pH 2.5 with *phosphoric acid R.*

Test solution Dissolve 25.0 mg of the substance to be examined in *methanol R* and dilute to 25.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *methanol R.* Dilute 1.0 mL of this solution to 10.0 mL with *methanol R.*

Reference solution (b) Dissolve 5 mg of *cathine hydrochloride R* (1*S*,2*S*-enantiomer of impurity A) in *methanol R* and dilute to 50 mL with the same solvent. Dilute 1 mL of the solution to 10 mL with *methanol R.* Dilute 1 mL of this solution to 10 mL with the test solution.

Column:

— *size:* l = 0.075 m, Ø = 3.0 mm;

— *stationary phase: octadecylsilyl silica gel for chromatography R* (1.8 µm);

— *temperature:* 40 °C.

Mobile phase:

— *mobile phase A: acetonitrile R1,* buffer solution (2:98 *V/V*);

— *mobile phase B: water for chromatography R, acetonitrile R1* (30:70 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 6	100	0
6 - 14	100 → 48	0 → 52
14 - 18	48	52

Flow rate 0.56 mL/min.

Detection Spectrophotometer at 206 nm.

Injection 1 µL.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity A.

Relative retention With reference to phenylpropanolamine (retention time = about 3 min): impurity A = about 1.2.

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to phenylpropanolamine.

Calculation of percentage contents:

— for each impurity, use the concentration of phenylpropanolamine hydrochloride in reference solution (a).

Limits:

— *unspecified impurities*: for each impurity, maximum 0.10 per cent;

— *total*: maximum 0.2 per cent;

— *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

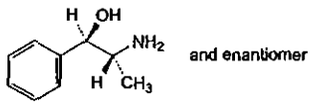
ASSAY

Dissolve 0.150 g in a mixture of 5.0 mL of *0.01 M hydrochloric acid* and 50 mL of *ethanol (96 per cent) R.* Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide.* Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 18.77 mg of $C_9H_{14}ClNO$.

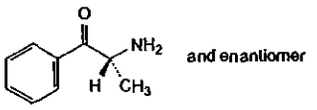
IMPURITIES

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, E.*



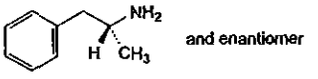
and enantiomer

A. (1*RS*,2*RS*)-2-amino-1-phenylpropan-1-ol (racemic cathine, racemic norpseudoephedrine),



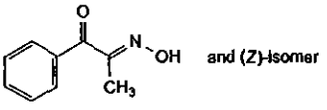
and enantiomer

B. (2*RS*)-2-amino-1-phenylpropan-1-one (racemic cathinone),



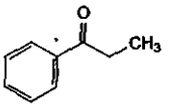
and enantiomer

C. (2*RS*)-1-phenylpropan-2-amine (amfetamine),



and (*Z*)-isomer

D. (2*EZ*)-2-(hydroxyimino)-1-phenylpropan-1-one (α-isonitrosopropiophenone),

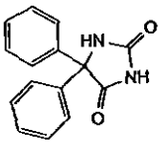


E. 1-phenylpropan-1-one (propiophenone).

Ph Eur

Phenytoin

(Ph. Eur. monograph 1253)



$C_{15}H_{12}N_2O_2$ 252.3 *57-41-0*

Action and use
Antiepileptic.

Preparation
Phenytoin Oral Suspension

Ph Eur

DEFINITION
5,5-Diphenylimidazolidine-2,4-dione.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, sparingly soluble in ethanol (96 per cent), very slightly soluble in methylene chloride. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *phenytoin CRS.*

TESTS
Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Dissolve 1.0 g in a mixture of 5 mL of *1 M sodium hydroxide* and 20 mL of *water R*.

Acidity or alkalinity
To 1.0 g add 45 mL of *water R* and boil for 2 min. Allow to cool and filter. Wash the filter with *carbon dioxide-free water R* and dilute the combined filtrate and washings to 50 mL with the same solvent. To 10 mL of the solution add 0.15 mL of *methyl red solution R*. Not more than 0.5 mL of *0.01 M hydrochloric acid* is required to change the colour of the indicator to red. To 10 mL of the solution add 0.15 mL of *bromothymol blue solution R1*. Not more than 0.5 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to blue.

Related substances
Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Test solution (b) Dilute 1.0 mL of test solution (a) to 20.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of test solution (a) to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 2 mg of *2,2-diphenylglycine R* (impurity C) in 100 mL of the mobile phase.

Reference solution (c) Dissolve 10 mg of *phenytoin for system suitability CRS* (containing impurities D and E) in the mobile phase, add 1 mL of reference solution (b) and dilute to 10 mL with the mobile phase.

Reference solution (d) Dissolve 50.0 mg of *phenytoin CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 20.0 mL with the mobile phase.

Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography compatible with 100 per cent aqueous mobile phases R* (5 µm).

Mobile phase Mix 20 volumes of *methanol R2*, 35 volumes of *acetonitrile R1* and 45 volumes of a 5.75 g/L solution of *ammonium dihydrogen phosphate R* adjusted to pH 2.5 with *phosphoric acid R*.

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µL of test solution (a) and reference solutions (a) and (c).

Run time 4 times the retention time of phenytoin.

Identification of impurities Use the chromatogram supplied with *phenytoin for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities C, D and E.

Relative retention With reference to phenytoin (retention time = about 4 min): impurity C = about 0.5; impurity D = about 0.6; impurity E = about 0.8.

System suitability Reference solution (c):
— *resolution*: minimum 3.5 between the peaks due to impurities D and E.

Limits:
— *correction factors*: for the calculation of content, multiply the peaks areas of the following impurities by the corresponding correction factor: impurity D = 1.7; impurity E = 1.4;
— *impurity E*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
— *impurity C*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *impurity D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

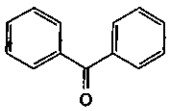
ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (d).

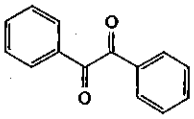
Calculate the percentage content of $C_{15}H_{12}N_2O_2$ taking into account the assigned content of *phenytoin CRS*.

IMPURITIES
Specified impurities *C, D, E.*

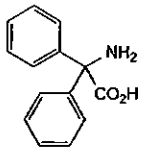
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, F.*



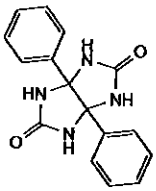
A. diphenylmethanone (benzophenone),



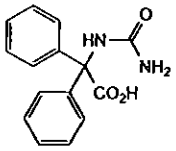
B. diphenylethanedione (benzil),



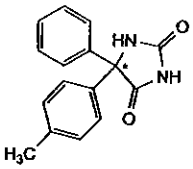
C. amino(diphenyl)acetic acid (2,2-diphenylglycine),



D. 3a,6a-diphenyltetrahydroimidazo[4,5-*d*]imidazole-2,5 (1*H*,3*H*)-dione,



E. (carbamoylamino)diphenylacetic acid,

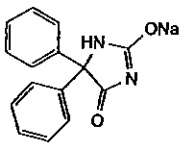


F. (5*E*)-5-(4-methylphenyl)-5-phenylimidazolidine-2,4-dione.

Ph Eur

Phenytoin Sodium

(Ph. Eur. monograph 0521)



$C_{15}H_{11}N_2NaO_2$ 274.3 *630-93-3*

Action and use
Antiepileptic.

Preparations
Phenytoin Capsules
Phenytoin Injection
Phenytoin Tablets

Ph Eur

DEFINITION
Sodium 4-oxo-5,5-diphenyl-4,5-dihydro-1*H*-imidazol-2-olate.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white, very hygroscopic, crystalline powder.

Solubility
Soluble in water and in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

First identification: A, C.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Suspend 0.1 g in 20 mL of *water R*. Acidify with *dilute hydrochloric acid R* and shake with 3 quantities, each of 30 mL, of *ethyl acetate R*. Wash the combined ethyl acetate layers with *water R*, evaporate to dryness and dry the residue at 100-105 °C (test residue). Repeat the operations using 0.1 g of *phenytoin sodium CRS* (reference residue). Examine as discs prepared using *potassium bromide R*.

Comparison *phenytoin sodium CRS*.

B. Thin-layer chromatography (*2.2.27*).

Solvent mixture *acetone R, methanol R* (50:50 *V/V*).

Test solution Dissolve 20 mg of the substance to be examined in the solvent mixture and dilute to 10 mL with the solvent mixture.

Reference solution Dissolve 20 mg of *phenytoin sodium CRS* in the solvent mixture and dilute to 10 mL with the solvent mixture.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *concentrated ammonia R, toluene R, 2-propanol R* (10:40:50 *V/V/V*).

Application 10 µL as bands of 8 mm.

Development Over 2/3 of the plate.

Drying At 80 °C for 5 min; allow to cool.

Detection Examine in ultraviolet light at 254 nm.

Results The principal zone in the chromatogram obtained with the test solution is similar in position and size to the principal zone in the chromatogram obtained with the reference solution.

C. Ignite 1 g and cool. Add 2 mL of *water R* to the residue and neutralise the solution with *hydrochloric acid R*. Filter and dilute the filtrate to 4 mL with *water R*. 0.1 mL of the solution gives reaction (b) of sodium (*2.3.1*).

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Suspend 1.0 g in 5 mL of *water R* and dilute to 20 mL with *0.1 M sodium hydroxide*.

Related substances
Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Test solution (b) Dilute 1.0 mL of test solution (a) to 20.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of test solution (a) to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 2 mg of *2,2-diphenylglycine R* (impurity C) in 100 mL of the mobile phase.

Reference solution (c) Dissolve 10 mg of *phenytoin for system suitability CRS* (containing impurities D and E) in the mobile phase, add 1 mL of reference solution (b) and dilute to 10 mL with the mobile phase.

Reference solution (d) Dissolve 50.0 mg of *phenytoin CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 20.0 mL with the mobile phase.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography compatible with 100 per cent aqueous mobile phases R* (5 µm).

Mobile phase Mix 20 volumes of *methanol R2*, 35 volumes of *acetonitrile R1* and 45 volumes of a 5.75 g/L solution of *ammonium dihydrogen phosphate R* adjusted to pH 2.5 with *phosphoric acid R*.

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µL of test solution (a) and reference solutions (a) and (c).

Run time 4 times the retention time of phenytoin.

Identification of impurities Use the chromatogram supplied with *phenytoin for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities C, D and E.

Relative retention With reference to phenytoin (retention time = about 4 min): impurity C = about 0.5; impurity D = about 0.6; impurity E = about 0.8.

System suitability Reference solution (c):
- *resolution*: minimum 3.5 between the peaks due to impurities D and E.

Limits:
- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity D = 1.7; impurity E = 1.4;
- *impurity E*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
- *impurity C*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *impurity D*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Free phenytoin
Dissolve 0.30 g in 10 mL of a mixture of equal volumes of *pyridine R* and *water R*. Add 0.5 mL of *phenolphthalein solution R* and 3 mL of *silver nitrate solution in pyridine R*. Not more than 1.0 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

Water (*2.5.12*)
Maximum 3.0 per cent, determined on 1.00 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (d).

Calculate the percentage content of $C_{15}H_{11}N_2NaO_2$ taking into account the assigned content of *phenytoin CRS*, and a conversion factor of 1.09.

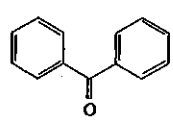
STORAGE

In an airtight container.

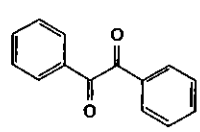
IMPURITIES

Specified impurities *C, D, E.*

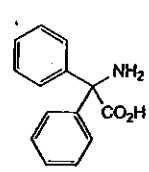
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, F.*



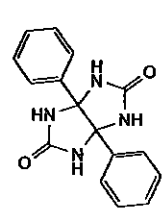
A. diphenylmethanone (benzophenone),



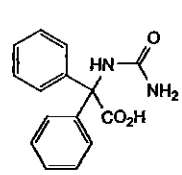
B. diphenylethanedione (benzil),



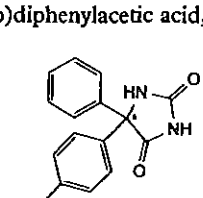
C. amino(diphenyl)acetic acid (2,2-diphenylglycine),



D. 3a,6a-diphenyltetrahydroimidazo[4,5-*d*]imidazole-2,5 (1*H*,3*H*)-dione,



E. (carbamoylamino)diphenylacetic acid,



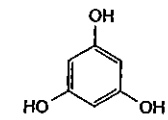
F. (5*Z*)-5-(4-methylphenyl)-5-phenylimidazolidine-2,4-dione.

Ph Eur

Phloroglucinol

Anhydrous Phloroglucinol

(Ph. Eur. monograph 2301)



$C_6H_6O_3$ 126.1 *108-73-6*

Action and use

Antispasmodic.

Ph Eur

DEFINITION

Benzene-1,3,5-triol.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Sparingly soluble in water, freely soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *anhydrous phloroglucinol CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.20 g of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 0.20 g of *anhydrous phloroglucinol CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *anhydrous formic acid R, hexane R, ethyl acetate R* (2:37.5:62.5 *V/V/V*).

Application 10 µL.

Development Over 2/3 of the plate.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. Loss on drying (see Tests).

TESTS

Solution S

Dissolve 2.50 g in *ethanol (96 per cent) R* and dilute to 25 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

pH (*2.2.3*)

4.0 to 6.0.

Dilute 10 mL of solution S to 100 mL with *carbon dioxide-free water R.*

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use and protect from light.*

Solvent mixture Mobile phase B, mobile phase A (10:90 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 6 mg each of *resorcinol R* (impurity B), *phloroglucide R* (impurity D) and *pyrogallol R* (impurity A) in 10 mL of the solvent mixture, add 2 mL of the test solution and dilute to 20 mL with the solvent mixture. Dilute 1 mL of this solution to 50 mL with the solvent mixture.

Reference solution (c) Dissolve 4 mg each of *benzene-1,2,4-triol R* (impurity E), *2,6-dichlorophenol R* (impurity I), *4-chlororesorcinol R* (impurity K) and *3,5-dichloroaniline R* (impurity L) in 10 mL of the solvent mixture and dilute to 20 mL with the solvent mixture.

Reference solution (d) Dissolve 10 mg of the substance to be examined in 10 mL of the solvent mixture, add 1 mL of reference solution (c) and dilute to 20 mL with the solvent mixture.

Column:
- *size: l* = 0.25 m, Ø = 4.0 mm;
- *stationary phase: polar end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A*: 1.36 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.0 with *phosphoric acid R*;
- *mobile phase B: acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 9	100	0
9 - 15	100 → 50	0 → 50
15 - 25	50 → 20	50 → 80
25 - 30	20	80

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 265 nm.

Injection 20 µL of the test solution and reference solutions (a), (b) and (d).

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B and D; use the chromatogram obtained with reference solution (d) to identify the peaks due to impurities E, I, K and L.

Relative retention With reference to phloroglucinol (retention time = about 12 min): impurity E = about 0.7; impurity A = about 0.9; impurity D = about 1.3; impurity B = about 1.35; impurity K = about 1.5; impurity I = about 1.8; impurity L = about 2.0.

System suitability Reference solution (b):
- *resolution*: minimum 2.5 between the peaks due to impurity A and phloroglucinol; minimum 4.0 between the peaks due to impurities D and B.

Limits:
- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.6; impurity D = 0.2; impurity E = 0.7; impurity I = 0.6; impurity K = 0.6; impurity L = 0.4;
- *impurities A, D, E, I, K, L*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Chlorides (*2.4.4*)
Maximum 200 ppm.

Dilute 2.5 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 500 ppm.

Dilute 3 mL of solution S to 15 mL with *distilled water R*.

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.500 g in 50 mL of *water R*. Titrate with *1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *1 M sodium hydroxide* is equivalent to 63.05 mg of $C_6H_6O_3$.

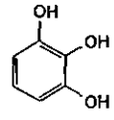
STORAGE

Protected from light.

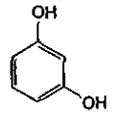
IMPURITIES

Specified impurities *A, D, E, I, K, L.*

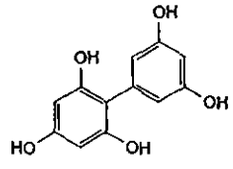
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, O.*



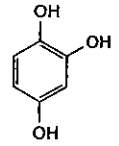
A. benzene-1,2,3-triol (pyrogallol),



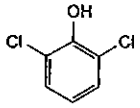
B. benzene-1,3-diol (resorcinol),



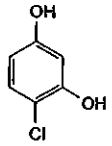
D. 2,3′,4,5′,6-biphenylpentol (phloroglucide),



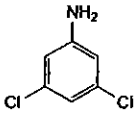
E. benzene-1,2,4-triol,



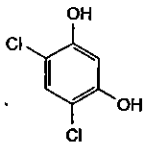
I. 2,6-dichlorophenol,



K. 4-chlorobenzene-1,3-diol (4-chlororesorcinol),



L. 3,5-dichloroaniline,

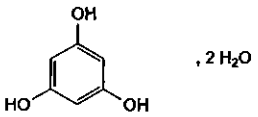


O. 4,6-dichlorobenzene-1,3-diol (4,6-dichlororesorcinol).

Ph Eur

Phloroglucinol Dihydrate

(Ph. Eur. monograph 2302)



$C_6H_6O_3,2H_2O$ 162.1 *6099-90-7*

Action and use
Antispasmodic.

Ph Eur

DEFINITION
Benzene-1,3,5-triol dihydrate.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Sparingly soluble in water, freely soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Previously dry the substance to be examined in an oven at 105 °C.

Comparison *anhydrous phloroglucinol CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.20 g of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 0.20 g of *anhydrous phloroglucinol CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *anhydrous formic acid R, hexane R, ethyl acetate R* (2:37.5:62.5 *V/V/V*).

Application 10 µL.

Development Over 2/3 of the plate.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. Loss on drying (see Tests).

TESTS

Solution S
Dissolve 2.50 g in *ethanol (96 per cent) R* and dilute to 25 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

pH (*2.2.3*)
4.0 to 6.0.

Dilute 10 mL of solution S to 100 mL with *carbon dioxide-free water R.*

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use and protect from light.*

Solvent mixture Mobile phase B, mobile phase A (10:90 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 6 mg each of *resorcinol R* (impurity B), *phloroglucide R* (impurity D) and *pyrogallol R* (impurity A) in 10 mL of the solvent mixture, add 2 mL of the test solution and dilute to 20 mL with the solvent mixture. Dilute 1 mL of this solution to 50 mL with the solvent mixture.

Reference solution (c) Dissolve 4 mg each of *benzene-1,2,4-triol R* (impurity E), *2,6-dichlorophenol R* (impurity I), *4-chlororesorcinol R* (impurity K) and *3,5-dichloroaniline R* (impurity L) in 10 mL of the solvent mixture and dilute to 20 mL with the solvent mixture.

Reference solution (d) Dissolve 10 mg of the substance to be examined in 10 mL of the solvent mixture, add 1 mL of reference solution (c) and dilute to 20 mL with the solvent mixture.

Column:
— *size*: *l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase: polar end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: 1.36 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.0 with *phosphoric acid R*;
— *mobile phase B: acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 9	100	0
9 - 15	100 → 50	0 → 50
15 - 25	50 → 20	50 → 80
25 - 30	20	80

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 265 nm.

Injection 20 µL of the test solution and reference solutions (a), (b) and (d).

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B and D; use the chromatogram obtained with reference solution (d) to identify the peaks due to impurities E, I, K and L.

Relative retention With reference to phloroglucinol (retention time = about 12 min): impurity E = about 0.7; impurity A = about 0.9; impurity D = about 1.3; impurity B = about 1.35; impurity K = about 1.5; impurity I = about 1.8; impurity L = about 2.0.

System suitability Reference solution (b):
— *resolution*: minimum 2.5 between the peaks due to impurity A and phloroglucinol; minimum 4.0 between the peaks due to impurities D and B.

Limits:
— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.6; impurity D = 0.2; impurity E = 0.7; impurity I = 0.6; impurity K = 0.6; impurity L = 0.4;
— *impurities A, D, E, I, K, L*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Chlorides (*2.4.4*)
Maximum 200 ppm.

Dilute 2.5 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 500 ppm.

Dilute 3 mL of solution S to 15 mL with *distilled water R*.

Loss on drying (*2.2.32*)
20.0 per cent to 23.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.600 g in 50 mL of *water R*. Titrate with *1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *1 M sodium hydroxide* is equivalent to 63.05 mg of $C_6H_6O_3$.

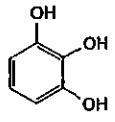
STORAGE

Protected from light.

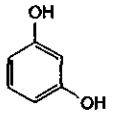
IMPURITIES

Specified impurities *A, D, E, I, K, L.*

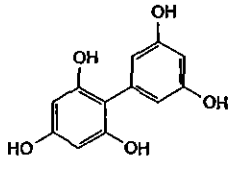
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, O.*



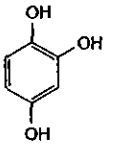
A. benzene-1,2,3-triol (pyrogallol),



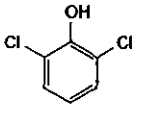
B. benzene-1,3-diol (resorcinol),



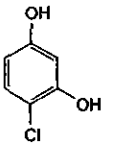
D. 2,3′,4,5′,6-biphenylpentol (phloroglucide),



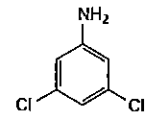
E. benzene-1,2,4-triol,



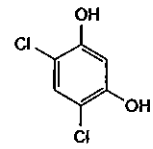
I. 2,6-dichlorophenol,



K. 4-chlorobenzene-1,3-diol (4-chlororesorcinol),



L. 3,5-dichloroaniline,



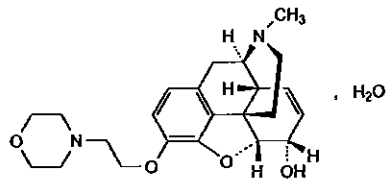
O. 4,6-dichlorobenzene-1,3-diol (4,6-dichlororesorcinol).

Ph Eur

Pholcodine Monohydrate

Pholcodine

(Ph. Eur. monograph 0522)



$C_{23}H_{30}N_2O_4,H_2O$ 416.5 *6254-99-5*

Action and use
Opioid receptor agonist; cough suppressant.

Preparations
Pholcodine Linctus
Strong Pholcodine Linctus

Ph Eur

DEFINITION
17-Methyl-3-[2-(morpholin-4-yl)ethoxy]-7,8-didehydro-4,5α-epoxymorphinan-6α-ol monohydrate.

Content
98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder or colourless crystals.

Solubility
Sparingly soluble in water, freely soluble in acetone and in ethanol (96 per cent). It dissolves in dilute mineral acids.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *pholcodine CRS*.

TESTS

Specific optical rotation (*2.2.7*)
−98 to −94 (dried substance).
Dissolve 1.000 g in *ethanol (96 per cent) R* and dilute to 50.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

0.02 M phosphate buffer solution To 80.0 mL of *0.2 M sodium hydroxide* add 100.0 mL of *0.2 M potassium dihydrogen phosphate R* and dilute to 1.0 L with *water R*.

Solvent mixture Dilute 80 mL of *acetonitrile R* to 1 L with the 0.02 M phosphate buffer solution.

Test solution Dissolve 50 mg of the substance to be examined in the solvent mixture and dilute to 50 mL with the solvent mixture.

Reference solution (a) Dissolve 10 mg of *codeine R* (impurity B) in the solvent mixture and dilute to 10 mL with the solvent mixture. To 0.5 mL of this solution add 0.5 mL of the test solution and dilute to 50 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 5 mg of *pholcodine for peak identification CRS* (containing impurities A, B and D) in the solvent mixture and dilute to 5 mL with the solvent mixture.

Reference solution (d) Dissolve 5 mg of *pholcodine for impurity G identification CRS* in the solvent mixture and dilute to 5 mL with the solvent mixture.

Column:
— *size*: *l* = 0.075 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped solid core phenylhexylsilyl silica gel for chromatography R* (2.6 µm);
— *temperature*: 35 °C.

Mobile phase To 50 mL of *tetrahydrofuran for chromatography R* add 75 mL of *acetonitrile R* and dilute to 1000 mL with the 0.02 M phosphate buffer solution; adjust to pH 7.9 ± 0.05 with *0.2 M sodium hydroxide*; the pH must not exceed 8.0.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 238 nm.

Injection 20 µL.

Run time 5 times the retention time of pholcodine.

Identification of impurities Use the chromatogram supplied with *pholcodine for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B and D; use the chromatogram obtained with reference solution (d) to identify the peak due to impurity G.

Relative retention With reference to pholcodine (retention time = about 10 min): impurity A = about 0.4; impurity B = about 0.8; impurity D = about 2.3; impurity G = about 4.2.

System suitability Reference solution (a):
— *resolution*: minimum 3.0 between the peaks due to impurity B and pholcodine.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity G by 0.5;
— *impurities A, B, D*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
— *impurity G*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

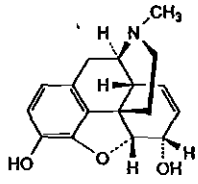
— *total*: not more than 7 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.7 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
3.9 per cent to 4.5 per cent, determined on 0.500 g by drying in an oven at 105 °C.

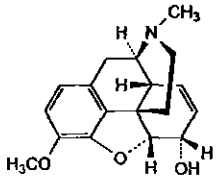
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.180 g in 50 mL of *anhydrous acetic acid R*, warming gently. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).
1 mL of *0.1 M perchloric acid* is equivalent to 19.93 mg of $C_{23}H_{30}N_2O_4$.

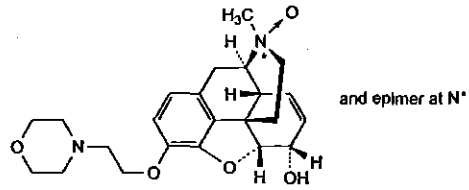
IMPURITIES
Specified impurities *A, B, D, G.*
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C, E, F.*



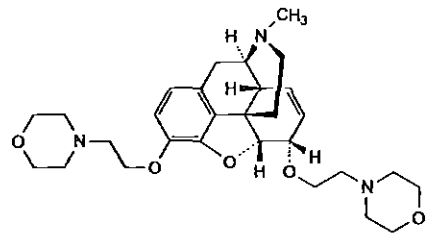
A. 17-methyl-7,8-didehydro-4,5α-epoxymorphinan-3,6α-diol (morphine),



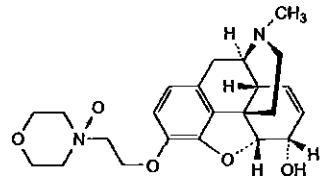
B. 3-methoxy-17-methyl-7,8-didehydro-4,5α-epoxymorphinan-6α-ol (codeine),



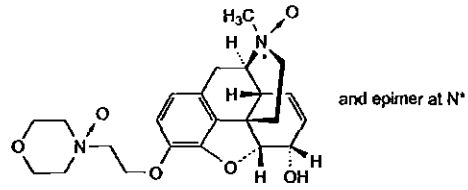
C. (17*RS*)-6α-hydroxy-17-methyl-3-[2-(morpholin-4-yl) ethoxy]-7,8-didehydro-4,5α-epoxymorphinan 17-oxide (pholcodine *N*-oxide),



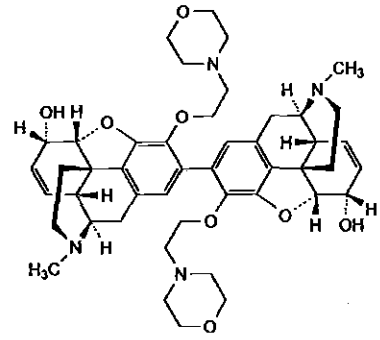
D. 17-methyl-3,6α-bis[2-(morpholin-4-yl)ethoxy]-7,8-didehydro-4,5α-epoxymorphinan,



E. 4-[2-[(6α-hydroxy-17-methyl-7,8-didehydro-4,5α-epoxymorphinan-3-yl)oxy]ethyl]morpholine 4-oxide (pholcodine *N'*-oxide),



F. (17*RS*)-6α-hydroxy-17-methyl-3-[2-(4-oxidomorpholin-4-ium-4-yl)ethoxy]-7,8-didehydro-4,5α-epoxymorphinan 17-oxide (pholcodine *N,N'*-dioxide),



G. 17,17'-dimethyl-3,3'-bis[2-(morpholin-4-yl)ethoxy]-7,7',8,8'-tetradehydro-4,5α:4',5'α-diepoxybimorphinan-6α,6'α-diol (pholcodine dimer).

Ph Eur

Phosphoric Acid

(Concentrated Phosphoric Acid, Ph. Eur. monograph 0004)

H_3PO_4 98.0 *7664-38-2*

Preparation
Phosphate Oral Solution

Ph Eur

DEFINITION
Content
84.0 per cent *m/m* to 90.0 per cent *m/m*.

CHARACTERS

Appearance
Clear, colourless, syrupy liquid, corrosive.

Solubility
Miscible with water and with ethanol (96 per cent).

When stored at a low temperature it may solidify into a mass of colourless crystals which do not melt at a temperature below 28 °C.

Relative density
About 1.7.

IDENTIFICATION

A. Dilute with *water R*. The solution is strongly acid (*2.2.4*).

B. Solution S (see Tests) neutralised with *dilute sodium hydroxide solution R* gives the reactions of phosphates (*2.3.1*).

TESTS

Solution S
Dilute 10.0 g to 150 mL with *water R*.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Substances precipitated with ammonia
To 10 mL of solution S add 8 mL of *dilute ammonia R1*. Any opalescence in the solution is not more intense than that in a mixture of 10 mL of solution S and 8 mL of *water R*.

Hypophosphorous acid and phosphorous acid
To 5 mL of solution S add 2 mL of *silver nitrate solution R2* and heat on a water-bath for 5 min. The solution shows no change in appearance.

Chlorides (*2.4.4*)
Maximum 50 ppm, determined on solution S.

Sulfates (*2.4.13*)
Maximum 100 ppm.

Dilute 1.5 g to 15 mL with *distilled water R*.

Iron (*2.4.9*)
Maximum 50 ppm.

Dilute 3 mL of solution S to 10 mL with *water R*.

ASSAY

Dilute 0.500 g in 30 mL of *water R*. Titrate with *1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Read the volume added at the 2nd inflexion point.

1 mL of *1 M sodium hydroxide* is equivalent to 49.00 mg of H_3PO_4.

STORAGE

In a glass container.

Ph Eur

Dilute Phosphoric Acid

(Ph. Eur. monograph 0005)

Ph Eur

DEFINITION

Content
9.5 per cent *m/m* to 10.5 per cent *m/m* of H_3PO_4 (M_r 98.0).

PREPARATION

To 885 g of *water R* add 115 g of concentrated phosphoric acid and mix.

IDENTIFICATION

A. It is strongly acid (*2.2.4*).

B. Solution S (see Tests), neutralised with *dilute sodium hydroxide solution R*, gives the reactions of phosphates (*2.3.1*).

TESTS

Solution S
Dilute 86 g to 150 mL with *water R*.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Substances precipitated with ammonia
To 10 mL of solution S add 8 mL of *dilute ammonia R1*. Any opalescence in the solution is not more intense than that in a mixture of 10 mL of solution S and 8 mL of *water R*.

Hypophosphorous acid and phosphorous acid
To 5 mL of solution S add 2 mL of *silver nitrate solution R2* and heat on a water-bath for 5 min. The solution shows no change in appearance.

Chlorides (*2.4.4*)
Maximum 6 ppm, determined on solution S.

Sulfates (*2.4.13*)
Maximum 10 ppm, determined on the preparation to be examined.

Iron (*2.4.9*)
Maximum 6 ppm.

Dilute 3 mL of solution S to 10 mL with *water R*.

ASSAY

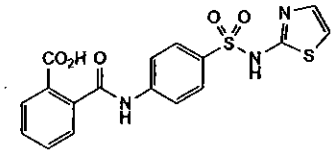
Dilute 4.30 g in 30 mL of *water R*. Titrate with *1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Read the volume added at the 2nd inflexion point.

1 mL of *1 M sodium hydroxide* is equivalent to 49.00 mg of H_3PO_4.

Ph Eur

Phthalylsulfathiazole

(Ph. Eur. monograph 0352)



$C_{17}H_{13}N_3O_5S_2$ 403.4 *85-73-4*

Action and use
Antibacterial.

Ph Eur

DEFINITION

2-[[4-(Thiazol-2-ylsulfamoyl)phenyl]carbamoyl]benzoic acid.

Content
98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance
White or yellowish-white, crystalline powder.

Solubility
Practically insoluble in water, freely soluble in dimethylformamide, slightly soluble in acetone and in ethanol (96 per cent).

IDENTIFICATION

First identification: A, B, E.

Second identification: B, C, D, E.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison phthalylsulfathiazole CRS.

B. To 1 g add 8.5 mL of *dilute sodium hydroxide solution R* and boil under a reflux condenser for 30 min. Cool and add 17.5 mL of *dilute hydrochloric acid R*. Shake vigorously and filter. Neutralise the filtrate with *dilute sodium hydroxide solution R*. Filter, wash the precipitate with *water R*, recrystallise from *water R* and dry the crystals at 100-105 °C. The crystals melt (*2.2.14*) at 200 °C to 203 °C.

C. To 0.1 g in a test-tube add 3 mL of *dilute sulfuric acid R* and 0.5 g of *zinc powder R*. Fumes are evolved which produce a black stain on *lead acetate paper R*.

D. To 0.1 g add 0.5 g of *resorcinol R* and 0.3 mL of *sulfuric acid R* and heat on a water-bath until a homogeneous mixture is obtained. Allow to cool. Add 5 mL of *dilute sodium hydroxide solution R*. Dilute 0.1 mL of this brownish-red mixture to 25 mL with *water R*. An intense green fluorescence appears which disappears on acidification.

E. Dissolve about 10 mg of the crystals obtained in identification test B in 200 mL of *0.1 M hydrochloric acid*. 2 mL of the solution gives the reaction of primary aromatic amines (*2.3.1*) with formation of an orange precipitate.

TESTS

Appearance of solution

The solution is clear (2.2.1) and not more intensely coloured than reference solution BY_5 (2.2.2, Method II).

Dissolve 1.0 g in *1 M sodium hydroxide* and dilute to 20 mL with the same solvent.

Acidity

To 2.0 g add 20 mL of *water R*, shake continuously for 30 min and filter. To 10 mL of the filtrate add 0.1 mL of *phenolphthalein solution R*. Not more than 0.2 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Sulfathiazole and other primary aromatic amines

Maximum 2.0 per cent.

Dissolve 5 mg in a mixture of 3.5 mL of *water R*, 6 mL of *dilute hydrochloric acid R* and 25 mL of *ethanol (96 per cent) R*, previously cooled to 15 °C. Place immediately in iced water and add 1 mL of a 2.5 g/L solution of *sodium nitrite R*. Allow to stand for 3 min, add 2.5 mL of a 40 g/L solution of *sulfamic acid R* and allow to stand for 5 min. Add 1 mL of a 4 g/L solution of *naphthylethylenediamine dihydrochloride R* and dilute to 50 mL with *water R*. Measured at 550 nm, the absorbance (*2.2.25*) is not greater than that of a standard prepared at the same time and in the same manner using a mixture of 1 mL of a 100 mL aqueous solution containing 10 mg of *sulfathiazole R* and 0.5 mL of *hydrochloric acid R*; 2.5 mL of *water R*; 6 mL of *dilute hydrochloric acid R*; and 25 mL of *ethanol (96 per cent) R*.

Loss on drying (*2.2.32*)

Maximum 2 per cent, determined on 1.00 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 40 mL of *dimethylformamide R*. Titrate with *0.1 M sodium hydroxide* until the colour becomes blue, using 0.2 mL of *thymolphthalein solution R* as indicator. Carry out a blank titration.

1 mL of *0.1 M sodium hydroxide* is equivalent to 20.17 mg of $C_{17}H_{13}N_3O_5S_2$.

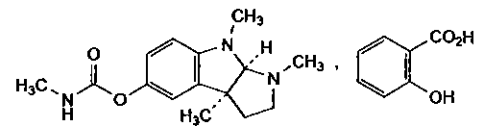
STORAGE

Protected from light.

Ph Eur

Physostigmine Salicylate

(Ph. Eur. monograph 0286)



$C_{22}H_{27}N_3O_5$ 413.5 *57-64-7*

Action and use

Cholinesterase inhibitor.

Ph Eur

DEFINITION

Physostigmine salicylate contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (3a*S*,8a*R*)-1,2,3,3a,8,8a-hexahydro-1,3a,8-trimethylpyrrolo[2,3-*b*]indol-5-yl methylcarbamate salicylate, calculated with reference to the dried substance.

CHARACTERS

Colourless or almost colourless crystals, sparingly soluble in water, soluble in alcohol. The crystals gradually become red when exposed to air and light; the colour develops more quickly when the crystals are also exposed to moisture. Aqueous solutions are unstable.

It melts at about 182 °C, with decomposition.

IDENTIFICATION

First identification: A, B.

Second identification: B, C, D.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *physostigmine salicylate CRS*.

B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Heat about 10 mg in a porcelain dish with a few drops of *dilute ammonia R1*. An orange colour develops. Evaporate the solution to dryness. The residue dissolves in *alcohol R* giving a blue solution. Add 0.1 mL of *glacial acetic acid R*. The colour becomes violet. Dilute with *water R*. An intense red fluorescence appears.

D. Solution S (see Tests) gives reaction (a) of salicylates (*2.3.1*).

TESTS

Solution S

Dissolve 0.900 g, without heating, in 95 mL of *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100.0 mL with the same solvent. Prepare immediately before use.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)
The pH of solution S is 5.1 to 5.9.

Specific optical rotation (*2.2.7*)
−90 to −94, determined on solution S and calculated with reference to the dried substance.

Related substances
Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution (a) Dissolve 0.2 g of the substance to be examined in *alcohol R* and dilute to 10 mL with the same solvent.

Test solution (b) Dilute 2.5 mL of test solution (a) to 50 mL with *alcohol R*.

Reference solution (a) Dissolve 10 mg of *physostigmine salicylate CRS* in *alcohol R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dilute 2 mL of reference solution (a) to 20 mL with *alcohol R*.

Apply to the plate 20 µL of each solution. Develop over a path of 15 cm using a mixture of 2 volumes of *concentrated ammonia R*, 23 volumes of *2-propanol R* and 100 volumes of *cyclohexane R*. Dry the plate in a current of cold air and carry out a second development in the same direction. Allow the plate to dry in air and spray with freshly prepared *potassium iodobismuthate solution R* and then with *dilute hydrogen peroxide solution R*. Examine the plate within 2 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Eseridine
To 5 mL of solution S add a few crystals of *potassium iodate R*, 0.05 mL of *dilute hydrochloric acid R* and 2 mL of *chloroform R*. Shake. No violet colour develops in the chloroform layer within 1 min.

Sulfates (*2.4.13*)
15 mL of solution S complies with the limit test for sulfates (0.1 per cent).

Loss on drying (*2.2.32*)
Not more than 1.0 per cent, determined on 1.00 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Not more than 0.1 per cent, determined on the residue obtained in the test for loss on drying.

ASSAY
Dissolve 0.350 g in 50 mL of a mixture of equal volumes of *anhydrous acetic acid R* and *chloroform R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (*2.2.20*).

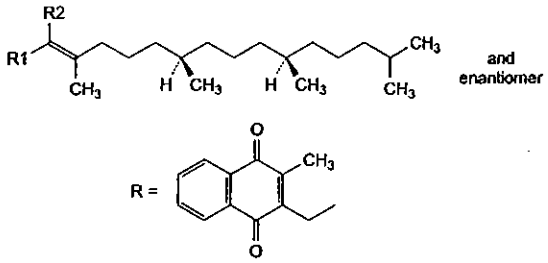
1 mL of *0.1 M perchloric acid* is equivalent to 41.35 mg of $C_{22}H_{27}N_3O_5$.

STORAGE
Store in an airtight container, protected from light.

Ph Eur

Phytomenadione

(Racemic Phytomenadione, Ph. Eur. monograph 3011)



Phytomenadione	R1	R2	Molecular formula	M_r
trans isomers	R	H	$C_{31}H_{46}O_2$	450.7
cis isomers	H	R	$C_{31}H_{46}O_2$	450.7

$C_{31}H_{46}O_2$ 450.7

Action and use
Vitamin K analogue.

Ph Eur

DEFINITION
Mixture of 2-methyl-3-[(2*E*,7*RS*,11*RS*)-3,7,11,15-tetramethylhexadec-2-en-1-yl]naphthalene-1,4-dione (*trans*-phytomenadione isomers) and 2-methyl-3-[(2*Z*,7*RS*,11*RS*)-3,7,11,15-tetramethylhexadec-2-en-1-yl]naphthalene-1,4-dione (*cis*-phytomenadione isomers).

Content
— *trans*-phytomenadione isomers: minimum 85.0 per cent;
— sum of *trans*-phytomenadione and *cis*-phytomenadione isomers: 97.0 per cent to 103.0 per cent.

CHARACTERS
Appearance
Clear, intense yellow, viscous, oily liquid.

Solubility
Practically insoluble in water, sparingly soluble in ethanol (96 per cent), miscible with fatty oils.

It decomposes on exposure to actinic light.

Refractive index
About 1.526.

IDENTIFICATION
Prepare the solutions immediately before use and protect from light.

A. Optical rotation (see Tests).

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution (a) Dissolve 10.0 mg of the substance to be examined in *trimethylpentane R* and dilute to 100.0 mL with the same solvent.

Test solution (b) Dilute 10.0 mL of test solution (a) to 50.0 mL with *trimethylpentane R*.

Spectral range 275-340 nm for test solution (a); 230-280 nm for test solution (b).

Absorption maxima 327 nm for test solution (a); 243 nm, 249 nm, 261 nm and 270 nm for test solution (b).

Absorption minimum 285 nm for test solution (a).

Specific absorbance at the absorption maximum at 327 nm
67 to 73 for test solution (a).

C. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (d).

TESTS

Appearance of solution

The solution is clear (*2.2.1*).

Dissolve 2.5 g in *trimethylpentane R* and dilute to 25 mL with the same solvent.

Optical rotation (*2.2.7*)

−0.05° to + 0.05°, measured at 25 °C.

Dissolve 0.25 g in *dioxan R* and dilute to 25.0 mL with the same solvent.

Acid value (*2.5.1*)

Maximum 2.0, determined on 2.00 g.

Impurity A

Thin-layer chromatography (*2.2.27*). *Prepare the solutions immediately before use and protect from light.*

Test solution Dissolve 0.40 g of the substance to be examined in *cyclohexane R* and dilute to 10.0 mL with the same solvent.

Reference solution Dissolve 4.0 mg of *menadione R* (impurity A) in *cyclohexane R* and dilute to 50.0 mL with the same solvent.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *cyclohexane R, toluene R* (20:80 *V/V*).

Application 10 µL.

Development Over 3/4 of the plate.

Drying In air for 5 min.

Detection Examine in ultraviolet light at 254 nm.

Relative retention With reference to racemic *trans*-phytomenadione (R_F = about 0.5): impurity A = about 0.4.

Limit:

— *impurity A*: any spot due to impurity A is not more intense than the spot in the chromatogram obtained with the reference solution (0.2 per cent).

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use and protect from light.*

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 25.0 mL with the mobile phase.

Reference solution (a) Dissolve 20.0 mg of *phytomenadione CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve the contents of a vial of *trans-epoxyphytomenadione CRS* (impurity B) in 1 mL of the mobile phase. Add 0.4 mL of the solution to 5 mL of reference solution (a) and dilute to 100 mL with the mobile phase.

Reference solution (c) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (d) Dilute 1.0 mL of reference solution (a) to 25.0 mL with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: spherical *silica gel for chromatography R* (5 µm).

Mobile phase *octanol R, di-isopropyl ether R, heptane R* (1:3.3:1000 *V/V/V*).

Flow rate 0.8 mL/min.

Detection Spectrophotometer at 254 nm.

Equilibration With the mobile phase for at least 24 h.

Injection 50 µL of the test solution and reference solutions (b) and (c).

Run time 1.6 times the retention time of *trans*-phytomenadione isomers.

Relative retention With reference to *trans*-phytomenadione isomers (retention time = about 27 min): impurity B = about 0.6; *cis*-phytomenadione isomers = about 0.65.

System suitability Reference solution (b):

— *resolution*: minimum 1.5 between the peaks due to impurity B and *cis*-phytomenadione isomers; minimum 4.0 between the peaks due to *cis*-phytomenadione isomers and *trans*-phytomenadione isomers.

Calculation of percentage contents:

— for each impurity, use the concentration of *trans*-phytomenadione isomers in reference solution (c), taking into account their content as determined in the assay.

Limits:

— *impurity B*: maximum 0.2 per cent;

— *unspecified impurities*: for each impurity, maximum 0.15 per cent;

— *total*: maximum 1.0 per cent;

— *reporting threshold*: 0.05 per cent.

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (d).

System suitability Reference solution (d):

— *repeatability*: maximum relative standard deviation of 1.0 per cent for the peak due to *trans*-phytomenadione isomers, determined on 6 injections.

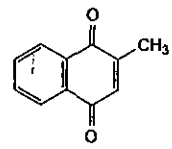
Calculate the percentage contents of *trans*-phytomenadione isomers and *cis*-phytomenadione isomers taking into account the assigned content of *phytomenadione CRS*.

STORAGE

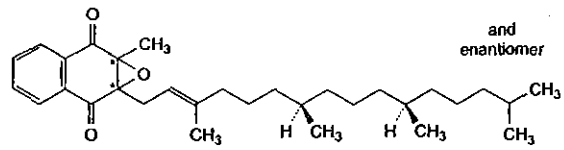
Protected from light.

IMPURITIES

Specified impurities *A, B.*



A. 2-methylnaphthalene-1,4-dione (menadione),



B. (1aΞ,7aΞ)-1a-methyl-7a-[(2*E*,7*RS*,11*RS*)-3,7,11,15-tetramethylhexadec-2-en-1-yl]-1a,7a-dihydronaphtho[2,3-*b*]oxirene-2,7-dione (*trans*-epoxyphytomenadione isomers).

Ph Eur

Phytosterol

(Ph. Eur. monograph 1911)

Ph Eur

DEFINITION

Natural mixture of sterols obtained from plants of the genera *Hypoxis*, *Pinus* and *Picea*.

Content

Minimum 70.0 per cent of β-sitosterol (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, soluble in tetrahydrofuran, sparingly soluble in ethyl acetate.

IDENTIFICATION

A. Mix 1 mL of *acetic anhydride R* with 0.5 mL of solution S (see Tests). After the addition of 0.1 mL of *sulfuric acid R* a red colour is produced, which changes rapidly to violet, then to blue and finally to green.

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the peak in the chromatogram obtained with reference solution (b).

TESTS

Solution S

Dissolve 1.0 g in *tetrahydrofuran R* and dilute to 20 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Acidity or alkalinity

Shake 0.20 g with a mixture of 4.0 mL of *ethyl acetate R* and 10.0 mL of *carbon dioxide-free water R* for 3 min. Allow the layers to separate. To the aqueous layer add 0.1 mL of *bromothymol blue solution R1*. If the solution is yellow, not more than 0.5 mL of *0.01 M sodium hydroxide* is required to change the colour to blue. If the solution is blue, not more than 0.5 mL of *0.01 M hydrochloric acid* is required to change the colour to yellow.

Specific optical rotation (*2.2.7*)

−28.0 to −15.0 (dried substance).

Dissolve 0.500 g in *ethyl acetate R* and dilute to 10.0 mL with the same solvent.

Acid value (*2.5.1*)

Maximum 1.0, determined on 2.0 g.

Peroxide value (*2.5.5*)

Maximum 10.0.

Saponification value (*2.5.6*)

Maximum 1.0.

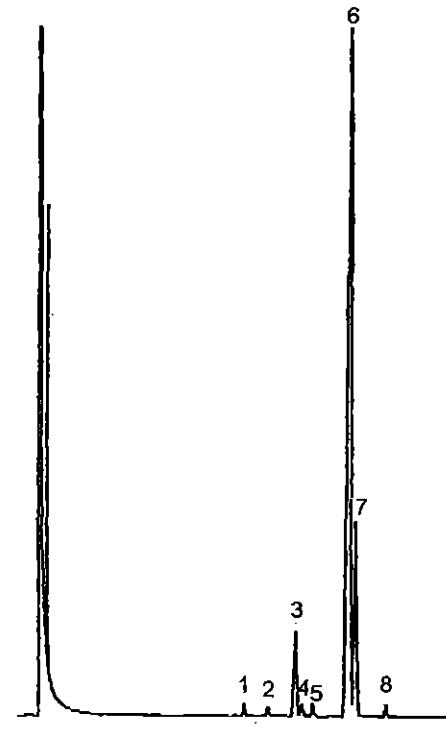
Carry out the test using 2.50 g of the substance to be examined, *0.1 M alcoholic potassium hydroxide*, *0.1 M hydrochloric acid*, and a factor of 5.61 (instead of 28.05).

Other sterols

Examine the chromatogram obtained with the test solution in the assay (Figure 1911.-1).

Composition of the other sterols:

— *cholesterol*: maximum 0.5 per cent;
— *brassicasterol*: maximum 0.5 per cent;
— *campesterol*: maximum 15.0 per cent;
— *campestanol*: maximum 5.0 per cent;
— *stigmasterol*: maximum 5.0 per cent;
— *sitostanol*: maximum 15.0 per cent;
— *Δ7-stigmastenol*: maximum 5.0 per cent.



1. cholesterol
2. brassicasterol
3. campesterol
4. campestanol
5. stigmasterol
6. β-sitosterol
7. sitostanol
8. Δ7-stigmastenol

Figure 1911.-1. – *Chromatogram for the assay of phytosterol (trimethylsilyl derivatives)*

Loss on drying (*2.2.32*)

Maximum 4.0 per cent, determined on 0.250 g by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*)

Maximum 0.5 per cent, determined on 1.0 g.

ASSAY

Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution Dissolve 0.100 g in *tetrahydrofuran R* and dilute to 10.0 mL with the same solvent. Introduce 100 µL of this solution into a 3 mL flask and evaporate to dryness under *nitrogen R*. Add 100 µL of a freshly prepared mixture of 50 µL of *1-methylimidazole R* and 1.0 mL of *heptafluoro-N-*

methyl-N-(trimethylsilyl)butanamide R, close the flask tightly and heat to 100 °C for 15 min. Allow to cool.

Reference solution (a) Dissolve 25 mg of *β-sitosterol R* and 25 mg of *sitostanol R* in *tetrahydrofuran R* and dilute to 10.0 mL with the same solvent. Introduce 100 µL of this solution into a 3 mL flask and evaporate to dryness under *nitrogen R*. Add 100 µL of a freshly prepared mixture of 50 µL of *1-methylimidazole R* and 1.0 mL of *heptafluoro-N-methyl-N-(trimethylsilyl)butanamide R*. Close the flask tightly and heat to 100 °C for 15 min. Allow to cool.

Reference solution (b) Dissolve 0.100 g of *β-sitosterol R* in *tetrahydrofuran R* and dilute to 10.0 mL with the same solvent. Introduce 100 µL of this solution into a 3 mL flask and evaporate to dryness under *nitrogen R*. Add 100 µL of a freshly prepared mixture of 50 µL of *1-methylimidazole R* and 1.0 mL of *heptafluoro-N-methyl-N-(trimethylsilyl)butanamide R*. Close the flask tightly and heat to 100 °C for 15 min. Allow to cool.

Column:
— *material*: quartz;
— *size*: *l* = 25 m, Ø = 0.3 mm;
— *stationary phase*: *vinyl(1)phenyl(5)methyl(94)polysiloxane R* (1 µm).

Carrier gas *hydrogen for chromatography R*.

Flow rate 2 mL/min.

Split ratio 1:20.

Temperature:
— *column*: 280 °C;
— *injection port and detector*: 300 °C.

Detection Flame ionisation.

Injection 1 µL.

Relative retentions With reference to β-sitosterol (retention time = about 16 min): cholesterol = about 0.7; brassicasterol = about 0.77; campesterol = about 0.84; campestanol = about 0.86; stigmasterol = about 0.9; sitostanol = about 1.02; Δ7-stigmastenol = about 1.1.

System suitability Reference solution (a):
— *resolution*: minimum 1.0 between the peaks due to β-sitosterol and sitostanol.

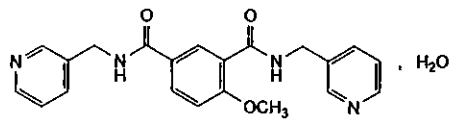
STORAGE

In an airtight container, protected from light.

Ph Eur

Picotamide Monohydrate

(Ph. Eur. monograph 1358)



$C_{21}H_{20}N_4O_3,H_2O$ 394.4

Action and use

Thromboxane Synthetase inhibitor; thromboxane receptor antagonist; antiplatelet agent.

Ph Eur

DEFINITION

Picotamide monohydrate contains not less than 98.0 per cent and not more than 101.0 per cent of 4-methoxy-*N*,*N'*-bis(pyridin-3-ylmethyl)benzene-1,3-dicarboxamide, calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white, crystalline powder, slightly soluble in water, soluble in ethanol and in methylene chloride. It dissolves in dilute mineral acids.

It shows polymorphism (*5.9*).

IDENTIFICATION

Examine by infrared spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *picotamide monohydrate CRS*. If the spectra obtained in the solid state shows differences, dissolve the substance to be examined and the reference substance separately in *acetone R*, evaporate to dryness and record new spectra using the residues.

TESTS

Appearance of solution

Dissolve 2.5 g in *methanol R* and dilute to 50 mL with the same solvent. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Related substances

Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel F_{254} plate R*.

Test solution Dissolve 0.5 g of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dilute 1 mL of the test solution to 10 mL with *methanol R*. Dilute 1 mL of the solution to 20 mL with *methanol R*.

Reference solution (b) Dilute 5 mL of reference solution (a) to 10 mL with *methanol R*.

Reference solution (c) Dissolve 0.5 g of the substance to be examined and 5 mg of *picotamide impurity A CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Apply to the plate 5 µL of each solution. Develop over a path of 15 cm using a mixture 0.8 volumes of *glacial acetic acid R*, 1 volume of *water R*, 2.5 volumes of *methanol R* and 8 volumes of *butanol R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the principal spot in the chromatogram obtained with reference solution (a) (0.5 per cent) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots.

Chlorides (*2.4.4*)

Dissolve 0.25 g in a mixture of 2.5 mL of *dilute nitric acid R* and 12.5 mL of *water R*. The solution complies with the limit test for chlorides (200 ppm).

Water (*2.5.12*)

4.5 per cent to 5.0 per cent, determined on 0.300 g by the semi-micro determination of water.

Sulfated ash (*2.4.14*)

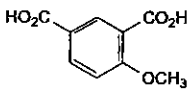
Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

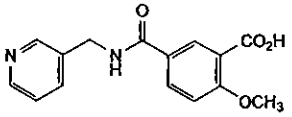
Dissolve 0.150 g in a mixture of 20 mL of *anhydrous acetic acid R* and 20 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 18.82 mg of $C_{21}H_{20}N_4O_3$.

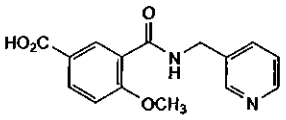
IMPURITIES



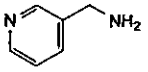
A. 4-methoxybenzene-1,3-dicarboxylic acid,



B. 2-methoxy-5-[[(pyridin-3-ylmethyl)amino] carbonyl]benzoic acid,



C. 4-methoxy-3-[[(pyridin-3-ylmethyl)amino] carbonyl]benzoic acid,

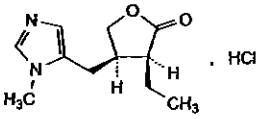


D. (pyridin-3-yl)methanamine.

Ph Eur

Pilocarpine Hydrochloride

(Ph. Eur. monograph 0633)



$C_{11}H_{17}ClN_2O_2$ 244.7 *54-71-7*

Action and use
Cholinoceptor agonist; treatment of glaucoma.

Preparation
Pilocarpine Hydrochloride Eye Drops

Ph Eur

DEFINITION
(3*S*,4*R*)-3-Ethyl-4-[(1-methyl-1*H*-imidazol-5-yl) methyl]dihydrofuran-2(3*H*)-one hydrochloride.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder or colourless crystals, hygroscopic.

Solubility
Very soluble in water and freely soluble in ethanol (96 per cent).

mp
About 203 °C.

IDENTIFICATION
First identification: A, B, D.

Second identification: C, D.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *pilocarpine hydrochloride CRS.*

If the substances are examined as discs, prepare them using *potassium chloride R.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 2 mL with the same solvent.

Reference solution Dissolve 10 mg of *pilocarpine hydrochloride CRS* in *methanol R* and dilute to 2 mL with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *concentrated ammonia R, methanol R, methylene chloride R* (1:14:85 *V/V/V*).

Application 2 µL.

Development Over a path of 15 cm.

Drying At 100-105 °C for 10 min, then allow to cool.

Detection Spray with *dilute potassium iodobismuthate solution R.*

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S
Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 50.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

pH (*2.2.3*)
3.5 to 4.5 for solution S.

Specific optical rotation (*2.2.7*)
+ 89 to + 93 (dried substance), determined on solution S.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in *water R* and dilute to 100.0 mL with the same solvent.

Reference solution (a) Dilute 5.0 mL of the test solution to 100.0 mL with *water R.* Dilute 2.0 mL of this solution to 20.0 mL with *water R.*

Reference solution (b) Dissolve 5.0 mg of *pilocarpine nitrate for system suitability CRS* (containing impurity A) in *water R* and dilute to 50.0 mL with the same solvent.

Reference solution (c) To 5 mL of the test solution, add 0.1 mL of *ammonia R* and heat the solution on a water-bath for 30 min, cool and dilute to 25 mL with *water R.* Dilute 3 mL of this solution to 25 mL with *water R.* Mainly pilocarpic acid (impurity B) is formed.

Column:
— *size: l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase: octadecylsilyl silica gel for chromatography R1* (5 µm) with a pore size of 10 nm and a carbon loading of 19 per cent.

Mobile phase Mix 55 volumes of *methanol R,* 60 volumes of *acetonitrile R* and 885 volumes of a 0.679 g/L solution of

tetrabutylammonium dihydrogen phosphate R previously adjusted to pH 7.7 with *dilute ammonia R2*.

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µL.

Run time Twice the retention time of pilocarpine.

Elution order Impurity B, impurity C, impurity A, pilocarpine.

Retention time Pilocarpine = about 20 min.

System suitability Reference solution (b):
— *resolution*: minimum 1.6 between the peaks due to impurity A and pilocarpine.

Limits:
— *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent);
— *sum of impurities A and B*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent);
— *sum of impurities other than A and B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *disregard limit*: 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent).

Iron (*2.4.9*)
Maximum 10 ppm, determined on solution S. Prepare the standard using 5 mL of *iron standard solution (1 ppm Fe) R* and 5 mL of *water R*.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

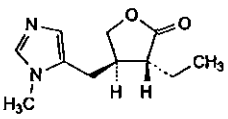
ASSAY
Dissolve 0.200 g in 50 mL of *ethanol (96 per cent) R* and add 5 mL of *0.01 M hydrochloric acid*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 24.47 mg of $C_{11}H_{17}ClN_2O_2$.

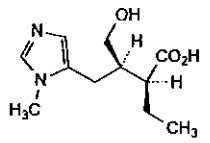
STORAGE
In an airtight container, protected from light.

IMPURITIES
Specified impurities *A, B.*

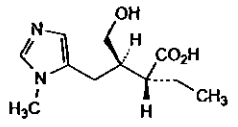
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C.*



A. (3*R*,4*R*)-3-ethyl-4-[(1-methyl-1*H*-imidazol-5-yl)methyl] dihydrofuran-2(3*H*)-one (isopilocarpine),



B. (2*S*,3*R*)-2-ethyl-3-(hydroxymethyl)-4-(1-methyl-1*H*-imidazol-5-yl)butanoic acid (pilocarpic acid),

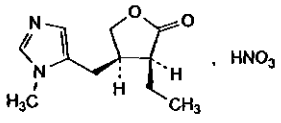


C. (2*R*,3*R*)-2-ethyl-3-(hydroxymethyl)-4-(1-methyl-1*H*-imidazol-5-yl)butanoic acid (isopilocarpic acid).

Ph Eur

Pilocarpine Nitrate

(Ph. Eur. monograph 0104)



$C_{11}H_{17}N_3O_5$ 271.3 *148-72-1*

Action and use
Cholinoceptor agonist; treatment of glaucoma.

Preparation
Pilocarpine Nitrate Eye Drops

Ph Eur

DEFINITION
(3*S*,4*R*)-3-Ethyl-4-[(1-methyl-1*H*-imidazol-5-yl) methyl]dihydrofuran-2(3*H*)-one nitrate.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder or colourless crystals.

Solubility
Freely soluble in water, sparingly soluble in ethanol (96 per cent).

It is sensitive to light.

mp
About 174 °C, with decomposition.

IDENTIFICATION
First identification: A, B, D.

Second identification: C, D.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *pilocarpine nitrate CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *water R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 10 mg of *pilocarpine nitrate CRS* in *water R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *concentrated ammonia R, methanol R, methylene chloride R* (1:14:85 *V/V/V*).

Application 10 µL.

Development Over a path of 15 cm.

Drying At 100-105 °C for 10 min and allow to cool.

Detection Spray with *potassium iodobismuthate solution R.*

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. It gives the reaction of nitrates (*2.3.1*).

TESTS

Solution S

Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 50.0 mL with the same solvent. Prepare immediately before use.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

pH (*2.2.3*)

3.5 to 4.5 for solution S.

Specific optical rotation (*2.2.7*)

+ 80 to + 83 (dried substance), determined on solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in *water R* and dilute to 100.0 mL with the same solvent.

Reference solution (a) Dilute 5.0 mL of the test solution to 100.0 mL with *water R*. Dilute 2.0 mL of this solution to 20.0 mL with *water R*.

Reference solution (b) Dissolve 5.0 mg of *pilocarpine nitrate for system suitability CRS* (containing impurity A) in *water R* and dilute to 50.0 mL with the same solvent.

Reference solution (c) To 5 mL of the test solution, add 0.1 mL of *ammonia R* and heat the solution on a water-bath for 30 min, cool and dilute to 25 mL with *water R*. Dilute 3 mL of this solution to 25 mL with *water R*. Mainly pilocarpic acid (impurity B) is formed.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R1* (5 µm) with a pore size of 10 nm and a carbon loading of 19 per cent.

Mobile phase Mix 55 volumes of *methanol R*, 60 volumes of *acetonitrile R* and 885 volumes of a 0.679 g/L solution of *tetrabutylammonium dihydrogen phosphate R* previously adjusted to pH 7.7 with *dilute ammonia R2*.

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µL.

Run time Twice the retention time of pilocarpine.

Elution order Impurity B, impurity C, impurity A, pilocarpine.

Retention time Pilocarpine = about 20 min.

System suitability Reference solution (b):

— *resolution*: minimum 1.6 between the peaks due to impurity A and pilocarpine.

Limits:

— *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent);

— *sum of impurities A and B*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent);

— *sum of impurities other than A and B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent); disregard any peak due to the nitrate ion with a relative retention with reference to pilocarpine of about 0.3.

Chlorides (*2.4.4*)

Maximum 70 ppm, determined on solution S.

Iron (*2.4.9*)

Maximum 10 ppm, determined on solution S. Prepare the standard using 5 mL of *iron standard solution (1 ppm Fe) R* and 5 mL of *water R*.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 30 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 27.13 mg of $C_{11}H_{17}N_3O_5$.

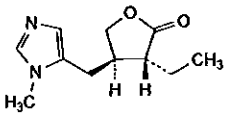
STORAGE

Protected from light.

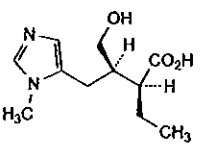
IMPURITIES

Specified impurities *A, B.*

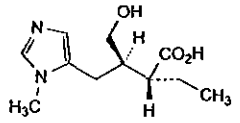
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C.*



A. (3*R*,4*R*)-3-ethyl-4-[(1-methyl-1*H*-imidazol-5-yl)methyl] dihydrofuran-2(3*H*)-one (isopilocarpine),



B. (2*S*,3*R*)-2-ethyl-3-(hydroxymethyl)-4-(1-methyl-1*H*-imidazol-5-yl)butanoic acid (pilocarpic acid),

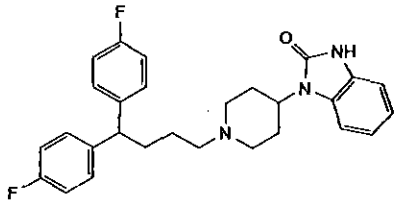


C. (2*R*,3*R*)-2-ethyl-3-(hydroxymethyl)-4-(1-methyl-1*H*-imidazol-5-yl)butanoic acid (isopilocarpic acid).

Ph Eur

Pimozide

(Ph. Eur. monograph 1254)



$C_{28}H_{29}F_2N_3O$ 461.6 *2062-78-4*

Action and use
Dopamine receptor antagonist; neuroleptic.

Preparation
Pimozide Tablets

Ph Eur

DEFINITION
1-[1-[4,4-Bis(4-fluorophenyl)butyl]piperidin-4-yl]-1,3-dihydro-2*H*-benzimidazol-2-one.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white powder.

Solubility
Practically insoluble in water, soluble in methylene chloride, sparingly soluble in methanol, slightly soluble in ethanol (96 per cent).

IDENTIFICATION
First identification: B.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 216 °C to 220 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *pimozide CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 30 mg of the substance to be examined in the mobile phase and dilute to 10 mL with the mobile phase.

Reference solution (a) Dissolve 30 mg of *pimozide CRS* in the mobile phase and dilute to 10 mL with the mobile phase.

Reference solution (b) Dissolve 30 mg of *pimozide CRS* and 30 mg of *benperidol CRS* in the mobile phase and dilute to 10 mL with the mobile phase.

Plate *TLC silica gel plate R.*

Mobile phase *acetone R, methanol R* (10:90 *V/V*).

Application 10 µL.

Development Over 3/4 of the plate.

Drying In a current of warm air for 15 min.

Detection Expose to iodine vapour until the spots appear.

System suitability Reference solution (b):
— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Mix about 5 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, then add 1 mL of *water R*, 0.05 mL of *phenolphthalein solution R1* and about 1 mL of *dilute hydrochloric acid R* to render the solution colourless. Filter. To a freshly prepared mixture of 0.1 mL of *alizarin S solution R* and 0.1 mL of *zirconyl nitrate solution R*, add 1.0 mL of the filtrate. Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank prepared in the same manner. The test solution is yellow and the blank is red.

TESTS
Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

Dissolve 0.2 g in *methanol R* and dilute to 20 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.10 g of the substance to be examined in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dissolve 5.0 mg of *pimozide CRS* and 2.0 mg of *mebendazole CRS* in *methanol R* and dilute to 100.0 mL with the same solvent.

Reference solution (b) Dilute 5.0 mL of the test solution to 100.0 mL with *methanol R*. Dilute 1.0 mL of this solution to 10.0 mL with *methanol R*.

Column:
— *size: l* = 0.1 m, Ø = 4.6 mm;
— *stationary phase: octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
— *mobile phase A*: solution containing 2.5 g/L of *ammonium acetate R* and 8.5 g/L of *tetrabutylammonium hydrogen sulfate R*;
— *mobile phase B: acetonitrile R;*

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	80 → 70	20 → 30
10 - 15	70	30
15 - 20	80	20

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 10 µL.

Relative retention With reference to pimozide (retention time = about 8 min): impurity A = about 0.1; mebendazole = about 0.88; impurity B = about 0.9; impurity C = about 0.95; impurity D = about 1.1; impurity E = about 1.3.

System suitability Reference solution (a):
— *resolution*: minimum 5.0 between the peaks due to mebendazole and pimozide.

Limits:
- — *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- — *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- — *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.75 per cent);
- — *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

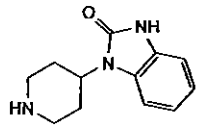
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY
Dissolve 0.300 g in 50 mL of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Titrate with *0.1 M perchloric acid*, using 0.2 mL of *naphtholbenzein solution R* as indicator.

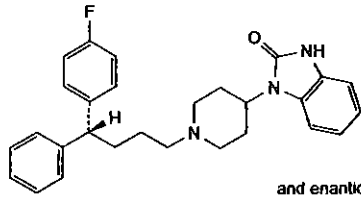
1 mL of *0.1 M perchloric acid* is equivalent to 46.16 mg of $C_{28}H_{29}F_2N_3O$.

STORAGE
Protected from light.

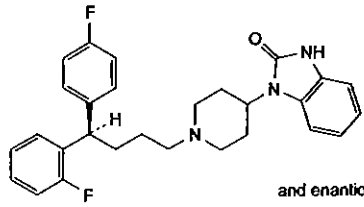
IMPURITIES
Specified impurities *A, B, C, D, E.*



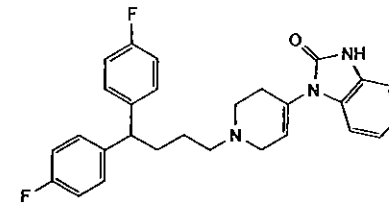
A. 1-(piperidin-4-yl)-1,3-dihydro-2*H*-benzimidazol-2-one,



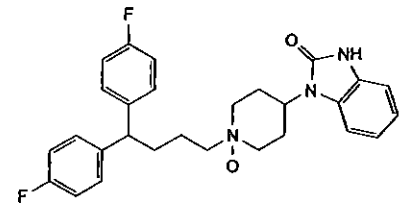
B. 1-[1-[(4*RS*)-4-(4-fluorophenyl)-4-phenylbutyl]piperidin-4-yl]-1,3-dihydro-2*H*-benzimidazol-2-one,



C. 1-[1-[(4*RS*)-4-(2-fluorophenyl)-4-(4-fluorophenyl) butyl]piperidin-4-yl]-1,3-dihydro-2*H*-benzimidazol-2-one,



D. 1-[1-[4,4-bis(4-fluorophenyl)butyl]-1,2,3,6-tetrahydropyridin-4-yl]-1,3-dihydro-2*H*-benzimidazol-2-one,

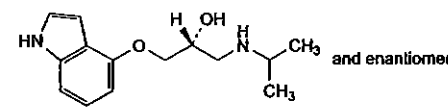


E. 1-[1-[4,4-bis(4-fluorophenyl)butyl]piperidin-4-yl 1-oxide]-1,3-dihydro-2*H*-benzimidazol-2-one.

Ph Eur

Pindolol

(Ph. Eur. monograph 0634)



$C_{14}H_{20}N_2O_2$ 248.3 *13523-86-9*

Action and use
Beta-adrenoceptor antagonist.

Preparation
Pindolol Tablets

Ph Eur

DEFINITION
Pindolol contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (2*RS*)-1-(1*H*-indol-4-yloxy)-3-[(1-methylethyl)amino]propan-2-ol, calculated with reference to the dried substance.

CHARACTERS
A white or almost white, crystalline powder, practically insoluble in water, slightly soluble in methanol. It dissolves in dilute mineral acids.

IDENTIFICATION
First identification: A, C.
Second identification: A, B, D.

A. Melting point (*2.2.14*): 169 °C to 174 °C.

B. Dissolve 20.0 mg in a 0.085 per cent *V/V* solution of *hydrochloric acid R* in *methanol R* and dilute to 100.0 mL with the same solution. Dilute 10.0 mL of the solution to 100.0 mL with a 0.085 per cent *V/V* solution of *hydrochloric acid R* in *methanol R*. Examined between 230 nm and 320 nm (*2.2.25*), the solution shows two absorption maxima, at 264 nm and at 287 nm, and a shoulder at 275 nm. The specific absorbance at the maxima are 330 to 350 and 170 to 190, respectively.

C. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *pindolol CRS*.

D. Examine in daylight the chromatograms on plate A obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution

Dissolve 0.5 g in *dilute acetic acid R* and dilute to 10 mL with the same acid. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 or B_5 (*2.2.2, Method II*).

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel GF_{254} R* as the coating substance. *Carry out all operations as rapidly as possible, protected from light.*

Test solution (a) Dissolve 0.10 g of the substance to be examined in a mixture of 1 volume of *anhydrous acetic acid R* and 99 volumes of *methanol R* and dilute to 5 mL with the same mixture of solvents. Prepare immediately before use and apply this solution to the plate last.

Test solution (b) Dilute 1 mL of test solution (a) to 10 mL with a mixture of 1 volume of *anhydrous acetic acid R* and 99 volumes of *methanol R*.

Reference solution (a) Dissolve 20 mg of *pindolol CRS* in a mixture of 1 volume of *anhydrous acetic acid R* and 99 volumes of *methanol R* and dilute to 10 mL with the same mixture of solvents.

Reference solution (b) Dilute 1.5 mL of reference solution (a) to 50 mL with a mixture of 1 volume of *anhydrous acetic acid R* and 99 volumes of *methanol R*.

A. Apply separately 5 µL of each solution. Develop the plate without delay over a path of 10 cm using a freshly prepared mixture of 4 volumes of *concentrated ammonia R*, 50 volumes of *ethyl acetate R* and 50 volumes of *methanol R*. Dry the plate briefly in a current of cold air. Spray the plate without delay with *dimethylaminobenzaldehyde solution R7* and heat to 50 °C for 20 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.3 per cent).

B. Apply separately 10 µL of each solution. Develop the plate without delay over a path of 10 cm using a freshly prepared mixture of 4 volumes of *concentrated ammonia R*, 50 volumes of *ethyl acetate R* and 50 volumes of *methanol R*. Dry the plate briefly in a current of cold air. Examine the plate without delay in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot and the spots detected on plate A, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.3 per cent).

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

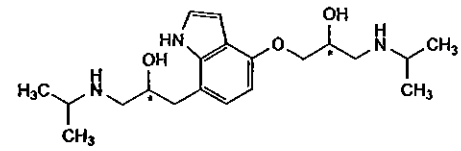
Dissolve 0.200 g in 80 mL of *methanol R*. Titrate with *0.1 M hydrochloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M hydrochloric acid* is equivalent to 24.83 mg of $C_{14}H_{20}N_2O_2$.

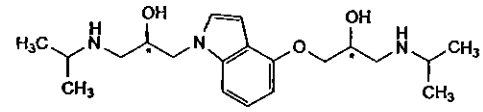
STORAGE

Store protected from light.

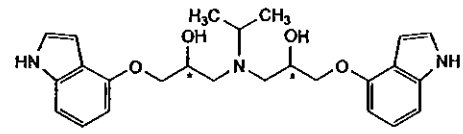
IMPURITIES



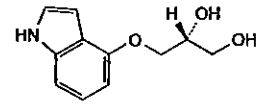
A. 1-[[7-[2-hydroxy-3-[(1-methylethyl)amino]propyl]-1*H*-indol-4-yl]oxy]-3-[(1-methylethyl)amino]propan-2-ol,



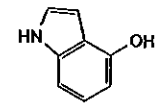
B. 1-[4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]-1*H*-indol-1-yl]-3-[(1-methylethyl)amino]propan-2-ol,



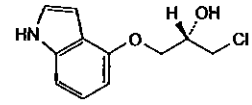
C. 1,1′-[(1-methylethyl)imino]bis[3-(1*H*-indol-4-yloxy) propan-2-ol],



D. (2*RS*)-3-(1*H*-indol-4-yloxy)propane-1,2-diol,



E. 1*H*-indol-4-ol,

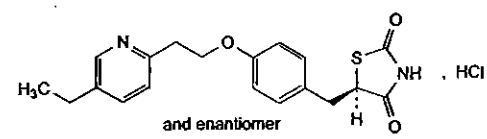


F. (2*RS*)-1-chloro-3-(1*H*-indol-4-yloxy)propan-2-ol.

Ph Eur

Pioglitazone Hydrochloride

(Ph. Eur. monograph 2601)



$C_{19}H_{21}ClN_2O_3S$ 392.9 *112529-15-4*

Action and use

Peroxisome proliferator-activated receptor (PPAR)-gamma agonist; treatment of diabetes mellitus.

Ph Eur

DEFINITION

(5*RS*)-5-[[4-[2-(5-Ethylpyridin-2-yl)ethoxy]phenyl]methyl] thiazolidine-2,4-dione hydrochloride.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white crystals or crystalline powder.

Solubility

Practically insoluble in water, slightly soluble to soluble in methanol, very slightly soluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *pioglitazone hydrochloride CRS.*

B. Dissolve 25 mg in 0.5 mL of *nitric acid R* and add 2 mL of *dilute nitric acid R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Specific optical rotation (*2.2.7*)

−0.2 to + 0.2.

Dissolve 2.5 g in *dimethylformamide R* and dilute to 50.0 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 20 mg of the substance to be examined in 20 mL of *methanol R* and dilute to 100.0 mL with the mobile phase.

Test solution (b) Dissolve 50.0 mg of the substance to be examined in *methanol R* and dilute to 100.0 mL with the same solvent. Dilute 2.0 mL of the solution to 20.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of test solution (a) to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Suspend 5 mg of *pioglitazone for system suitability CRS* (containing impurities B and C) in 5 mL of *methanol R*. Heat at 60 °C for about 30 s, cool to room temperature and dilute to 25.0 mL with the mobile phase. Filter through a membrane filter (nominal pore size 0.45 µm).

Reference solution (c) Dissolve 50.0 mg of *pioglitazone hydrochloride CRS* in *methanol R* and dilute to 100.0 mL with the same solvent. Dilute 2.0 mL of the solution to 20.0 mL with the mobile phase.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *glacial acetic acid R, acetonitrile R,* 7.71 g/L solution of *ammonium acetate R* (1:25:25 *V/V/V*).

Flow rate 0.7 mL/min.

Detection Spectrophotometer at 269 nm.

Injection 40 µL of test solution (a) and reference solutions (a) and (b).

Run time 4 times the retention time of pioglitazone.

Identification of impurities Use the chromatogram supplied with *pioglitazone for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B and C.

Relative retention With reference to pioglitazone (retention time = about 7 min): impurity B = about 1.4; impurity C = about 3.0.

System suitability Reference solution (b):

— *resolution*: minimum 5.0 between the peaks due to pioglitazone and impurity B.

Limits:

— *impurities B, C*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.32*)

Maximum 0.5 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

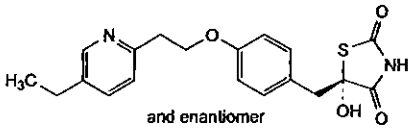
Injection 20 µL of test solution (b) and reference solution (c).

Calculate the percentage content of $C_{19}H_{21}ClN_2O_3S$ taking into account the assigned content of *pioglitazone hydrochloride CRS.*

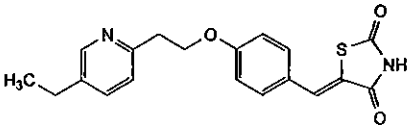
IMPURITIES

Specified impurities *B, C.*

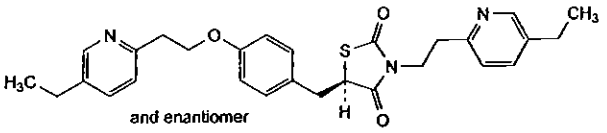
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, D, E.*



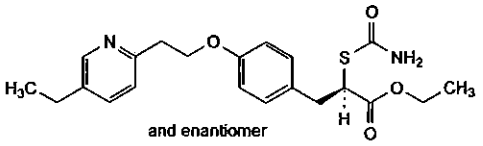
A. (5*RS*)-5-[[4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl] methyl]-5-hydroxythiazolidine-2,4-dione,



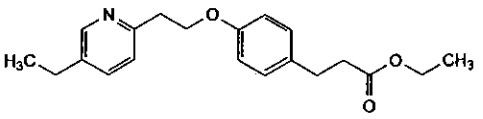
B. (5*Z*)-5-[[4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl] methylene]thiazolidine-2,4-dione,



C. (5*RS*)-5-[[4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl]methyl]-3-[2-(5-ethylpyridin-2-yl)ethyl]thiazolidine-2,4-dione,



D. ethyl (2*RS*)-2-(carbamoylsulfanyl)-3-[4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl]propanoate,

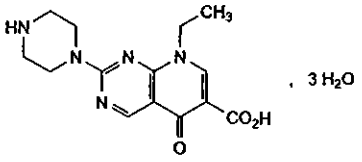


E. ethyl 3-[4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl]propanoate.

Ph Eur

Pipemidic Acid Trihydrate

(Ph. Eur. monograph 1743)



$C_{14}H_{17}N_5O_3,3H_2O$ 357.4 *72571-82-5*

Action and use
Antibacterial.

Ph Eur

DEFINITION

8-Ethyl-5-oxo-2-(piperazin-1-yl)-5,8-dihydropyrido[2,3-*d*]pyrimidine-6-carboxylic acid trihydrate.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
Pale yellow or yellow, crystalline powder.

Solubility
Very slightly soluble in water and in methylene chloride, practically insoluble in ethanol (96 per cent). It dissolves in dilute solutions of acids and of alkali hydroxides.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *pipemidic acid trihydrate CRS.*

TESTS

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 20 mg of the substance to be examined in 10 mL of the mobile phase and dilute to 20.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 10.0 mL with the mobile phase.

Reference solution (a) Dilute 2.0 mL of the test solution to 10.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve 1.0 mg of *ethyl parahydroxybenzoate R* in 2.0 mL of the test solution and dilute to 20.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R1* (5 µm).

Mobile phase Mix 20 volumes of *acetonitrile R*, 20 volumes of *methanol R* and 60 volumes of a solution containing 5.7 g/L of *citric acid monohydrate R* and 1.7 g/L of *sodium decanesulfonate R*.

Flow rate 0.8 mL/min.

Detection Spectrophotometer at 275 nm.

Injection 20 µL.

Run time 2.5 times the retention time of pipemidic acid.

Relative retention With reference to pipemidic acid (retention time = about 15 min): ethyl parahydroxybenzoate = about 0.8.

System suitability Reference solution (b):
— *resolution*: minimum 4.0 between the peaks due to ethyl parahydroxybenzoate and pipemidic acid.

Limits:
— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
14.0 per cent to 16.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.240 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

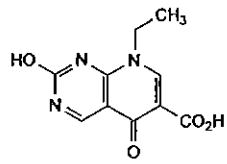
1 mL of *0.1 M perchloric acid* is equivalent to 30.33 mg of $C_{14}H_{17}N_5O_3$.

STORAGE

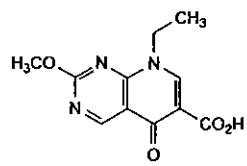
Protected from light.

IMPURITIES

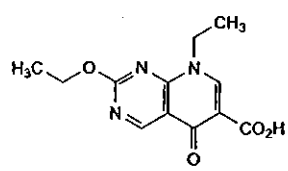
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, E, F.*



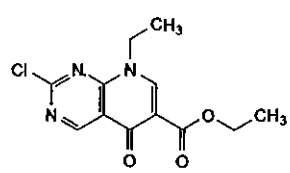
A. 8-ethyl-2-hydroxy-5-oxo-5,8-dihydropyrido[2,3-*d*] pyrimidine-6-carboxylic acid,



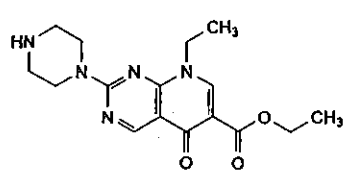
B. 8-ethyl-2-methoxy-5-oxo-5,8-dihydropyrido[2,3-*d*] pyrimidine-6-carboxylic acid,



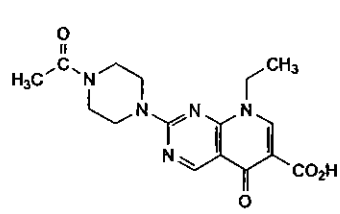
C. 2-ethoxy-8-ethyl-5-oxo-5,8-dihydropyrido[2,3-*d*] pyrimidine-6-carboxylic acid,



D. ethyl 2-chloro-8-ethyl-5-oxo-5,8-dihydropyrido[2,3-*d*] pyrimidine-6-carboxylate,



E. ethyl 8-ethyl-5-oxo-2-(piperazin-1-yl)-5,8-dihydropyrido [2,3-*d*]pyrimidine-6-carboxylate,



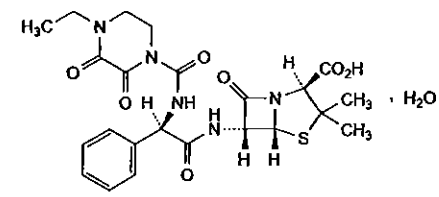
F. 2-(4-acetylpiperazin-1-yl)-8-ethyl-5-oxo-5,8-dihydropyrido [2,3-*d*]pyrimidine-6-carboxylic acid (acetylpipemidic acid).

Ph Eur

Piperacillin Monohydrate

Piperacillin

(Ph. Eur. monograph 1169)



$C_{23}H_{27}N_5O_7S,H_2O$ 535.6 *66258-76-2*

Action and use
Penicillin antibacterial.

Preparation
Piperacillin Infusion

Ph Eur

DEFINITION

(2*S*,5*R*,6*R*)-6-[(2*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid monohydrate.

Semi-synthetic product derived from a fermentation product.

Content
95.5 per cent to 102.0 per cent (anhydrous substance).

PRODUCTION

The manufacturing process is evaluated to determine the potential presence of *N*,*N*-dimethylaniline. Where necessary, the manufacturing process is validated to demonstrate that the piperacillin monohydrate complies with the following test:

***N*,*N*-Dimethylaniline** *(2.4.26, Method A)*
Maximum 20 ppm.

CHARACTERS

Appearance
White or almost white powder.

Solubility
Slightly soluble in water, freely soluble in methanol, slightly soluble in ethyl acetate.

IDENTIFICATION

Infrared absorption spectrophotometry *(2.2.24)*.

Comparison *piperacillin CRS.*

TESTS

Appearance of solution
The solution is not more opalescent than reference suspension II *(2.2.1)* and its absorbance *(2.2.25)* at 430 nm is not greater than 0.10.

Dissolve 2.50 g in *sodium carbonate solution R* and dilute to 25 mL with the same solvent.

Related substances
Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.120 g of the substance to be examined in mobile phase B and dilute to 20.0 mL with mobile phase B.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase B.

Reference solution (b) Dissolve 6 mg of *piperacillin impurity I CRS* in mobile phase B and dilute to 20 mL with mobile phase B.

Reference solution (c) Dissolve 6 mg of *anhydrous ampicillin CRS* (impurity A) in mobile phase B and dilute to 20 mL with mobile phase B.

Reference solution (d) To 2 mL of reference solution (b), add 1 mL of reference solution (c) and dilute to 10 mL with mobile phase B.

Reference solution (e) Dissolve 6 mg of *piperacillin for peak identification CRS* (containing impurities A, B, C, D, E, F, G, I, J, K, L, M, O, P, Q, R, S and T) in mobile phase B and dilute to 1 mL with mobile phase B.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl amorphous organosilica polymer for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase:
- *mobile phase A*: mix 3 mL of a 320 g/L solution of *tetrabutylammonium hydroxide R*, 100 mL of a 27.6 g/L solution of *sodium dihydrogen phosphate R*, 275 mL of *methanol R1* and 622 mL of *water for chromatography R*; adjust the apparent pH to 5.5 with *phosphoric acid R*;
- *mobile phase B*: mix 3 mL of a 320 g/L solution of *tetrabutylammonium hydroxide R*, 100 mL of a 27.6 g/L solution of *sodium dihydrogen phosphate R*, 282 mL of *water for chromatography R* and 615 mL of *methanol R1*; adjust the apparent pH to 5.5 with *phosphoric acid R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 6	100	0
6 - 55	100 → 71	0 → 29
55 - 73	71 → 10	29 → 90
73 - 85	10	90

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 220 nm.

Autosampler Set at 4 °C.

Injection 10 µL of the test solution and reference solutions (a), (d) and (e).

Identification of impurities Use the chromatogram supplied with *piperacillin for peak identification CRS* and the chromatogram obtained with reference solution (e) to identify the peaks due to impurities A, B, C, D, E, F, G, I, J, K, L, M, O, P, Q, R, S and T.

Relative retention With reference to piperacillin (retention time = about 54 min): impurity E = about 0.05; impurity I = about 0.12; impurity A = about 0.14; impurity G = about 0.30; impurity J = about 0.36; impurity F = about 0.57; impurity K = about 0.60; impurity L = about 0.65; impurity B (isomer 1) = about 0.71; impurity M = about 0.75; impurity B (isomer 2) = about 0.83; impurity C (isomer 1) = about 0.87; impurity C (isomer 2) = about 0.92; impurity O = about 1.23; impurity P = about 1.26; impurity Q = about 1.31; impurity R = about 1.36; impurity S = about 1.38; impurity T = about 1.41; impurity D = about 1.54.

System suitability Reference solution (d):
- *resolution*: minimum 1.5 between the peaks due to impurities I and A.

Calculation of percentage contents:
- *correction factors*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.3; impurity E = 0.4; impurity I = 3.2;
- for each impurity, use the concentration of piperacillin monohydrate in reference solution (a).

Limits:
- *impurity G*: maximum 1.5 per cent;
- *impurities B (sum of isomers), D*: for each impurity, maximum 1.0 per cent;
- *impurity F*: maximum 0.8 per cent;
- *impurity C (sum of isomers)*: maximum 0.7 per cent;
- *impurity S*: maximum 0.5 per cent;
- *impurity T*: maximum 0.3 per cent;
- *impurities A, E, I, J, K, L, M, O, P, Q, R*: for each impurity, maximum 0.2 per cent;
- *any other impurity*: for each impurity, maximum 0.15 per cent;
- *total*: maximum 2.5 per cent;
- *reporting threshold*: 0.05 per cent.

Water (*2.5.12*)
2.0 per cent to 4.0 per cent, determined on 0.500 g.

ASSAY

Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R*, 31.2 g/L solution of *sodium dihydrogen phosphate R* (25:75 *V/V*).

Test solution Dissolve 0.100 g of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture. Dilute 5.0 mL of the solution to 50.0 mL with the solvent mixture.

Reference solution (a) Dissolve 50.0 mg of *piperacillin CRS* in the solvent mixture and dilute to 50.0 mL with the solvent mixture. Dilute 10.0 mL of the solution to 50.0 mL with the solvent mixture.

Reference solution (b) Dissolve 0.1 g of the substance to be examined in the solvent mixture and dilute to 50 mL with the solvent mixture.

Reference solution (c) Dissolve 5 mg of *piperacillin impurity N CRS* in the solvent mixture and dilute to 25 mL with the solvent mixture.

Reference solution (d) To 5 mL of reference solution (b), add 0.1 mL of reference solution (c) and dilute to 50 mL with the solvent mixture.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl amorphous organosilica polymer for chromatography R* (3.5 µm);
- *temperature*: 40 °C.

Mobile phase:
- *mobile phase A*: mix 24 mL of an 80 g/L solution of *tetrabutylammonium hydroxide R*, 200 mL of *acetonitrile for chromatography R*, 200 mL of a 31.2 g/L solution of *sodium dihydrogen phosphate R* and 576 mL of *water for chromatography R*; adjust the apparent pH to 5.5 with *dilute phosphoric acid R* or *dilute sodium hydroxide solution R*;
- *mobile phase B*: mix 24 mL of an 80 g/L solution of *tetrabutylammonium hydroxide R*, 126 mL of *water for chromatography R*, 200 mL of a 31.2 g/L solution of *sodium dihydrogen phosphate R* and 650 mL of *acetonitrile for chromatography R*; adjust the apparent pH to 5.5 with *dilute phosphoric acid R* or *dilute sodium hydroxide solution R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3.5	100	0
3.5 - 4	100 → 92	0 → 8
4 - 14	92 → 86	8 → 14
14 - 15	86 → 0	14 → 100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µL of the test solution and reference solutions (a) and (d).

Relative retention With reference to piperacillin (retention time = about 13 min): impurity N = about 0.96.

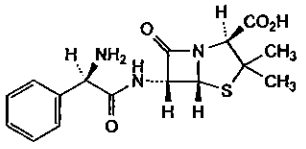
System suitability Reference solution (d):
— *resolution*: minimum 1.5 between the peaks due to impurity N and piperacillin.

Calculate the percentage content of $C_{23}H_{27}N_5O_7S$ using the chromatogram obtained with reference solution (a) and taking into account the assigned content of *piperacillin CRS*.

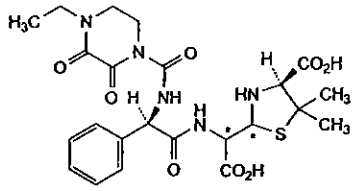
IMPURITIES

Specified impurities *A, B, C, D, E, F, G, I, J, K, L, M, O, P, Q, R, S, T.*

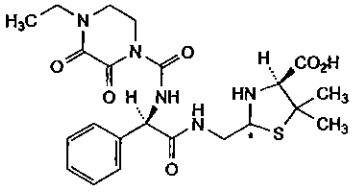
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *H, N.*



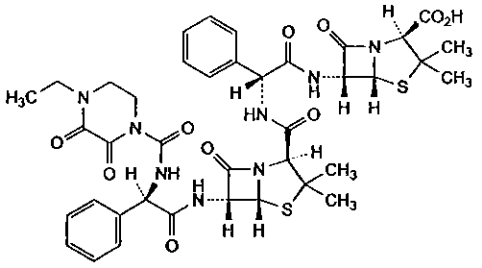
A. (2*S*,5*R*,6*R*)-6-[(2*R*)-2-amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (ampicillin),



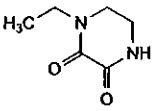
B. (2*Ξ*,4*S*)-2-[(*Ξ*)-carboxy[(2*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid (penicilloic acids of piperacillin),



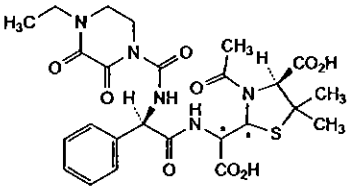
C. (2*Ξ*,4*S*)-2-[[(2*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid (penilloic acids of piperacillin),



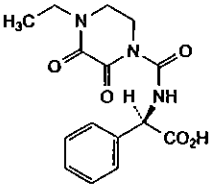
D. (2*S*,5*R*,6*R*)-6-[(2*R*)-2-[(2*S*,5*R*,6*R*)-6-[(2*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (piperacillinylampicillin),



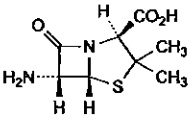
E. 1-ethylpiperazine-2,3-dione,



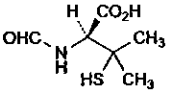
F. (2*Ξ*,4*S*)-3-acetyl-2-[(*Ξ*)-carboxy[(2*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid (acetylated penicilloic acids of piperacillin),



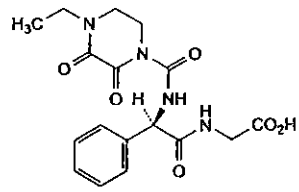
G. (*R*)-(4-ethyl-2,3-dioxopiperazine-1-carboxamido) phenylacetic acid,



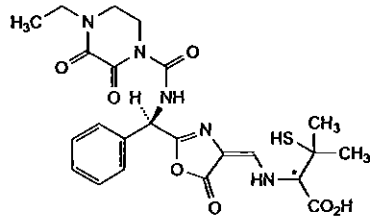
H. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillamic acid),



I. (2*S*)-2-formamido-3-methyl-3-sulfanylbutanoic acid (*N*-formylpenicillamine),

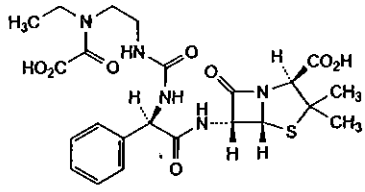


J. [(2*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]acetic acid,

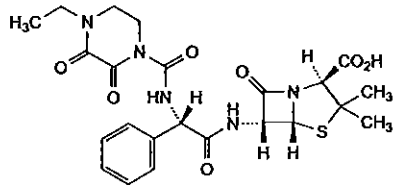


K. (2Ξ)-2-[[(*E*)-[2-[(*R*)-[(4-ethyl-2,3-dioxopiperazine-1-carboxamido)phenylmethyl]-5-oxo-1,3-oxazol-4(5*H*)-ylidene]methyl]amino]-3-methyl-3-sulfanylbutanoic acid (penicillenic acid),

L. unknown structure,

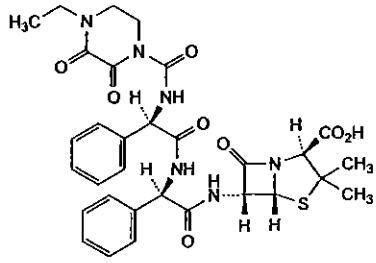


M. (2*S*,5*R*,6*R*)-6-[(2*R*)-2-[[[2-[ethyl(oxalo)amino]ethyl]carbamoyl]amino]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid,



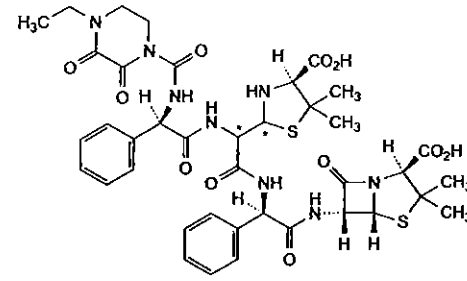
N. (2*S*,5*R*,6*R*)-6-[(2*S*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (L-piperacillin),

O. unknown structure,

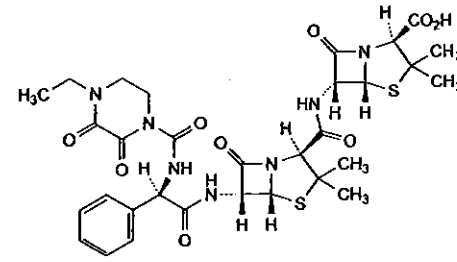


P. (2*S*,5*R*,6*R*)-6-[(2*R*)-2-[(2*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid,

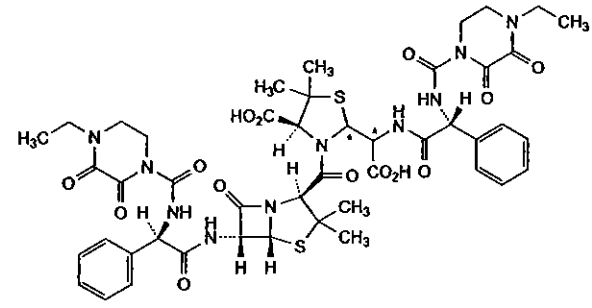
Q. unknown structure,



R. (2*S*,5*R*,6*R*)-6-[(2*R*)-2-[(2Ξ)-2-[(2Ξ,4*S*)-4-carboxy-5,5-dimethyl-1,3-thiazolidin-2-yl]-2-[(2*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]acetamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid,



S. (2*S*,5*R*,6*R*)-6-[(2*S*,5*R*,6*R*)-6-[(2*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid,

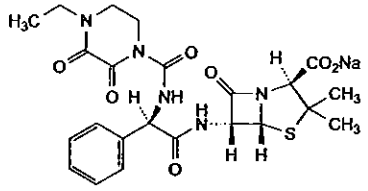


T. (2Ξ,4*S*)-2-[(Ξ)-carboxy[(2*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-3-[(2*S*,5*R*,6*R*)-6-[(2*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carbonyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid.

Ph Eur

Piperacillin Sodium

(Ph. Eur. monograph 1168)



$C_{23}H_{26}N_5NaO_7S$ 539.5 *59703-84-3*

Action and use
Penicillin antibacterial.

Ph Eur

DEFINITION

Sodium (2*S*,5*R*,6*R*)-6-[(2*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate.

Semi-synthetic product derived from a fermentation product.

Content
95.5 per cent to 102.0 per cent (anhydrous substance).

PRODUCTION

The manufacturing process is evaluated to determine the potential presence of *N*,*N*-dimethylaniline. Where necessary, the manufacturing process is validated to demonstrate that the piperacillin sodium complies with the following test:

***N*,*N*-Dimethylaniline** *(2.4.26, Method A)*
Maximum 20 ppm.

CHARACTERS

Appearance
White or almost white, hygroscopic powder.

Solubility
Freely soluble in water and in methanol, practically insoluble in ethyl acetate.

IDENTIFICATION

A. Infrared absorption spectrophotometry *(2.2.24)*.

Preparation Dissolve 0.250 g in *water R*, add 0.5 mL of *dilute hydrochloric acid R* and 5 mL of *ethyl acetate R*; stir and allow to stand for 10 min in iced water. Filter the crystals through a small sintered-glass filter (40), applying suction. Wash with 5 mL of *water R* and 5 mL of *ethyl acetate R*, then dry in an oven at 60 °C for 60 min.

Comparison *piperacillin CRS*.

B. It gives reaction (a) of sodium *(2.3.1)*.

TESTS

Solution S
Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent.

Appearance of solution
Solution S is clear *(2.2.1)* and its absorbance *(2.2.25)* at 430 nm is not greater than 0.10.

pH *(2.2.3)*
5.0 to 7.0 for solution S.

Related substances
Liquid chromatography *(2.2.29)*.

Test solution Dissolve 0.120 g of the substance to be examined in *water R* and dilute to 20.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase B.

Reference solution (b) Dissolve 6 mg of *piperacillin impurity I CRS* in mobile phase B and dilute to 20 mL with mobile phase B.

Reference solution (c) Dissolve 6 mg of *anhydrous ampicillin CRS* (impurity A) in mobile phase B and dilute to 20 mL with mobile phase B.

Reference solution (d) To 2 mL of reference solution (b), add 1 mL of reference solution (c) and dilute to 10 mL with mobile phase B.

Reference solution (e) Dissolve 6 mg of *piperacillin for peak identification CRS* (containing impurities A, B, C, D, E, F, I, J, K, L, M, O, P, Q, R, S and T) in mobile phase B and dilute to 1 mL with mobile phase B.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl amorphous organosilica polymer for chromatography R* (5 µm);
— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: mix 3 mL of a 320 g/L solution of *tetrabutylammonium hydroxide R*, 100 mL of a 27.6 g/L solution of *sodium dihydrogen phosphate R*, 275 mL of *methanol R1* and 622 mL of *water for chromatography R*; adjust the apparent pH to 5.5 with *phosphoric acid R*;
— *mobile phase B*: mix 3 mL of a 320 g/L solution of *tetrabutylammonium hydroxide R*, 100 mL of a 27.6 g/L solution of *sodium dihydrogen phosphate R*, 282 mL of *water for chromatography R* and 615 mL of *methanol R1*; adjust the apparent pH to 5.5 with *phosphoric acid R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 6	100	0
6 - 55	100 → 71	0 → 29
55 - 73	71 → 10	29 → 90
73 - 85	10	90

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 220 nm.

Autosampler Set at 4 °C.

Injection 10 µL of the test solution and reference solutions (a), (d) and (e).

Identification of impurities Use the chromatogram supplied with *piperacillin for peak identification CRS* and the chromatogram obtained with reference solution (e) to identify the peaks due to impurities A, B, C, D, E, F, I, J, K, L, M, O, P, Q, R, S and T.

Relative retention With reference to piperacillin (retention time = about 54 min): impurity E = about 0.05; impurity I = about 0.12; impurity A = about 0.14; impurity J = about 0.36; impurity F = about 0.57; impurity K = about 0.60; impurity L = about 0.65; impurity B (isomer 1) = about 0.71; impurity M = about 0.75; impurity B (isomer 2) = about 0.83; impurity C (isomer 1) = about 0.87; impurity C (isomer 2) = about 0.92; impurity O = about 1.23; impurity P = about 1.26; impurity Q = about 1.31; impurity R = about 1.36; impurity S = about 1.38; impurity T = about 1.41; impurity D = about 1.54.

System suitability Reference solution (d):
— *resolution*: minimum 1.5 between the peaks due to impurities I and A.

Calculation of percentage contents:
— *correction factors*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.3; impurity E = 0.4; impurity I = 3.2;
— for each impurity, use the concentration of piperacillin sodium in reference solution (a).

Limits:
— *impurities B (sum of isomers), D*: for each impurity, maximum 1.0 per cent;
— *impurity F*: maximum 0.8 per cent;
— *impurity C (sum of isomers)*: maximum 0.7 per cent;
— *impurities L, T*: for each impurity, maximum 0.3 per cent;
— *impurities A, E, I, J, K, M, O, P, Q, R, S*: for each impurity, maximum 0.2 per cent;
— *any other impurity*: for each impurity, maximum 0.15 per cent;
— *total*: maximum 2.5 per cent;
— *reporting threshold*: 0.05 per cent.

Water (*2.5.12*)
Maximum 2.0 per cent, determined on 0.500 g.

ASSAY

Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R*, 31.2 g/L solution of *sodium dihydrogen phosphate R* (25:75 *V/V*).

Test solution Dissolve 0.100 g of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture. Dilute 5.0 mL of the solution to 50.0 mL with the solvent mixture.

Reference solution (a) Dissolve 50.0 mg of *piperacillin CRS* in the solvent mixture and dilute to 50.0 mL with the solvent mixture. Dilute 10.0 mL of the solution to 50.0 mL with the solvent mixture.

Reference solution (b) Dissolve 0.1 g of the substance to be examined in the solvent mixture and dilute to 50 mL with the solvent mixture.

Reference solution (c) Dissolve 5 mg of *piperacillin impurity N CRS* in the solvent mixture and dilute to 25 mL with the solvent mixture.

Reference solution (d) To 5 mL of reference solution (b), add 0.1 mL of reference solution (c) and dilute to 50 mL with the solvent mixture.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl amorphous organosilica polymer for chromatography R* (3.5 µm);
— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: mix 24 mL of an 80 g/L solution of *tetrabutylammonium hydroxide R*, 200 mL of *acetonitrile for chromatography R*, 200 mL of a 31.2 g/L solution of *sodium dihydrogen phosphate R* and 576 mL of *water for chromatography R*; adjust the apparent pH to 5.5 with *dilute phosphoric acid R* or *dilute sodium hydroxide solution R*;
— *mobile phase B*: mix 24 mL of an 80 g/L solution of *tetrabutylammonium hydroxide R*, 126 mL of *water for chromatography R*, 200 mL of a 31.2 g/L solution of *sodium dihydrogen phosphate R* and 650 mL of *acetonitrile for chromatography R*; adjust the apparent pH to 5.5 with *dilute phosphoric acid R* or *dilute sodium hydroxide solution R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3.5	100	0
3.5 - 4	100 → 92	0 → 8
4 - 14	92 → 86	8 → 14
14 - 15	86 → 0	14 → 100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µL of the test solution and reference solutions (a) and (d).

Relative retention With reference to piperacillin (retention time = about 13 min): impurity N = about 0.96.

System suitability Reference solution (d):
— *resolution*: minimum 1.5 between the peaks due to impurity N and piperacillin.

Calculate the percentage content of $C_{23}H_{26}N_5NaO_7S$ using the chromatogram obtained with reference solution (a) and taking into account the assigned content of *piperacillin CRS* and a conversion factor of 1.042.

STORAGE

In an airtight container. If the substance is sterile, the container is also sterile and tamper-evident.

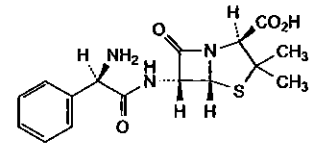
LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

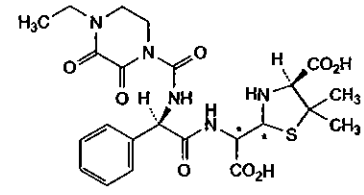
IMPURITIES

Specified impurities *A, B, C, D, E, F, I, J, K, L, M, O, P, Q, R, S, T.*

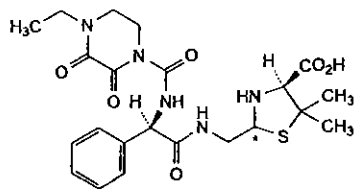
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *G, H, N.*



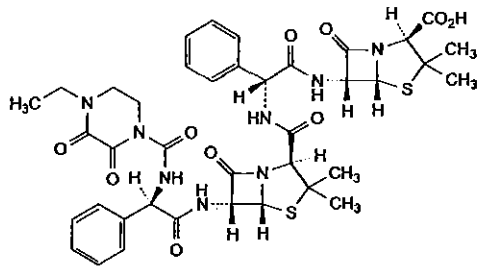
A. (2*S*,5*R*,6*R*)-6-[(2*R*)-2-amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (ampicillin),



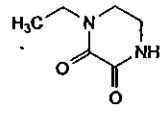
B. (2*Ξ*,4*S*)-2-[(*Ξ*)-carboxy[(2*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid (penicilloic acids of piperacillin),



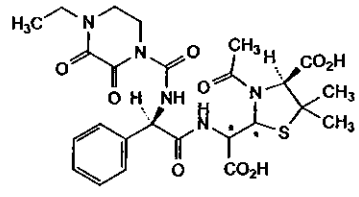
C. (2*E*,4*S*)-2-[[(2*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid (penilloic acids of piperacillin),



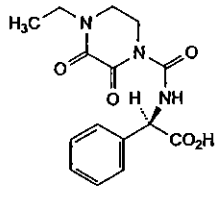
D. (2*S*,5*R*,6*R*)-6-[(2*R*)-2-[(2*S*,5*R*,6*R*)-6-[(2*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (piperacillinylampicillin),



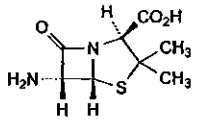
E. 1-ethylpiperazine-2,3-dione,



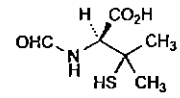
F. (2*E*,4*S*)-3-acetyl-2-[(*E*)-carboxy[(2*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid (acetylated penicilloic acids of piperacillin),



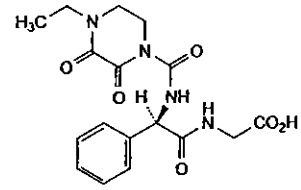
G. (*R*)-(4-ethyl-2,3-dioxopiperazine-1-carboxamido) phenylacetic acid,



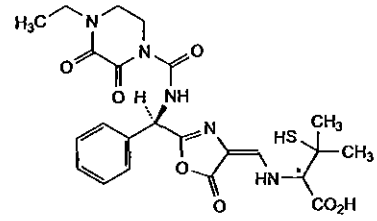
H. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillamic acid),



I. (2*S*)-2-formamido-3-methyl-3-sulfanylbutanoic acid (*N*-formylpenicillamine),

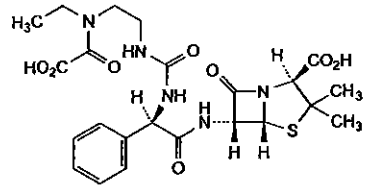


J. [(2*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]acetic acid,

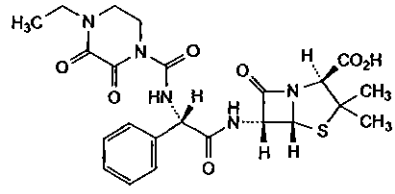


K. (2*E*)-2-[[(*E*)-[2-[(*R*)-[(4-ethyl-2,3-dioxopiperazine-1-carboxamido)phenylmethyl]-5-oxo-1,3-oxazol-4(5*H*)-ylidene]methyl]amino]-3-methyl-3-sulfanylbutanoic acid (penicillenic acid),

L. unknown structure,

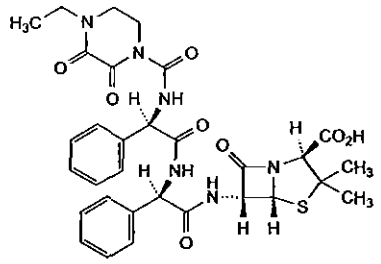


M. (2*S*,5*R*,6*R*)-6-[(2*R*)-2-[[[2-[ethyl(oxalo)amino]ethyl]carbamoyl]amino]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid,



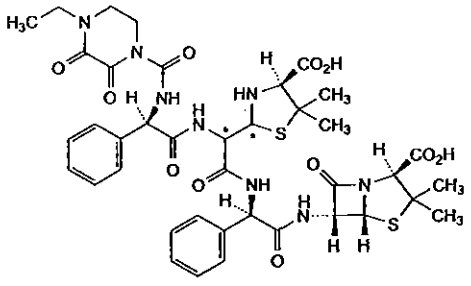
N. (2*S*,5*R*,6*R*)-6-[(2*S*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (L-piperacillin),

O. unknown structure,

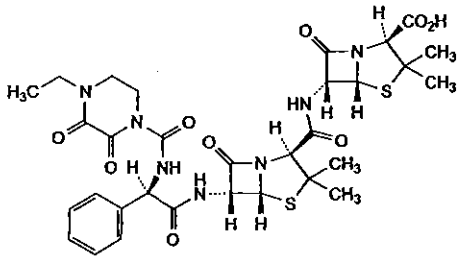


P. (2*S*,5*R*,6*R*)-6-[(2*R*)-2-[(2*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylic acid,

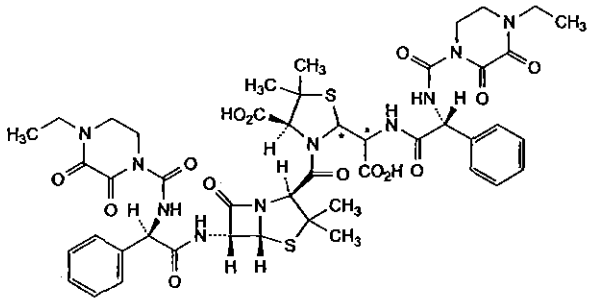
Q. unknown structure,



R. (2*S*,5*R*,6*R*)-6-[(2*R*)-2-[(2*E*)-2-[(2*E*,4*S*)-4-carboxy-5,5-dimethyl-1,3-thiazolidin-2-yl]-2-[(2*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido] acetamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid,



S. (2*S*,5*R*,6*R*)-6-[(2*S*,5*R*,6*R*)-6-[(2*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylic acid,

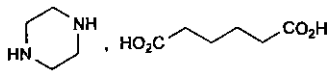


T. (2*E*,4*S*)-2-[(*E*)-carboxy[(2*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido] methyl]-3-[(2*S*,5*R*,6*R*)-6-[(2*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carbonyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid.

Ph Eur

Piperazine Adipate

(Ph. Eur. monograph 0423)



$C_{10}H_{20}N_2O_4$ 232.3 *142-88-1*

Action and use
Anthelminthic.

Ph Eur

DEFINITION
Piperazine adipate contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of piperazine hexanedioate, calculated with reference to the anhydrous substance.

CHARACTERS
A white or almost white crystalline powder, soluble in water, practically insoluble in alcohol. It melts at about 250 °C, with decomposition.

IDENTIFICATION
First identification: A.

Second identification: B, C.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *piperazine adipate CRS*. Examine the substances prepared as discs.

B. Examine the chromatograms obtained in the test for related substances after spraying with the ninhydrin solutions. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. To 10 mL of solution S (see Tests) add 5 mL of *hydrochloric acid R* and shake with three quantities, each of 10 mL, of *ether R*. Evaporate the combined ether layers to dryness. The residue, washed with 5 mL of *water R* and dried at 100 °C to 105 °C, melts (*2.2.14*) at 150 °C to 154 °C.

TESTS
Solution S
Dissolve 2.5 g in *water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_8 (*2.2.2, Method II*).

Related substances
Examine by thin-layer chromatography (*2.2.27*), using a suitable silica gel as the coating substance.

Test solution (a) Dissolve 1.0 g of the substance to be examined in 6 mL of *concentrated ammonia R* and dilute to 10 mL with *ethanol R*.

Test solution (b) Dilute 1 mL of test solution (a) to 10 mL with a mixture of 2 volumes of *ethanol R* and 3 volumes of *concentrated ammonia R*.

Reference solution (a) Dissolve 0.1 g of *piperazine adipate CRS* in a mixture of 2 volumes of *ethanol R* and 3 volumes of *concentrated ammonia R* and dilute to 10 mL with the same mixture of solvents.

Reference solution (b) Dissolve 25 mg of *ethylenediamine R* in a mixture of 2 volumes of *ethanol R* and 3 volumes of *concentrated ammonia R* and dilute to 100 mL with the same mixture of solvents.

Reference solution (c) Dissolve 25 mg of *triethylenediamine R* in a mixture of 2 volumes of *ethanol R* and 3 volumes of *concentrated ammonia R* and dilute to 100 mL with the same mixture of solvents.

Reference solution (d) Dissolve 12.5 mg of *triethylenediamine R* in 5.0 mL of test solution (a) and dilute to 50 mL with a mixture of 2 volumes of *ethanol R* and 3 volumes of *concentrated ammonia R*.

Apply separately to the plate 5 µL of each solution. Develop over a path of 15 cm using a freshly prepared mixture of 20 volumes of *concentrated ammonia R* and 80 volumes of *acetone R*. Dry the plate at 105 °C and spray successively with a 3 g/L solution of *ninhydrin R* in a mixture of 3 volumes of *anhydrous acetic acid R* and 100 volumes of *butanol R* and a 1.5 g/L solution of *ninhydrin R* in *ethanol R*. Dry the plate at 105 °C for 10 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent). Spray the plate with *0.05 M iodine* and allow to stand for about 10 min. Any spot corresponding to triethylenediamine in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.25 per cent). The test is not valid unless the chromatogram obtained with reference solution (d) shows two clearly separated spots. Disregard any spots remaining on the line of application.

Water (*2.5.12*)
Not more than 0.5 per cent, determined on 1.00 g by the semi-micro determination of water.

Sulfated ash (*2.4.14*)
Not more than 0.1 per cent, determined on 1.0 g.

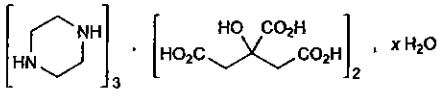
ASSAY
Dissolve 0.100 g in 10 mL of *anhydrous acetic acid R* with gentle heating and dilute to 70 mL with the same acid. Titrate with *0.1 M perchloric acid* using 0.25 mL of *naphtholbenzein solution R* as indicator until the colour changes from brownish-yellow to green.

1 mL of *0.1 M perchloric acid* is equivalent to 11.61 mg of $C_{10}H_{20}N_2O_4$.

Ph Eur

Piperazine Citrate

(Ph. Eur. monograph 0424)



$C_{24}H_{46}N_6O_{14},xH_2O$ 643
(anhydrous substance)

Action and use
Anthelminthic.

Preparation
Piperazine Citrate Elixir

Ph Eur

DEFINITION
Piperazine citrate contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of tripiperazine bis(2-hydroxy-propane-1,2,3-tricarboxylate), calculated with reference to the anhydrous substance.
It contains a variable quantity of water.

CHARACTERS
A white or almost white granular powder, freely soluble in water, practically insoluble in ethanol (96 per cent).

After drying at 100 °C to 105 °C, it melts at about 190 °C.

IDENTIFICATION
First identification: A.

Second identification: B, C.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *piperazine citrate CRS*. Dry the substance to be examined and the reference substance at 120 °C for 5 h, powder the substances avoiding uptake of water, prepare discs and record the spectra without delay.

B. Examine the chromatograms obtained in the test for related substances after spraying with the ninhydrin solutions. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 0.5 g in *water R* and dilute to 5 mL with the same solvent. The solution gives the reaction of citrates (*2.3.1*).

TESTS

Solution S
Dissolve 1.25 g in *water R* and dilute to 25 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_8 (*2.2.2, Method II*).

Related substances
Examine by thin-layer chromatography (*2.2.27*), using a suitable silica gel as the coating substance.

Test solution (a) Dissolve 1.0 g of the substance to be examined in 6 mL of *concentrated ammonia R* and dilute to 10 mL with *anhydrous ethanol R*.

Test solution (b) Dilute 1 mL of test solution (a) to 10 mL with a mixture of 2 volumes of *anhydrous ethanol R* and 3 volumes of *concentrated ammonia R*.

Reference solution (a) Dissolve 0.1 g of *piperazine citrate CRS* in a mixture of 2 volumes of *anhydrous ethanol R* and 3 volumes of *concentrated ammonia R* and dilute to 10 mL with the same mixture of solvents.

Reference solution (b) Dissolve 25 mg of *ethylenediamine R* in a mixture of 2 volumes of *anhydrous ethanol R* and 3 volumes of *concentrated ammonia R* and dilute to 100 mL with the same mixture of solvents.

Reference solution (c) Dissolve 25 mg of *triethylenediamine R* in a mixture of 2 volumes of *anhydrous ethanol R* and 3 volumes of *concentrated ammonia R* and dilute to 100 mL with the same mixture of solvents.

Reference solution (d) Dissolve 12.5 mg of *triethylenediamine R* in 5.0 mL of test solution (a) and dilute to 50 mL with a mixture of 2 volumes of *anhydrous ethanol R* and 3 volumes of *concentrated ammonia R*.

Apply separately to the plate 5 µL of each solution. Develop over a path of 15 cm using a freshly prepared mixture of 20 volumes of *concentrated ammonia R* and 80 volumes of *acetone R*. Dry the plate at 105 °C and spray successively with a 3 g/L solution of *ninhydrin R* in a mixture of 3 volumes of *anhydrous acetic acid R* and 100 volumes of *butanol R* and a 1.5 g/L solution of *ninhydrin R* in *anhydrous ethanol R*. Dry the plate at 105 °C for 10 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent). Spray the plate with *0.05 M iodine* and allow to stand for about 10 min. Any spot corresponding to triethylenediamine in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.25 per cent). The test is not valid unless the chromatogram obtained with reference solution (d) shows 2 clearly separated spots. Disregard any spots remaining on the line of application.

Water (*2.5.12*)
10.0 per cent to 14.0 per cent, determined on 0.300 g by the semi-micro determination of water.

Sulfated ash (*2.4.14*)
Not more than 0.1 per cent, determined on 1.0 g.

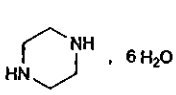
ASSAY
Dissolve 0.100 g in 10 mL of *anhydrous acetic acid R* with gentle heating and dilute to 70 mL with the same acid. Titrate with *0.1 M perchloric acid* using 0.25 mL of *naphtholbenzein solution R* as indicator until the colour changes from brownish-yellow to green.

1 mL of *0.1 M perchloric acid* is equivalent to 10.71 mg of $C_{24}H_{46}N_6O_{14}$.

Ph Eur

Piperazine Hydrate

(Ph. Eur. monograph 0425)



$C_4H_{10}N_2,6H_20$ 194.2 *142-63-2*

Action and use
Anthelminthic.

Ph Eur

DEFINITION
Piperazine hexahydrate.

Content
98.0 per cent to 101.0 per cent.

CHARACTERS
Appearance
Colourless, deliquescent crystals.

Solubility
Freely soluble in water and in ethanol (96 per cent).

mp
About 43 °C.

IDENTIFICATION
First identification: A.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Dry the substance to be examined and the reference substance *in vacuo* (*2.2.32*) for 48 h, powder the substances avoiding uptake of water, prepare discs and record the spectra without delay.

Comparison *piperazine hydrate CRS*.

B. Examine the chromatograms obtained in the test for related substances after spraying with the ninhydrin solutions.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 0.5 g in 5 mL of *dilute sodium hydroxide solution R*. Add 0.2 mL of *benzoyl chloride R* and mix. Continue to add *benzoyl chloride R* in portions of 0.2 mL until no further precipitate is formed. Filter and wash the precipitate with a total of 10 mL of *water R* added in small portions. Dissolve the precipitate in 2 mL of hot *ethanol (96 per cent) R* and pour the solution into 5 mL of *water R*. Allow to stand for 4 h, filter, wash the crystals with *water R* and dry at 100-105 °C. The crystals melt (*2.2.14*) at 191 °C to 196 °C.

TESTS
Solution S
Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_8 (*2.2.2, Method II*).

pH (*2.2.3*)
10.5 to 12.0 for solution S.

Related substances
Thin-layer chromatography (*2.2.27*).

Solvent mixture *anhydrous ethanol R, concentrated ammonia R* (40:60 *V/V*).

Test solution (a) Dissolve 1.0 g of the substance to be examined in 6 mL of *concentrated ammonia R* and dilute to 10 mL with *anhydrous ethanol R*.

Test solution (b) Dilute 1 mL of test solution (a) to 10 mL with the solvent mixture.

Reference solution (a) Dissolve 0.1 g of *piperazine hydrate CRS* in the solvent mixture and dilute to 10 mL with the solvent mixture.

Reference solution (b) Dissolve 25 mg of *ethylenediamine R* in the solvent mixture and dilute to 100 mL with the solvent mixture.

Reference solution (c) Dissolve 25 mg of *triethylenediamine R* in the solvent mixture and dilute to 100 mL with the solvent mixture.

Reference solution (d) Dissolve 12.5 mg of *triethylenediamine R* in 5.0 mL of test solution (a) and dilute to 50 mL with the solvent mixture.

Plate Suitable silica gel as the coating substance.

Mobile phase *concentrated ammonia R, acetone R* (20:80 *V/V*); use a freshly prepared mixture.

Application 5 µL.

Development Over a path of 15 cm.

Drying At 105 °C.

Detection A Spray successively with a 3 g/L solution of *ninhydrin R* in a mixture of 3 volumes of *anhydrous acetic*

acid R and 100 volumes of *butanol R* and a 1.5 g/L solution of *ninhydrin R* in *anhydrous ethanol R* and dry the plate at 105 °C for 10 min.

Limits A Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent).

Detection B Spray with *0.05 M iodine* and allow to stand for about 10 min.

Limits B Any spot corresponding to triethylenediamine in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.25 per cent).

System suitability Reference solution (d):
— the chromatogram shows 2 clearly separated spots.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

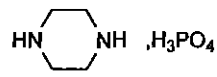
ASSAY
Dissolve 80.0 mg in 10 mL of *anhydrous acetic acid R* with gentle heating and dilute to 70 mL with the same acid. Titrate with *0.1 M perchloric acid* using 0.25 mL of *naphtholbenzein solution R* as indicator until the colour changes from brownish-yellow to green.

1 mL of *0.1 M perchloric acid* is equivalent to 9.705 mg of $C_4H_{10}N_2,6H_20$.

STORAGE
In an airtight container, protected from light.

Ph Eur

Piperazine Phosphate



$C_4H_{10}N_2,H_3PO_4,H_2O$ 202.1 *18534-18-4*

Action and use
Anthelminthic.

Preparations
Piperazine Phosphate Chewable Tablets
Piperazine Phosphate Tablets

DEFINITION
Piperazine Phosphate contains not less than 98.5% and not more than 100.5% of $C_4H_{10}N_2,H_3PO_4$, calculated with reference to the anhydrous substance.

CHARACTERISTICS
A white, crystalline powder; odourless or almost odourless.

Sparingly soluble in *water*; practically insoluble in *ethanol (96%)*.

IDENTIFICATION
A. Dissolve 0.1 g in 5 mL of *water*, add 0.5 g of *sodium hydrogen carbonate*, 0.5 mL of a 5% w/v solution of *potassium hexacyanoferrate(III)* and 0.1 mL of *mercury*. Shake vigorously for 1 minute and allow to stand for 20 minutes. A reddish colour is produced slowly.

B. Dissolve 0.2 g in 5 mL of *2M hydrochloric acid*, add with stirring 1 mL of a 50% w/v solution of *sodium nitrite* and cool in ice for 15 minutes, stirring if necessary to induce crystallisation. The *melting point* of the crystals, after washing with 10 mL of iced *water* and drying at 105°, is about 159°, Appendix V A.

C. A solution yields the reactions characteristic of *phosphates*, Appendix VI.

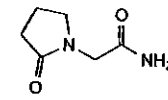
TESTS
Acidity
pH of a 1% w/v solution, 6.0 to 6.5, Appendix V L.

Water
8.0 to 9.5% w/w, Appendix IX C. Use 0.25 g.

ASSAY
Dissolve 0.2 g in a mixture of 3.5 mL of 0.5M *sulfuric acid* and 10 mL of *water*. Add 100 mL of *picric acid solution R1*, heat on a water bath for 15 minutes and allow to stand for 1 hour. Filter through a sintered-glass filter (ISO 4793, porosity grade 4, is suitable) and wash the residue with successive 10 mL quantities of a mixture of equal volumes of a saturated solution of *picric acid* and *water* until the washings are free from sulfate. Wash the residue with five 10 mL quantities of *absolute ethanol* and dry to constant weight at 100° to 105°. Each g of residue is equivalent to 0.3382 g of $C_4H_{10}N_2,H_3PO_4$.

Piracetam

(Ph. Eur. monograph 1733)



$C_6H_{10}N_2O_2$ 142.2 *7491-74-9*

Action and use
Nootropic; cortical myoclonus.

Ph Eur

DEFINITION
2-(2-Oxopyrrolidin-1-yl)acetamide.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white powder.

Solubility
Freely soluble in water, soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison piracetam CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *ethanol (96 per cent) R*, evaporate to dryness on a water-bath and record new spectra using the residues.

TESTS
Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 2.0 g in *water R* and dilute to 10 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R, water R* (10:90 *V/V*).

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Test solution (b) Dilute 10.0 mL of test solution (a) to 50.0 mL with the solvent mixture.

Reference solution (a) Dissolve 5 mg of the substance to be examined and 10 µL of *2-pyrrolidone R* (impurity A) in the solvent mixture and dilute to 100 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 20.0 mL with the solvent mixture.

Reference solution (c) Dissolve 50.0 mg of *piracetam CRS* in the solvent mixture and dilute to 100.0 mL with the solvent mixture. Dilute 10.0 mL of the solution to 50.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography compatible with 100 per cent aqueous mobile phases R* (5 µm).

Mobile phase Mix 10 volumes of *acetonitrile R1* and 90 volumes of a 1.0 g/L solution of *dipotassium hydrogen phosphate R*; adjust to pH 6.0 with *dilute phosphoric acid R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 205 nm.

Injection 20 µL of test solution (a) and reference solutions (a) and (b).

Run time 8 times the retention time of piracetam.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity A.

Relative retention With reference to piracetam (retention time = about 4 min): impurity A = about 1.15;.

System suitability Reference solution (a):
— *resolution*: minimum 3.0 between the peaks due to piracetam and impurity A;
— *symmetry factor*: maximum 2.0 for the peak due to piracetam.

Calculation of percentage contents:
— for each impurity, use the concentration of piracetam in reference solution (b).

Limits:
— *unspecified impurities*: for each impurity, maximum 0.05 per cent;
— *total*: maximum 0.3 per cent;
— *reporting threshold*: 0.03 per cent.

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

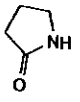
Injection Test solution (b) and reference solution (c).

Run time 1.5 times the retention time of piracetam.

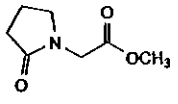
Calculate the percentage content of $C_6H_{10}N_2O_2$ taking into account the assigned content of *piracetam CRS*.

STORAGE
Protected from light.

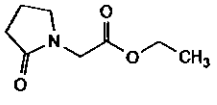
IMPURITIES
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D.*



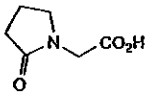
A. pyrrolidin-2-one (2-pyrrolidone),



B. methyl (2-oxopyrrolidin-1-yl)acetate,



C. ethyl (2-oxopyrrolidin-1-yl)acetate,

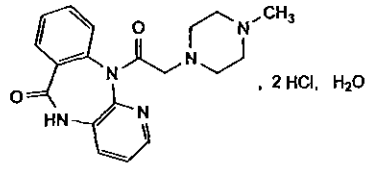


D. (2-oxopyrrolidin-1-yl)acetic acid.

Ph Eur

Pirenzepine Hydrochloride

(Pirenzepine Dihydrochloride Monohydrate, Ph. Eur. monograph 2001)



$C_{19}H_{23}Cl_2N_5O_2,H_2O$ 442.3

Action and use
Muscarinic M_3 receptor antagonist.

Ph Eur

DEFINITION
11-[(4-Methylpiperazin-1-yl)acetyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one dihydrochloride monohydrate.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or yellowish, crystalline powder.

Solubility

Freely soluble in water, slightly soluble in methanol, very slightly soluble in ethanol, practically insoluble in methylene chloride.

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Dissolve 30.0 mg in *methanol R* and dilute to 100.0 mL with the same solvent. Dilute 10.0 mL of the solution to 100.0 mL with *methanol R*. Examined between 240 nm and 360 nm (*2.2.25*), the solution shows an absorption maximum at 283 nm. The specific absorbance at the maximum is 190 to 205 (anhydrous substance).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *pirenzepine dihydrochloride monohydrate CRS.*

C. Examine the chromatograms obtained in the test for impurity D.

Results The principal zone obtained in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal zone in the chromatogram obtained with reference solution (d).

D. To 0.2 mL of solution S (see Tests) add 1.8 mL of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution GY_5 (*2.2.2, Method II*).

pH (*2.2.3*)

1.0 to 2.0 for solution S.

Impurity D

Thin-layer chromatography (*2.2.27*).

Test solution (a) To 0.10 g add 0.1 mL of *concentrated ammonia R* and dilute to 10 mL with *methanol R*.

Test solution (b) Dilute 1 mL of test solution (a) to 10 mL with *methanol R*.

Reference solution (a) To 0.1 g of *pirenzepine dihydrochloride monohydrate CRS* add 0.1 mL of *concentrated ammonia R* and dilute to 10 mL with *methanol R*.

Reference solution (b) Dissolve 25 mg of methylpiperazine R in *methanol R* and dilute to 25 mL with the same solvent. Dilute 2.0 mL of the solution to 100 mL with *methanol R*.

Reference solution (c) Dilute 5 mL of test solution (a) to 100 mL with *methanol R*. Dilute 4 mL of this solution to 100 mL with *methanol R*. Mix 1 mL with 1 mL of reference solution (b).

Reference solution (d) Dilute 1 mL of reference solution (a) to 10 mL with *methanol R*.

Plate *TLC silica gel plate R.*

Mobile phase *concentrated ammonia R, methanol R, ethyl acetate R, toluene R* (7:25:28:40 *V/V/V/V*).

Application 20 µL as zones of 20 mm by 2 mm.

Development Over 2/3 of the plate.

Drying In air.

Detection Expose the plate to iodine vapour until the zone in the chromatogram obtained with reference solution (b) is clearly visible (at most 60 min).

System suitability The test is not valid unless the chromatogram obtained with reference solution (c) shows 2 clearly separated zones.

Limit:

— *impurity D*: any zone corresponding to impurity D in the chromatogram obtained with test solution (a) is not more intense than the zone in the chromatogram obtained with reference solution (b) (0.2 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.30 g of the substance to be examined in *water R* and dilute to 10.0 mL with the same solvent. To 1.0 mL of the solution add 5 mL of *methanol R* and dilute to 10.0 mL with mobile phase A.

Reference solution (a) Dilute 2.0 mL of the test solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

Reference solution (b) Dissolve 0.1 g of *1-phenylpiperazine R* in *methanol R* and dilute to 10 mL with the same solvent. Mix 1 mL of the solution with 1 mL of the test solution, add 5 mL of *methanol R* and dilute to 10 mL with mobile phase A.

Column:

— *size*: l = 0.125 m, Ø = 4.6 mm,

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: dissolve 2.0 g of *sodium dodecyl sulfate R* in *water R*, adjust to pH 3.2 with *acetic acid R* and dilute to 1000 mL with *water R*,

— *mobile phase B*: *methanol R*,

— *mobile phase C*: *acetonitrile R*,

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Mobile phase C (per cent *V/V*)
0 - 15	55 → 25	30	15 → 45
15 - 18	25 → 20	30 → 0	45 → 80

Flow rate 1 mL/min.

Detection Spectrophotometer at 283 nm.

Injection 10 µL.

System suitability Reference solution (b):

— *resolution*: minimum 5.0 between the peaks due to pirenzepine and 1-phenylpiperazine.

Limits:

— *any impurity*: not more than the peak area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent),

— *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.04 per cent).

Water (*2.5.12*)

3.5 per cent to 5.0 per cent, determined on 0.250 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

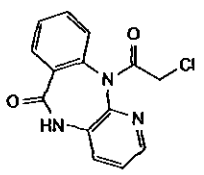
Dissolve 0.300 g in 50 mL of *water R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume at the first point of inflection.

1 mL of *0.1 M sodium hydroxide* is equivalent to 42.43 mg of $C_{19}H_{23}Cl_2N_5O_2$.

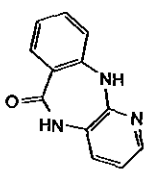
STORAGE

Protected from light.

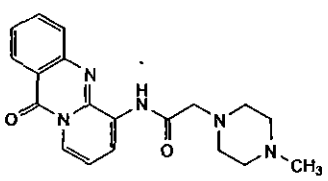
IMPURITIES



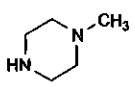
A. 11-(chloroacetyl)-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one,



B. 5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one,



C. 6-[[(4-methylpiperazin-1-yl)acetyl]amino]-11*H*-pyrido[2,1-*b*]quinazolin-11-one,

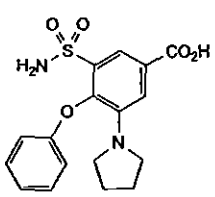


D. 1-methylpiperazine.

Ph Eur

Piretanide

(Ph. Eur. monograph 1556)



$C_{17}H_{18}N_2O_5S$ 362.4 *55837-27-9*

Action and use
Thiazide diuretic.

Ph Eur

DEFINITION

4-Phenoxy-3-(pyrrolidin-1-yl)-5-sulfamoylbenzoic acid.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
Yellowish-white or yellowish powder.

Solubility
Very slightly soluble in water, sparingly soluble in anhydrous ethanol.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *piretanide CRS*.

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *acetone R*, evaporate to dryness and record new spectra using the residues.

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution GY_4 (*2.2.2, Method II*).

Dissolve 0.1 g in *methanol R* and dilute to 10 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture *anhydrous ethanol R, acetonitrile R, water R* (10:45:45 *V/V/V*).

Test solution Dissolve 20 mg of the substance to be examined in the solvent mixture and dilute to 20.0 mL with the solvent mixture.

Reference solution (a) Dissolve 5 mg of *piretanide for system suitability CRS* (containing impurities A, B and C) in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (b) Dilute 0.3 mL of the test solution to 100.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.125 m, Ø = 4 mm;
— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase A mixture of 35 volumes of *acetonitrile R1* and 65 volumes of a solution prepared as follows: add 1 mL of *trifluoroacetic acid R* to 500 mL of *water for chromatography R*, add 1 mL of *triethylamine R* and dilute to 1000 mL with *water for chromatography R*.

Flow rate 1 mL/min.

Detection Spectrophotometer at 232 nm.

Injection 10 µL.

Run time 5 times the retention time of piretanide.

Identification of impurities Use the chromatogram supplied with *piretanide for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B and C.

Relative retention With reference to piretanide (retention time = about 10 min): impurity A = about 0.8; impurity B = about 3.1; impurity C = about 4.1.

System suitability Reference solution (a):
— *resolution*: minimum 2 between the peaks due to impurity A and piretanide.

Limits:
- — *impurities A, B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
- — *unspecified impurities*: for each impurity, not more than 0.33 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- — *total*: not more than 3.33 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
- — *disregard limit*: 0.17 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

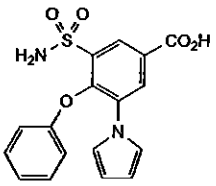
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.300 g in 25 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

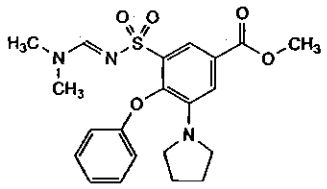
1 mL of *0.1 M perchloric acid* is equivalent to 36.24 mg of $C_{17}H_{18}N_2O_5S$.

STORAGE
Protected from light.

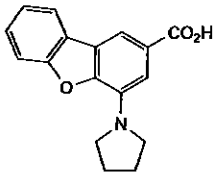
IMPURITIES
Specified impurities *A, B, C.*



A. 4-phenoxy-3-(1*H*-pyrrol-1-yl)-5-sulfamoylbenzoic acid,



B. methyl-3-[[(dimethylamino)methylidene]sulfamoyl]-4-phenoxy-5-(pyrrolidin-1-yl)benzoate,

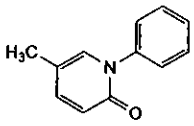


C. 4-(pyrrolidin-1-yl)dibenzo[*b,d*]furan-2-carboxylic acid.

Ph Eur

Pirfenidone

(Ph. Eur. monograph 2856)



$C_{12}H_{11}NO$ 185.2 *53179-13-8*

Action and use
Treatment of idiopathic pulmonary fibrosis.

Ph Eur

DEFINITION
5-Methyl-1-phenylpyridin-2(1*H*)-one.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS
Appearance
White or pale yellow, crystalline powder.

Solubility
Sparingly soluble in water, freely soluble in ethanol (96 per cent), slightly soluble in heptane.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *pirfenidone CRS.*

TESTS
Related substances
Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Test solution (b) Dilute 5.0 mL of test solution (a) to 50.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of test solution (b) to 10.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 20.0 mL with the mobile phase.

Reference solution (b) Dissolve 5 mg of *5-methylpyridin-2-amine R* (impurity A) and 5 mg of *5-methylpyridin-2(1H)-one R* (impurity B) in *methanol R* and dilute to 100 mL with the same solvent. Dilute 1 mL of the solution to 100 mL with the mobile phase.

Reference solution (c) Dissolve 50.0 mg of *pirfenidone CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 5.0 mL of the solution to 50.0 mL with the mobile phase.

Column:
- — *size*: *l* = 0.25 m, Ø = 4.6 mm;
- — *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm);
- — *temperature*: 40 °C.

Mobile phase Add 0.9 mL of *triethylamine R2* to 650 mL of *water for chromatography R* and adjust to pH 3.0 with *phosphoric acid R*. Mix 650 mL of this solution, 130 mL of *methanol R2* and 220 mL of *acetonitrile R1*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 15 µL of test solution (a) and reference solutions (a) and (b).

Run time 4 times the retention time of pirfenidone.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and B.

Relative retention With reference to pirfenidone (retention time = about 10 min): impurity A = about 0.35; impurity B = about 0.4.

System suitability Reference solution (b):
— *resolution*: minimum 3.0 between the peaks due to impurities A and B.

Calculation of percentage contents:
— for each impurity, use the concentration of pirfenidone in reference solution (a).

Limits:
— *unspecified impurities*: for each impurity, maximum 0.05 per cent;
— *total*: maximum 0.2 per cent;
— *reporting threshold*: 0.03 per cent.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying at 80 °C at a pressure not exceeding 75 kPa for 4 h.

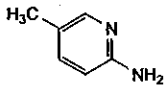
Sulfated ash (*2.4.14*)
Maximum 0.2 per cent, determined on 1.0 g.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

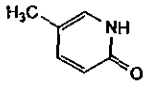
Injection Test solution (b) and reference solution (c).

Calculate the percentage content of $C_{12}H_{11}NO$ taking into account the assigned content of *pirfenidone CRS*.

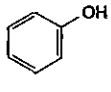
IMPURITIES
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D.*



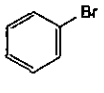
A. 5-methylpyridin-2-amine,



B. 5-methylpyridin-2(1*H*)-one,



C. phenol,

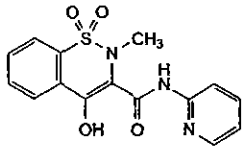


D. bromobenzene.

Ph Eur

Piroxicam

(Ph. Eur. monograph 0944)



$C_{15}H_{13}N_3O_4S$ 331.4 *36322-90-4*

Action and use
Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Preparations
Piroxicam Capsules
Piroxicam Gel

Ph Eur

DEFINITION
4-Hydroxy-2-methyl-*N*-(pyridin-2-yl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or slightly yellow, crystalline powder.

Solubility
Practically insoluble in water, soluble in methylene chloride, slightly soluble in anhydrous ethanol.

It shows polymorphism (*5.9*).

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *piroxicam CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *methylene chloride R*, evaporate to dryness on a water-bath and record new spectra using the residues.

TESTS
Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 75 mg of the substance to be examined in *acetonitrile R1*, warming slightly if necessary, and dilute to 50.0 mL with the same solvent.

Reference solution (a) Dissolve 7 mg of *piroxicam for system suitability CRS* (containing impurities A, B, D, G and J) in *acetonitrile R1* and dilute to 5.0 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of the test solution to 10.0 mL with *acetonitrile R1*. Dilute 1.0 mL of this solution to 50.0 mL with *acetonitrile R1*.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 50 °C.

Mobile phase Mix 30 volumes of *acetonitrile R1* and 70 volumes of a 6.81 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.0 with *phosphoric acid R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µL.

Run time 5 times the retention time of piroxicam.

Identification of impurities Use the chromatogram supplied with *piroxicam for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, D, G and J.

Relative retention With reference to piroxicam (retention time = about 16 min): impurity A = about 0.1; impurity D = about 0.6; impurity G = about 0.7; impurity B = about 0.8; impurity J = about 1.8.

System suitability Reference solution (a):
— *resolution*: minimum 1.5 between the peaks due to impurities G and B.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity A by 0.6;
— *impurities A, B, D, G, J*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent);
— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 105 °C for 4 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 60 mL of a mixture of equal volumes of *acetic anhydride R* and *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 33.14 mg of $C_{15}H_{13}N_3O_4S$.

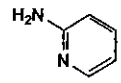
STORAGE

Protected from light.

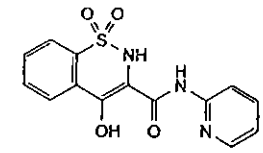
IMPURITIES

Specified impurities *A, B, D, G, J.*

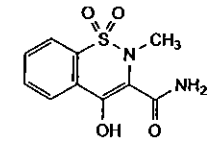
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C, E, F, H, I, K, L.*



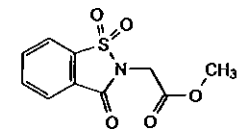
A. pyridin-2-amine,



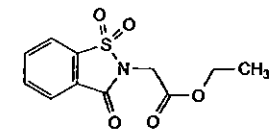
B. 4-hydroxy-*N*-(pyridin-2-yl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide,



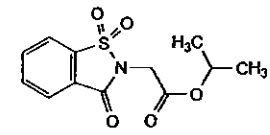
C. 4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide,



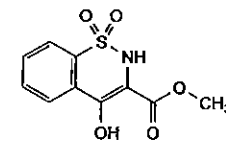
D. methyl (1,1-dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl) acetate,



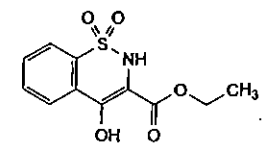
E. ethyl (1,1-dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl) acetate,



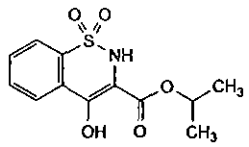
F. 1-methylethyl (1,1-dioxido-3-oxo-1,2-benzisothiazol-2 (3*H*)-yl)acetate,



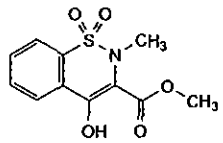
G. methyl 4-hydroxy-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide,



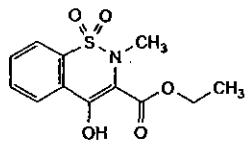
H. ethyl 4-hydroxy-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide,



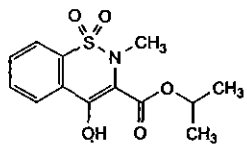
I. 1-methylethyl 4-hydroxy-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide,



J. methyl 4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide,



K. ethyl 4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide,

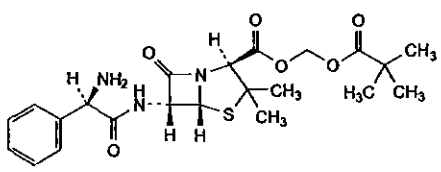


L. 1-methylethyl 4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide.

Ph Eur

Pivampicillin

(Ph. Eur. monograph 0852)



$C_{22}H_{29}N_3O_6S$ 463.6 *33817-20-8*

Action and use
Antibacterial.

Ph Eur

DEFINITION
Methylene (2*S*,5*R*,6*R*)-6-[[(2*R*)-2-amino-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 2,2-dimethylpropanoate.

Semi-synthetic product derived from a fermentation product.

Content
95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, freely soluble in methanol, soluble in anhydrous ethanol. It dissolves in dilute acids.

IDENTIFICATION

First identification: A.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *pivampicillin CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in 2 mL of *methanol R.*

Reference solution (a) Dissolve 10 mg of *pivampicillin CRS* in 2 mL of *methanol R.*

Reference solution (b) Dissolve 10 mg of *bacampicillin hydrochloride CRS,* 10 mg of *pivampicillin CRS* and 10 mg of *talampicillin hydrochloride CRS* in 2 mL of *methanol R.*

Plate *TLC silanised silica gel plate R.*

Mobile phase Mix 10 volumes of a 272 g/L solution of *sodium acetate R* adjusted to pH 5.0 with *glacial acetic acid R,* 40 volumes of *water R* and 50 volumes of *ethanol (96 per cent) R.*

Application 1 µL.

Development Over a path of 15 cm.

Drying In a current of warm air.

Detection Spray with *ninhydrin solution R1* and heat at 60 °C for 10 min.

System suitability Reference solution (b):
— the chromatogram shows 3 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Place about 2 mg in a test-tube about 150 mm long and 15 mm in diameter. Moisten with 0.05 mL of *water R* and add 2 mL of *sulfuric acid-formaldehyde reagent R.* Mix the contents of the tube by swirling; the solution is almost colourless. Place the test-tube in a water-bath for 1 min; a dark yellow colour develops.

TESTS

Appearance of solution
The solution is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution B_7 (*2.2.2, Method I*).

Dissolve 50 mg in 12 mL of *0.1 M hydrochloric acid.*

Specific optical rotation (*2.2.7*)
+ 208 to + 222 (anhydrous substance).

Dissolve 0.100 g in 5.0 mL of *ethanol (96 per cent) R* and dilute to 10.0 mL with *0.1 M hydrochloric acid.*

Triethanolamine
Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.100 g of the substance to be examined in 1.0 mL of a mixture of 1 volume of *water R* and 9 volumes of *acetonitrile R.*

Reference solution Dissolve 5.0 mg of *triethanolamine R* in a mixture of 1 volume of *water R* and 9 volumes of *acetonitrile R* and dilute to 100 mL with the same mixture of solvents.

Plate *TLC silica gel plate R.*

Mobile phase *methanol R, butanol R, phosphate buffer solution pH 5.8 R, glacial acetic acid R, butyl acetate R* (5:15:24:40:80 *V/V/V/V/V*).

Application 10 µL.

Development Over a path of 12 cm.

Drying At 110 °C for 10 min and allow to cool.

Chlorine treatment Place at the bottom of a chromatographic tank an evaporating dish containing a mixture of 1 volume of *hydrochloric acid R1*, 1 volume of *water R* and 2 volumes of a 15 g/L solution of *potassium permanganate R*; close the tank and allow to stand for 15 min; place the dried plate in the tank and close the tank; leave the plate in contact with the chlorine vapour in the tank for 15-20 min; withdraw the plate and allow it to stand in air for 2-3 min.

Detection Spray with *tetramethyldiaminodiphenylmethane reagent R*.

Limit:

— *triethanolamine*: any spot due to triethanolamine is not more intense than the corresponding spot in the chromatogram obtained with the reference solution (0.05 per cent).

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 50.0 mg of the substance to be examined in 10.0 mL of *acetonitrile R* and dilute to 20 mL with a 1 g/L solution of *phosphoric acid R*.

Reference solution Mix 2.0 mL of the test solution with 9.0 mL of *acetonitrile R* and 9.0 mL of a 1 g/L solution of *phosphoric acid R*.

Column:

— *size*: *l* = 0.125 m, Ø = 4 mm;

— *stationary phase*: *end-capped octylsilyl silica gel for chromatography R*.

Mobile phase:

— *mobile phase A*: mix 50 volumes of a 1.32 g/L solution of *ammonium phosphate R*, adjusted to pH 2.5 with a 100 g/L solution of *phosphoric acid R*, and 50 volumes of *acetonitrile R*;

— *mobile phase B*: mix 15 volumes of a 1.32 g/L solution of *ammonium phosphate R*, adjusted to pH 2.5 with a 100 g/L solution of *phosphoric acid R*, and 85 volumes of *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	100	0
10 - 12	0	100
12 - 17	100	0

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 50 µL.

Retention time Pivampicillin dimer = about 5 min.

System suitability Reference solution:

— *ratio of the mass distribution ratio*: minimum 12 for the peak due to pivampicillin dimer to that of the peak due to pivampicillin (principal peak).

Limits:

— *total*: not more than 0.3 times the area of the principal peak in the chromatogram obtained with the reference solution (3 per cent);

— *disregard limit*: 0.01 times the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent).

***N,N*-Dimethylaniline** (*2.4.26, Method B*)

Maximum 20 ppm.

Test solution To 1.00 g of the substance to be examined in a ground-glass-stoppered tube add 10 mL of *0.5 M sulfuric acid*. Heat the tube for 10 min in a water-bath, cool and add 15 mL of *1 M sodium hydroxide* and 1.0 mL of the internal standard solution. Stopper the tube and shake vigorously for 1 min. Centrifuge if necessary and use the upper layer.

Water (*2.5.12*)

Maximum 1.0 per cent, determined on 0.30 g.

Sulfated ash (*2.4.14*)

Maximum 0.5 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*). *Use the solutions within 2 h of preparation.*

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 10.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *pivampicillin CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 10.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution (b) Dissolve 25.0 mg of *propyl parahydroxybenzoate CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 10.0 mL of the solution to 50.0 mL with the mobile phase. Mix 5.0 mL of this solution with 5.0 mL of reference solution (a).

Column:

— *size*: *l* = 0.125 m, Ø = 4 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 40 volumes of *acetonitrile R* and 60 volumes of a 2.22 g/L solution of *phosphoric acid R* adjusted to pH 2.5 with *triethylamine R*.

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µL.

System suitability:

— *resolution*: minimum 5.0 between the peaks due to pivampicillin (1st peak) and propyl parahydroxybenzoate (2nd peak) in the chromatogram obtained with reference solution (b);

— *symmetry factor*: maximum 2.0 for the peak due to pivampicillin in the chromatogram obtained with reference solution (b);

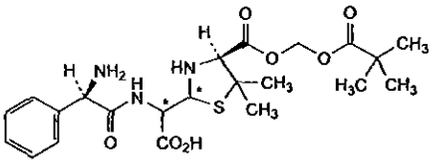
— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections of reference solution (a).

Calculate the percentage content of $C_{22}H_{29}N_3O_6S$ from the declared content of *pivampicillin CRS*.

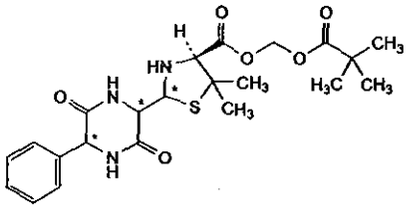
STORAGE

In an airtight container.

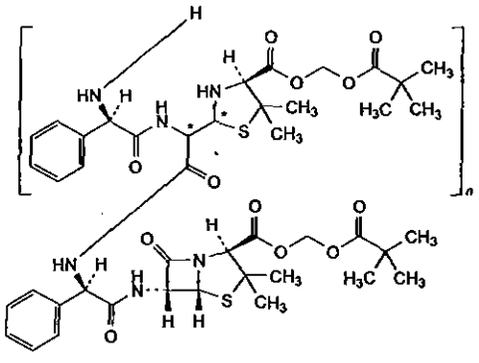
IMPURITIES



A. 2-[[(2*R*)-2-amino-2-phenylacetyl]amino]-2-[(4*S*)-4-[[[(2,2-dimethylpropanoyl)oxy]methoxy]carbonyl]-5,5-dimethylthiazolidin-2-yl]acetic acid (penicilloic acids of pivampicillin),



B. methylene (4*S*)-5,5-dimethyl-2-(3,6-dioxo-5-phenylpiperazin-2-yl)thiazolidine-4-carboxylate 2,2-dimethylpropanoate (diketopiperazines of pivampicillin),

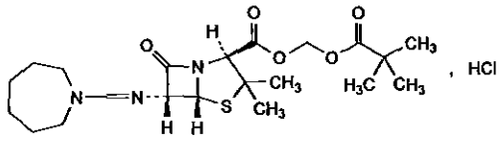


C. co-oligomers of pivampicillin and of penicilloic acids of pivampicillin.

Ph Eur

Pivmecillinam Hydrochloride

(Ph. Eur. monograph 1359)



$C_{21}H_{34}ClN_3O_5S$ 476.0 *32887-03-9*

Action and use
Antibacterial.

Ph Eur

DEFINITION
Methylene 2,2-dimethylpropanoate (2*S*,5*R*,6*R*)-6-[[(hexahydro-1*H*-azepin-1-yl)methylene]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate hydrochloride.

Semi-synthetic product derived from a fermentation product.

Content
97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water, in anhydrous ethanol and in methanol, slightly soluble in acetone.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *pivmecillinam hydrochloride CRS.*

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Appearance of solution
The solution is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution B_8 (*2.2.2, Method I*).

Dissolve 0.5 g in *water R* and dilute to 10 mL with the same solvent.

pH (*2.2.3*)
2.8 to 3.8.

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Solvent mixture To 45 volumes of *acetonitrile R* add 55 volumes of a 13.5 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.0 with *dilute phosphoric acid R.*

Test solution (a) Dissolve 20.0 mg of the substance to be examined in the solvent mixture and dilute to 200.0 mL with the solvent mixture.

Test solution (b) Dissolve 25.0 mg of the substance to be examined in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

Reference solution (a) Dissolve 20.0 mg of *pivmecillinam hydrochloride CRS* in the solvent mixture and dilute to 200.0 mL with the solvent mixture.

Reference solution (b) Dilute 5.0 mL of reference solution (a) to 50.0 mL with the solvent mixture.

Reference solution (c) Dissolve 5 mg of *pivmecillinam hydrochloride CRS* and 5 mg of *pivmecillinam impurity C CRS* in the solvent mixture, and dilute to 50 mL with the solvent mixture.

Column:
— *size:* l = 0.25 m, Ø = 4.0 mm;
— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 0.55 g of *tetraethylammonium hydrogen sulfate R* and 1.0 g of *tetramethylammonium hydrogen sulfate R* in the solvent mixture and dilute to 1000 mL with the solvent mixture.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µL of test solution (b) and reference solutions (b) and (c).

Run time 3 times the retention time of pivmecillinam.

System suitability Reference solution (c):
— *resolution*: minimum 3.5 between the peaks due to pivmecillinam (1st peak) and impurity C (2nd peak).

Limits:
— *any impurity*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent);
— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3 per cent);
— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

N,N-Dimethylaniline (*2.4.26, Method A*)
Maximum 20 ppm.

Test solution Prepare as described in the general method but heat at about 27 °C after the addition of *strong sodium hydroxide solution R*, to dissolve the precipitate formed, then add the *trimethylpentane R*.

Water (*2.5.12*)
Maximum 0.5 per cent, determined on 1.00 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

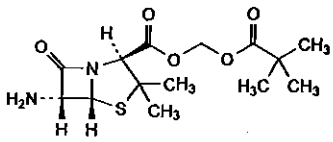
Injection Test solution (a) and reference solution (a).

System suitability Reference solution (a):
— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections.

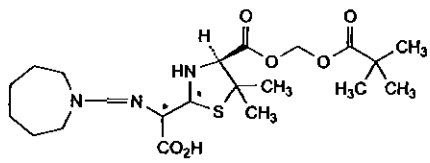
Calculate the percentage content of $C_{21}H_{34}ClN_3O_5S$ from the declared content of *pivmecillinam hydrochloride CRS*.

STORAGE
Protected from light, at a temperature of 2 °C to 8 °C.

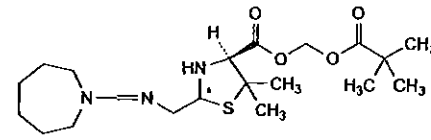
IMPURITIES



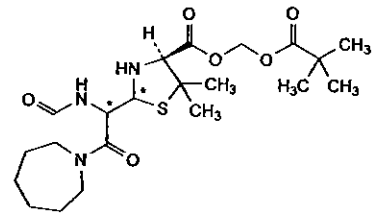
A. methylene (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 2,2-dimethylpropanoate (pivaloyloxymethyl 6-aminopenicillanate),



B. 2-[[(hexahydro-1*H*-azepin-1-yl)methylene]amino]-2-[(4*S*)-4-[[[(2,2-dimethylpropanoyl)oxy]methoxy]carbonyl]-5,5-dimethylthiazolidin-2-yl]acetic acid (penicilloic acids of pivmecillinam),



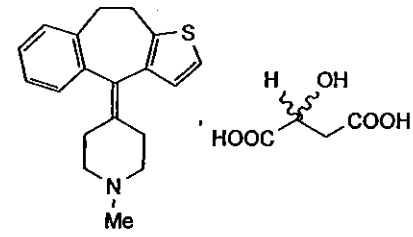
C. methylene 2,2-dimethylpropanoate (2*RS*,4*S*)-2-[[[(hexahydro-1*H*-azepin-1-yl)methylene]amino]methyl]-5,5-dimethylthiazolidin-4-carboxylate,



D. methylene 2,2-dimethylpropanoate (4*S*)-2-[1-(formylamino)-2-(hexahydro-1*H*-azepin-1-yl)-2-oxoethyl]-5,5-dimethylthiazolidin-4-carboxylate.

Ph Eur

Pizotifen Malate



$C_{19}H_{21}NS,C_4H_6O_5$ 429.5 *5189-11-7*

Action and use
Serotonin (5HT) receptor partial agonist; prophylaxis of migraine.

Preparation
Pizotifen Tablets

DEFINITION
Pizotifen Malate is 9,10-dihydro-4-(1-methylpiperidin-4-ylidene)-4*H*-benzo[4,5]cyclohepta[1,2-*b*]thiophene hydrogen malate. It contains not less than 98.5% and not more than 101.5% of $C_{19}H_{21}NS,C_4H_6O_5$, calculated with reference to the dried substance.

CHARACTERISTICS
A white or slightly yellowish white, crystalline powder.

Very slightly soluble in *water*, slightly soluble in *ethanol (96%)*; sparingly soluble in *methanol*.

IDENTIFICATION
A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of pizotifen malate *(RS 277)*.

B. In the test for Related substances, the principal spot in the chromatogram obtained with solution (2) corresponds to that in the chromatogram obtained with solution (5).

C. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 70 volumes of *isopropyl ether*, 25 volumes of *anhydrous formic acid* and 5 volumes of *water* as the mobile

phase. Apply separately to the plate 5 µL of each of the following solutions. For solution (1) dissolve 30 mg of the substance being examined in 1 mL of *ethanol (80%)*, heating if necessary. Solution (2) contains 1% w/v of *malic acid* in *ethanol (80%)*. After removal of the plate, dry it at 100° for 30 minutes, cool, spray with 0.02M *potassium permanganate* and dry in a current of warm air for about 1 minute.
The chromatogram obtained with solution (1) exhibits a spot corresponding in position, colour and size to the spot in the chromatogram obtained with solution (2).

TESTS

Clarity and colour of solution
A 1.0% w/v solution in a mixture of equal volumes of *ethanol (96%)* and *water* is *clear*, Appendix IV A, and not more intensely coloured than *reference solution* BY_6, Appendix IV B, Method II.

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 100 volumes of *toluene*, 60 volumes of *butan-1-ol*, 40 volumes of *hexane* and 3 volumes of 13.5M *ammonia* as the mobile phase. Apply separately to the plate 5 µL of each of five solutions in a mixture of 9 volumes of *dichloromethane* and 1 volume of *methanol* containing (1) 2.0% w/v, (2) 0.20% w/v, (3) 0.010% w/v and (4) 0.0050% w/v of the substance being examined and (5) 0.20% w/v of *pizotifen malate BPCRS*. After removal of the plate, dry it in a current of cold air for 5 minutes, spray with a mixture of 1 volume of *potassium iodobismuthate solution* and 10 volumes of 2M *acetic acid* and then with *hydrogen peroxide solution (10 vol)*, cover immediately with a glass plate and examine in daylight. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (4). Disregard any yellow spot or band remaining on the line of application.

Loss on drying
When dried to constant weight at 100° to 105°, loses not more than 0.5% of its weight. Use 1 g.

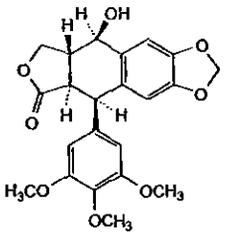
Sulfated ash
Not more than 0.2%, Appendix IX A, Method II. Use the residue obtained in the test for Loss on drying.

ASSAY
Dissolve 0.35 g in 60 mL of *anhydrous acetic acid* and carry out Method I for *non-aqueous titration*, Appendix VIII A, determining the end point potentiometrically. Each mL of *0.1M perchloric acid VS* is equivalent to 42.95 mg of $C_{19}H_{21}NS,C_4H_6O_5$.

STORAGE
Pizotifen Malate should be protected from light.

Podophyllotoxin

(Ph. Eur. monograph 2750)



$C_{22}H_{22}O_8$ 414.4 *518-28-5*

Action and use
Inhibitor of DNA topoisomerase type II.

Ph Eur

DEFINITION
(5*R*,5a*R*,8a*R*,9*R*)-9-Hydroxy-5-(3,4,5-trimethoxyphenyl)-5,8,8a,9-tetrahydrofuro[3′,4′:6,7]naphtho[2,3-*d*][1,3]dioxol-6(5a*H*)-one.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, very soluble in acetone, soluble in methanol.

It shows polymorphism (*5.9*).

mp
About 184 °C.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *podophyllotoxin CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *acetone R*, evaporate to dryness and record new spectra using the residues.

TESTS

Specific optical rotation (*2.2.7*)
−112 to −107 (anhydrous substance).

Dissolve 0.200 g in *anhydrous ethanol R* and dilute to 20.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in 10.0 mL of the mobile phase using sonication for 30 min and dilute to 25.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 50.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 20.0 mL with the mobile phase.

Reference solution (b) Dissolve 5 mg of *podophyllotoxin for system suitability CRS* (containing impurities A and C) in 5 mL of the mobile phase.

Reference solution (c) Dissolve 25.0 mg of *podophyllotoxin CRS* in 10.0 mL of the mobile phase using sonication for 30 min and dilute to 25.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 25 °C.

Mobile phase Mix 40 volumes of a 1.0 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.5 with *phosphoric acid R*, and 60 volumes of *methanol R1*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 240 nm.

Injection 5 µL of the test solution and reference solutions (a) and (b).

Run time Twice the retention time of podophyllotoxin.

Identification of impurities Use the chromatogram supplied with *podophyllotoxin for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and C.

Relative retention With reference to podophyllotoxin (retention time = about 8 min): impurity A = about 0.9; impurity C = about 1.1.

System suitability Reference solution (b):
— *resolution*: minimum 3.0 between the peaks due to podophyllotoxin and impurity C.

Calculation of percentage contents:
— *correction factors*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.7; impurity C = 0.4;
— for each impurity, use the concentration of podophyllotoxin in reference solution (a).

Limits:
— *impurities A, C*: for each impurity, maximum 0.3 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 1.0 per cent;
— *reporting threshold*: 0.05 per cent.

Water (*2.5.12*)
Maximum 1.0 per cent, determined on 0.300 g.

Sulfated ash (*2.4.14*)
Maximum 0.2 per cent, determined on 1.0 g.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

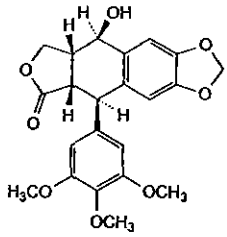
Injection Test solution and reference solution (c).

Calculate the percentage content of $C_{22}H_{22}O_8$ taking into account the assigned content of *podophyllotoxin CRS*.

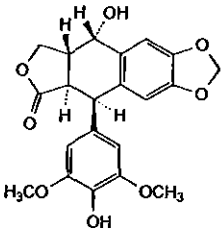
STORAGE
Protected from light, at a temperature of 2 °C to 8 °C.

IMPURITIES
Specified impurities *A, C.*

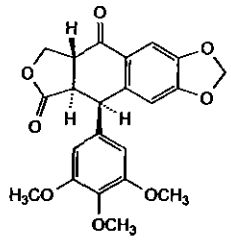
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B.*



A. (5*R*,5a*S*,8a*R*,9*R*)-9-hydroxy-5-(3,4,5-trimethoxyphenyl)-5,8,8a,9-tetrahydrofuro[3′,4′:6,7]naphtho[2,3-*d*][1,3]dioxol-6(5a*H*)-one (picropodophyllotoxin),



B. (5*R*,5a*R*,8a*R*,9*S*)-9-hydroxy-5-(4-hydroxy-3,5-dimethoxyphenyl)-5,8,8a,9-tetrahydrofuro[3′,4′:6,7]naphtho[2,3-*d*][1,3]dioxol-6(5a*H*)-one (4′-demethylepipodophyllotoxin),



C. (5a*R*,8a*R*,9*R*)-9-(3,4,5-trimethoxyphenyl)-5a,6,8a,9-tetrahydrofuro[3′,4′:6,7]naphtho[2,3-*d*][1,3]dioxol-5,8-dione (podophyllotoxone).

Ph Eur

Pollens for Allergen Products

(Ph. Eur. monograph 2627)

Ph Eur

DEFINITION
Pollens for allergen products come primarily from anemophilous plants and secondarily from a few entomophilous plants. They may be processed (e.g. defatted with organic solvents) or unprocessed pollen and they contain soluble substances, including proteins, whose functional activity is unrelated to any antigenic and allergenic properties they may have.

PRODUCTION
Pollens for allergen products are defined by their species and geographic location. Species identification is carried out on the parent plant from which the pollen is collected. The field characteristics and any treatments are specified for cultivated species. Where pollen is collected from wild species, the nature of collection areas is specified. The methods of collection are described and must ensure the origin, quality, consistency and traceability of the pollen. Where applicable, reference is made to good agricultural and collection practice

(GACP). The collection, handling and further processing of the pollen are such that a consistent composition of the source material is ensured from batch to batch.

Pollen may be defatted using organic solvents. The content of relevant pesticides, heavy metals and residual solvents is monitored and determined on a number of batches according to a justified sampling plan. Pesticides, heavy metals and residual solvents are limited according to the principles defined in general chapters *2.8.13. Pesticide residues*, *2.4.27. Heavy metals in herbal drugs and herbal drug preparations* and *2.4.24. Identification and control of residual solvents*, respectively.

Where major changes to the production of the pollens for allergen products take place (e.g. when a new process or supplier of pollen source material is introduced), such changes are qualified.

Microbial contamination of the pollen may be unavoidable and should be monitored on a representative number of batches of pollens for allergen products (e.g. defined by collection year and location) according to a justified sampling plan and each time a new supplier and/or a new process for production is introduced; if a determination of microbial contamination is not applicable, this must be justified. Microbial contamination values and potential increases in microbial contamination are monitored during stability studies, in order to assess this aspect along with the pollen characteristics upon storage.

Control methods and acceptance criteria relating to identity and purity of the pollen are established. The acceptance criteria must ensure the consistency of the pollens for allergen products from a qualitative and quantitative point of view. Pollens for allergen products are stored under controlled conditions justified by stability data.
The collection and production, as well as the handling of the pollen, are such that consistent quality is ensured from batch to batch.

POLLENS FOR ALLERGEN PRODUCTS REFERENCE BATCH

An appropriate reference batch is established for each species. The nature of the reference batch depends on the testing approach to verify batch-to-batch consistency and to establish acceptable quality. The reference batch may be, for example, an internal reference preparation (if available), a source material extract or a sample of a production batch. Its characterisation must be described. The extent of characterisation of the reference batch depends on the pollen, knowledge of the allergenic components and availability of suitable reagents. The reference batch is stored under controlled conditions ensuring its stability.

BATCH-TO-BATCH CONSISTENCY

To establish batch-to-batch consistency, one or more of the following tests are performed on each batch. If the microbial contamination test is performed, at least one other test is to be used. The choice of tests must be justified. Variations between different batches are to be expected due to, for example, geographic location of the harvest areas or different climates during the growth period.

Total protein (*2.5.33*)

Protein profile
Determined by using suitable electrophoresis methods (*2.2.31*, *2.2.54*).

Allergen profile
Relevant allergenic components are identified by means of suitable techniques using allergen-specific antibodies.

Major allergen content
Determined by using suitable immunochemical methods (*2.7.1*) such as enzyme-linked immunosorbent assay (ELISA).

Total allergenic activity
Determined by testing inhibition of the binding capacity of specific immunoglobulin E antibodies or by a suitable equivalent *in vitro* method.

Microbial contamination (*2.6.12*)

CHARACTERS

Pollens for allergen products are supplied as coloured powders and consist of grains in a great variety of shapes, densities and sizes depending on the species. Detailed specifications for each type of pollen grain are established.

IDENTIFICATION

Tests on identity are performed on each individual batch of pollen using adequate methods (e.g. microscopy). Specific characteristics, such as colour, size, shape, number and position of apertures, are described and compared with those of a reference batch or reference documents.

Additional tests may be performed as complementary identification determination.

TESTS

Foreign pollen
The content of pollen from other species is limited to 1 per cent of total mixed pollens and 0.5 per cent of any individual pollen as determined by a microscopic particle count.

Foreign matter
Particles of plant origin (determined by microscopic examination), other than pollen are kept to a minimum but in any case do not exceed 10 per cent unless otherwise justified and authorised. The sum of all non-plant particles (e.g. soil) must not exceed 1 per cent. Detectable mould spores must not exceed 1 per cent.

Water (*2.5.12* or *2.5.32*) **or loss on drying** (*2.2.32*)
The water content of dried material is determined; specification limits must be supported by batch analysis and stability data.

STORAGE

Pollens for allergen products are stored under controlled conditions justified by stability data.

LABELLING

The label states the species of the pollen.

Ph Eur

Poloxamers

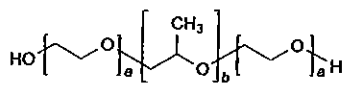
(Ph. Eur. monograph 1464)

Action and use
Non-ionic surfactant.

Ph Eur

DEFINITION

Synthetic block copolymer of ethylene oxide (oxirane) and propylene oxide (methyloxirane), represented by the following general formula:



Poloxamer type	Ethylene oxide units *a*	Propylene oxide units *b*	Content of oxyethylene units (per cent)	Average relative molecular mass
124	10 - 15	18 - 23	44.8 - 48.6	2090 - 2360
188	75 - 85	25 - 30	79.9 - 83.7	7680 - 9510
237	60 - 68	35 - 40	70.5 - 74.3	6840 - 8830
338	137 - 146	42 - 47	81.4 - 84.9	12 700 - 17 400
407	95 - 105	54 - 60	71.5 - 74.9	9840 - 14 600

A suitable antioxidant may be added.

CHARACTERS

Appearance
— *poloxamer 124*: colourless or almost colourless liquid;
— *poloxamers 188, 237, 338, 407*: white or almost white, waxy powder, microbeads or flakes.

Solubility
— *poloxamers 124, 237, 338, 407*: very soluble in water and in ethanol (96 per cent), practically insoluble in light petroleum (bp: 50-70 °C);
— *poloxamer 188*: soluble in water and in ethanol (96 per cent).

mp
About 50 °C for poloxamers 188, 237, 338 and 407.

IDENTIFICATION

First identification: A, B.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. chemical reference substance corresponding to the type of poloxamer to be examined.

B. Average relative molecular mass (see Tests).

C. Oxypropylene:oxyethylene ratio (see Tests).

TESTS

Solution S
Dissolve 10.0 g in *carbon dioxide-free water R* and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is not more intensely coloured than reference solution BY_7 (*2.2.2, Method II*).

pH (*2.2.3*)
5.0 to 7.5 for solution S.

Ethylene oxide, propylene oxide and dioxan
Head-space gas chromatography (*2.2.28*).

Ethylene oxide stock solution Introduce 0.5 mL of ethylene oxide stock solution R2 into a vial and dilute to 50.0 mL with *dimethyl sulfoxide R1*. Mix carefully.

Ethylene oxide solution Dilute 1.0 mL of the ethylene oxide stock solution to 250 mL with *dimethyl sulfoxide R1*.

Propylene oxide stock solution Introduce about 7 mL of *methylene chloride R* into a volumetric flask, add 0.500 g (*m*) of *propylene oxide R* and dilute to 10.0 mL with *methylene chloride R*. Dilute 0.5 mL of this solution to 50.0 mL with *dimethyl sulfoxide R1*. Mix carefully. Calculate the exact concentration of propylene oxide, in milligrams per millilitre, using the following expression:

$$\frac{m \times 1000 \times 0.5}{10 \times 50}$$

Propylene oxide solution Dilute 1.0 mL of the propylene oxide stock solution to 50.0 mL with *dimethyl sulfoxide R1*.

Calculate the exact concentration of propylene oxide, in micrograms per millilitre, using the following expression:

$$\frac{C \times 1000 \times 1}{50}$$

C = concentration of the propylene oxide stock solution, in milligrams per millilitre.

Dioxan solution Introduce 0.100 g (*m*) of *dioxan R* into a flask and dilute to 50.0 mL with *dimethyl sulfoxide R1*. Dilute 2.50 mL of this solution to 100.0 mL with *dimethyl sulfoxide R1*.

Calculate the exact concentration of dioxan, in micrograms per millilitre, using the following expression:

$$\frac{m \times 2.50 \times 1000 \times 1000}{50 \times 100}$$

Mixture solution Dilute a mixture of 6.0 mL of the ethylene oxide solution, 6.0 mL of the propylene oxide solution and 2.5 mL of the dioxan solution to 25.0 mL with *dimethyl sulfoxide R1*.

Test solution To 1.000 g of the substance to be examined in a head-space vial, add 4.0 mL of *dimethyl sulfoxide R1* and close the vial immediately.

Reference solution To 1.000 g of the substance to be examined in a head-space vial, add 2.0 mL of *dimethyl sulfoxide R1* and 2.0 mL of the mixture solution. Close the vial immediately.

Column:
— *material*: fused silica;
— *size*: l = 50 m, Ø = 0.32 mm;
— *stationary phase*: *phenyl(5)methyl(95)polysiloxane R* (film thickness 5 µm).

Carrier gas *helium for chromatography R.*

Flow rate 1.4 mL/min.

Static head-space conditions:
— *equilibrium temperature*: 110 °C;
— *equilibration time*: 30 min;
— *transfer-line temperature*: 140 °C;
— *pressurisation time*: 1 min;
— *injection time*: 0.05 min.

Temperature:

	Time (min)	Temperature (°C)
	0 - 10	70
Column	10 - 27	70 → 240
Injection port		250
Detector		250

Detection Flame ionisation.

Injection Inject a suitable volume of the gaseous phase, for example 1 mL.

Relative retention With reference to ethylene oxide (retention time = about 6 min): propylene oxide = about 1.3; methylene chloride = about 1.6; dioxan = about 3.0; dimethyl sulfoxide = about 3.7.

Limits:

- *ethylene oxide*: not more than 0.5 times the area of the corresponding peak in the chromatogram obtained with the reference solution (1 ppm);
- *propylene oxide*: not more than 0.5 times the area of the corresponding peak in the chromatogram obtained with the reference solution (5 ppm);
- *dioxan*: not more than 0.5 times the area of the corresponding peak in the chromatogram obtained with the reference solution (10 ppm).

Average relative molecular mass

Weigh 15 g (*m*) of the substance to be examined into a 250 mL ground-glass-stoppered flask, add 25.0 mL of *phthalic anhydride solution R* and a few glass beads and swirl to dissolve. Boil gently under a reflux condenser for 1 h, allow to cool and add 2 quantities, each of 10 mL, of *pyridine R*, through the condenser. Add 10 mL of *water R*, mix and allow to stand for 10 min. Add 40.0 mL of *0.5 M sodium hydroxide* and 0.5 mL of a 10 g/L solution of *phenolphthalein R* in *pyridine R*. Titrate with *0.5 M sodium hydroxide* to a light pink endpoint that persists for 15 s and record the volume of sodium hydroxide used (*S*). Prepare a blank. Record the volume of sodium hydroxide used (*B*).

Calculate the average relative molecular mass using the following expression:

$$\frac{4000m}{B-S}$$

Oxypropylene:oxyethylene ratio

Nuclear magnetic resonance spectrometry (*2.2.33*).

Use a 100 g/L solution of the substance to be examined in *deuterated chloroform R*. Record the average area of the doublet appearing at about 1.08 ppm due to the methyl groups of the oxypropylene units (A_1) and the average area of the composite band from 3.2 ppm to 3.8 ppm due to CH_2O groups of both the oxyethylene and oxypropylene units and the CHO groups of the oxypropylene units (A_2) with reference to the internal standard.

Calculate the percentage of oxyethylene, by weight, in the sample being examined using the following expression:

$$\frac{3300\alpha}{33\alpha+58}$$

where $\alpha = \frac{A_2}{A_1} - 1$

Water (*2.5.12*)

Maximum 1.0 per cent, determined on 1.000 g.

Total ash (*2.4.16*)

Maximum 0.4 per cent, determined on 1.0 g.

STORAGE

In an airtight container.

LABELLING

The label states the type of poloxamer.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristic may be relevant for poloxamers used as dispersing agents or vehicles (type 124).

Viscosity (*2.2.9*)

About 400 mPa·s, determined at 25 °C, for the undiluted poloxamer.

The following characteristics may be relevant for poloxamers used as solubilising agents (types 188 and 237).

Viscosity (*2.2.9*)

Typically less than 50 mPa·s, determined at 25 °C.

Dissolve 25.0 g in 100.0 mL of *water R*.

Oxypropylene:oxyethylene ratio

(see Tests).

The following characteristics may be relevant for poloxamers used as solubilising agents (type 407).

Viscosity (*2.2.9*)

Typically less than 75 mPa·s, determined at 5 °C.

Dissolve 25.0 g in 100.0 mL of *water R*.

Oxypropylene:oxyethylene ratio

(see Tests).

The following characteristics may be relevant for poloxamers used as wetting agents (types 188 and 407).

Particle-size distribution (*2.9.31*)

Oxypropylene:oxyethylene ratio

(see Tests).

The following characteristic may be relevant for poloxamers used as viscosity-increasing agents or suspending agents (types 338 and 407).

Gel formation

Dissolve 25.0 g in 100 mL of cold *water R* (5-8 °C) and stir overnight in a refrigerator until dissolution is complete. The viscous solution obtained forms a thermoreversible gel after heating to 37 °C and does not flow in a capillary tube (*2.2.9*) or flows only very slowly (flow time greater than 10 min).

Ph Eur

Polyacrylate Dispersion (30 per cent)

(Ph. Eur. monograph 0733)

Action and use
Excipient.

Ph Eur

DEFINITION
Dispersion in water of a copolymer of ethyl acrylate and methyl methacrylate having a mean relative molecular mass of about 800 000.

Content
28.5 per cent to 31.5 per cent (residue on evaporation).

It may contain a suitable emulsifier.

CHARACTERS
Appearance
Opaque, white or almost white, slightly viscous liquid.

Solubility
Miscible with water, soluble in acetone, in anhydrous ethanol and in 2-propanol.

IDENTIFICATION
First identification: A.

Second identification: B, C, D, E.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of polyacrylate.

B. To 1 g add 5 mL of *water R* and mix; the mixture remains opaque. Take 3 portions of 1 g and mix separately with 5 g of *anhydrous ethanol R*, 5 g of *acetone R* and 5 g of *2-propanol R*. Transparent solutions are obtained.

C. To 1 g add 10 mL of *0.1 M sodium hydroxide*. The mixture remains opaque.

D. Appearance of a film (see Tests).

E. Dry 4 g in a Petri dish at 60 °C in an oven for 4 h and transfer the resulting clear film to a small test-tube (100 mm × 12 mm). Heat over a flame and collect the fumes that evolve in a 2nd test-tube held over the mouth of the 1st tube. The condensate gives the reaction of esters (*2.3.1*).

TESTS
Relative density (*2.2.5*)
1.037 to 1.047.

Viscosity (*2.2.10*)
Maximum 50 mPa·s, determined using a rotating viscometer at 20 °C and a shear rate of 10 s^{-1}.

Appearance of a film
Pour 1 mL on a glass plate and allow to dry. A clear elastic film is formed.

Particulate matter
Filter 100.0 g through a tared stainless steel sieve (90). Rinse with *water R* until a clear filtrate is obtained and dry at 80 °C to constant mass. The residue weighs not more than 0.500 g.

Residual monomers
Liquid chromatography (*2.2.29*).

Test solution Dissolve 1.00 g of the substance to be examined in *tetrahydrofuran R* and dilute to 50.0 mL with the same solvent. To 5.0 mL of a 35 g/L solution of *sodium perchlorate R* add 10.0 mL of the solution dropwise whilst stirring continuously. Centrifuge and filter the clear supernatant. Dilute 5.0 mL of this solution to 10.0 mL with *water R*.

Reference solution Dissolve 10 mg of *ethyl acrylate R* and 10 mg of *methyl methacrylate R* in *tetrahydrofuran R* and dilute to 50.0 mL with the same solvent. Dilute 1.0 mL of this solution to 100.0 mL with *tetrahydrofuran R*. To 10.0 mL of the solution add 5.0 mL of a 35 g/L solution of *sodium perchlorate R* and mix. Dilute 5.0 mL of the mixture to 10.0 mL with *water R*.

Column:
— *size: l* = 0.12 m, Ø = 4.6 mm;
— *stationary phase: octadecylsilyl silica gel for chromatography R* (5-10 µm).

Mobile phase *acetonitrile R1, water for chromatography R* (15:85 *V/V*).

Flow rate 2 mL/min.

Detection Spectrophotometer at 205 nm.

Injection About 50 µL.

Limit:
— *residual monomers*: maximum 100 ppm.

Sulfated ash (*2.4.14*)
Maximum 0.4 per cent, determined on 1.0 g.

Microbial contamination
TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*).

TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

ASSAY
Dry 1.000 g at 110 °C for 3 h and weigh the residue.

STORAGE
At a temperature of 5 °C to 25 °C, protected from freezing.

FUNCTIONALITY-RELATED CHARACTERISTICS
This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for polyacrylate dispersion 30 per cent used as film former or matrix former in prolonged-release dosage forms.

Viscosity
(see Tests).

Appearance of a film
(see Tests).

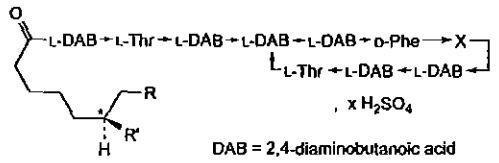
Solubility of a film
Take a piece of the film obtained in the test for appearance of a film and place it in a flask containing a 10.3 g/L solution of *hydrochloric acid R* with stirring. It does not dissolve within 2 h. Take another piece of the film and place it in a flask containing *0.33 M phosphate buffer solution pH 7.5 R* with stirring. It also does not dissolve within 2 h.

Ph Eur

Polymyxin B Sulfate

Polymyxin B Sulphate

(Ph. Eur. monograph 0203)



Polymyxin	R	R'	X	Molecular formula	M_r
B1	CH_3	CH_3	L-Leu	$C_{56}H_{98}N_{16}O_{13}$	1203
B2	H	CH_3	L-Leu	$C_{55}H_{96}N_{16}O_{13}$	1189
B3	CH_3	H	L-Leu	$C_{55}H_{96}N_{16}O_{13}$	1189
B1-I	CH_3	CH_3	L-Ile	$C_{56}H_{98}N_{16}O_{13}$	1203

Action and use
Antibacterial.

Preparation
Polymyxin and Bacitracin Ointment

Ph Eur

DEFINITION

Mixture of the sulfates of polypeptides produced by the growth of certain strains of *Paenibacillus polymyxa*, the main component being polymyxin B1.

Potency
Minimum 6500 IU/mg (dried substance).

CHARACTERS

Appearance
White or almost white, hygroscopic powder.

Solubility
Freely soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 5 mg of the substance to be examined in 1 mL of a mixture of equal volumes of *hydrochloric acid R* and *water R*. Heat at 135 °C in a sealed tube for 5 h. Evaporate to dryness on a water-bath and continue the heating until the hydrochloric acid has evaporated. Dissolve the residue in 0.5 mL of *water R*.

Reference solution (a) Dissolve 20 mg of *leucine R* in *water R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 20 mg of *threonine R* in *water R* and dilute to 10 mL with the same solvent.

Reference solution (c) Dissolve 20 mg of *phenylalanine R* in *water R* and dilute to 10 mL with the same solvent.

Reference solution (d) Dissolve 20 mg of *serine R* in *water R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel G plate R.*

Carry out the following procedures protected from light.

Mobile phase *water R, phenol R* (25:75 *V/V*).

Application 5 µL as bands of 10 mm, then place the plate in the chromatographic tank so that it is not in contact with the mobile phase, and allow it to become impregnated with the vapour of the mobile phase for at least 12 h.

Development Over 2/3 of the plate using the same mobile phase.

Drying At 100-105 °C.

Detection Spray with *ninhydrin solution R1* and heat at 110 °C for 5 min.

Results The chromatogram obtained with the test solution shows zones corresponding to those in the chromatograms obtained with reference solutions (a), (b) and (c), but shows no zone corresponding to that in the chromatogram obtained with reference solution (d); the chromatogram obtained with the test solution also shows a zone with a very low R_F value (2,4-diaminobutyric acid).

B. Examine the chromatograms obtained in the test for composition.

Results The peaks due to polymyxins B1, B2, B3 and B1-I in the chromatogram obtained with the test solution are similar in retention time to the corresponding peaks in the chromatogram obtained with reference solution (a).

C. Dissolve about 2 mg in 5 mL of *water R* and add 5 mL of a 100 g/L solution of *sodium hydroxide R*. Shake and add dropwise 0.25 mL of a 10 g/L solution of *copper sulfate pentahydrate R*, shaking after each addition. A reddish-violet colour develops.

D. It gives reaction (a) of sulfates (*2.3.1*).

TESTS

pH (*2.2.3*)
5.0 to 7.0.

Dissolve 0.2 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Composition
Liquid chromatography (*2.2.29*): use the normalisation procedure.

Solvent mixture *acetonitrile R, water R* (20:80 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Reference solution (a) Dissolve the contents of a vial of *polymyxin B sulfate CRS* in the solvent mixture and dilute to 50 mL with the solvent mixture.

Reference solution (b) Dilute 1 mL of reference solution (a) to 100 mL with the solvent mixture.

Column:
— *size:* l = 0.25 m, Ø = 4.6 mm;
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature:* 30 °C.

Mobile phase Mix 20 volumes of *acetonitrile R1* and 80 volumes of a solution prepared as follows: dissolve 4.46 g of *anhydrous sodium sulfate R* in 900 mL of *water for chromatography R*, adjust to pH 2.3 with *dilute phosphoric acid R* and dilute to 1000 mL with *water for chromatography R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µL.

Run time 1.4 times the retention time of polymyxin B1.

Identification of peaks Use the chromatogram supplied with *polymyxin B sulfate CRS* and the chromatogram obtained with

reference solution (a) to identify the peaks due to polymyxins B1, B2, B3 and B1-I.

Relative retention With reference to polymyxin B1 (retention time = about 35 min): polymyxin B2 = about 0.5; polymyxin B3 = about 0.6; polymyxin B1-I = about 0.8.

System suitability:
- *resolution*: minimum 3.0 between the peaks due to polymyxin B2 and polymyxin B3 in the chromatogram obtained with reference solution (a);
- *signal-to-noise ratio*: minimum 20 for the peak due to polymyxin B1 in the chromatogram obtained with reference solution (b).

Limits:
- *polymyxin B1-I*: maximum 15.0 per cent;
- *polymyxin B3*: maximum 6.0 per cent;
- *sum of polymyxins B1, B2, B3 and B1-I*: minimum 80.0 per cent;
- *reporting threshold*: 0.40 per cent.

Related substances

Liquid chromatography (*2.2.29*) as described in the test for composition.

Limits:
- *any impurity*: for each impurity, maximum 3.0 per cent;
- *total*: maximum 17.0 per cent;
- *reporting threshold*: 0.40 per cent.

Sulfate

15.5 per cent to 17.5 per cent (dried substance).

Dissolve 0.250 g in 100 mL of *water R* and adjust the solution to pH 11 with *concentrated ammonia R*. Add 10.0 mL of *0.1 M barium chloride* and about 0.5 mg of *phthalein purple R*. Titrate with *0.1 M sodium edetate*, adding 50 mL of *ethanol (96 per cent) R* when the colour of the solution begins to change and continuing the titration until the violet-blue colour disappears.

1 mL of *0.1 M barium chloride* is equivalent to 9.606 mg of SO_4.

Loss on drying (*2.2.32*)

Maximum 6.0 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C at a pressure not exceeding 0,7 kPa for 3 h.

Sulfated ash (*2.4.14*)

Maximum 0.75 per cent, determined on 1.0 g.

Pyrogens (*2.6.8*)

If intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of pyrogens, it complies with the test for pyrogens. Inject, per kilogram of the rabbit's mass, 1 mL of a solution in *water for injections R* containing 1.5 mg of the substance to be examined per millilitre.

ASSAY

Carry out the microbiological assay of antibiotics (*2.7.2*). Use *polymyxin B sulfate for microbiological assay CRS* as the chemical reference substance.

STORAGE

In an airtight container, protected from light. If the substance is sterile, the container is also sterile and tamper-evident.

LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

Ph Eur

Polyoxypropylene Stearyl Ether

(Ph. Eur. monograph 2602)

Ph Eur

DEFINITION

Mixture of ethers of polyoxypropylene with linear alcohols, mainly stearyl alcohol, obtained by the reaction of stearyl alcohol with propylene oxide. It may contain some free polyoxypropylene and various amounts of free stearyl alcohol. The number of moles of propylene oxide reacted per mole of stearyl alcohol is 11 (nominal value). A suitable antioxidant may be added.

CHARACTERS

Appearance

Colourless or pale yellow, clear or slightly turbid liquid.

Solubility

Practically insoluble in water, soluble in ethanol (96 per cent), in mineral oils and in 2-propanol, practically insoluble in propylene glycol and in glycerol.

Relative density

About 0.94 at 25 °C.

Refractive index

About 1.448 at 25 °C.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *polyoxypropylene stearyl ether CRS*.

B. Hydroxyl value (see Tests).

C. Saponification value (see Tests).

D. Viscosity (*2.2.9*): 83 mPa·s to 95 mPa·s.

TESTS

Acid value (*2.5.1*)

Maximum 2.0.

Hydroxyl value (*2.5.3, Method A*)

60 to 77.

Iodine value (*2.5.4, Method A*)

Maximum 3.0.

Peroxide value (*2.5.5, Method A*)

Maximum 5.0.

Saponification value (*2.5.6*)

Maximum 3.0.

Propylene oxide

Head-space gas chromatography (*2.2.28*).

Propylene oxide stock solution Weigh 45 g of cold *macrogol 200 R1* and add 1.000 g (*m*) of *propylene oxide R*. Mix carefully by swirling to ensure a homogeneous solution. Add more *macrogol 200 R1* until the total weight is 50.0 g and mix again. [NOTE: the solution is stable for 1 month if stored at −20 °C]. Allow to reach room temperature. Dilute 0.50 g of this solution to 100.0 mL with *water R*.

Propylene oxide standard solution Dilute 10.0 mL of propylene oxide stock solution to 100.0 mL with *water R*.

Propionaldehyde stock solution Weigh 0.1 g of *propionaldehyde R* into a volumetric flask and dilute to 100.0 mL with *water R*.

Test solution (a) Weigh 1.00 g of the substance to be examined into a 20 mL head-space vial. Add 0.5 mL of cold *water R* and seal the vial immediately with a polytetrafluoroethylene-coated silicon membrane and an aluminium cap. Mix carefully.

Test solution (b) Weigh 1.00 g of the substance to be examined into a 20 mL head-space vial. Add 0.5 mL of cold propylene oxide standard solution and seal the vial immediately with a polytetrafluoroethylene-coated silicon membrane and an aluminium cap. Mix carefully.

Reference solution Introduce 50.0 mL of cold propylene oxide standard solution into a volumetric flask, add 0.5 mL of cold propionaldehyde stock solution and dilute to 100.0 mL with *water R*. Introduce 0.5 mL of this solution into a 20 mL head-space vial.

Column:
— *material*: fused silica;
— *size*: *l* = 60 m, Ø = 0.32 mm;
— *stationary phase*: *methylpolysiloxane R* (film thickness 5 µm).

Carrier gas *helium for chromatography R*.

Flow rate 2.6 mL/min.

Split ratio 10:1.

Static head-space conditions that may be used:
— *equilibration temperature*: 90 °C;
— *equilibration time*: 45 min.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 5	50
	5 - 31	50 → 180
	31 - 32.7	180 → 230
	32.7 - 37.7	230
Injection port		150
Detector		250

Detection Flame ionisation.

Injection A suitable volume, for example 1.0 mL, of the gaseous phase of test solutions (a) and (b) and of the reference solution.

Identification of peaks Use the chromatogram obtained with the reference solution to identify the peaks due to propylene oxide and propionaldehyde.

Relative retention With reference to propylene oxide (retention time = about 10.4 min): propionaldehyde = about 0.96.

System suitability Reference solution:
— *resolution*: minimum 1.5 between the peaks due to propionaldehyde and propylene oxide;
— *signal-to-noise ratio*: minimum 10 for the peak due to propylene oxide.

Calculate the content of propylene oxide in parts per million using the following expression:

$$\frac{A_1 \times m \times 5}{(A_2 \times M_1) - (A_1 \times M_2)}$$

A_1 = area of the peak due to propylene oxide in the chromatogram obtained with test solution (a);
A_2 = area of the peak due to propylene oxide in the chromatogram obtained with test solution (b);
M_1 = mass of the substance to be examined in test solution (a), in grams;
M_2 = mass of the substance to be examined in test solution (b), in grams;
m = mass of propylene oxide used to prepare the propylene oxide stock solution, in grams.

Limit:
— *propylene oxide*: maximum 5 ppm.

Water (*2.5.12*)
Maximum 0.7 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*)
Maximum 0.3 per cent.

Ignite a suitable crucible at 600 ± 25 °C for 30 min, allow to cool in a desiccator over silica gel or other suitable desiccant and weigh. Place 1.0 g of the substance to be examined in the crucible and weigh. Carefully ignite and char the substance using a gas burner in a fume cupboard. Carry out the test for sulfated ash (*2.4.14*) on the residue obtained, starting from "Moisten the substance to be examined...".

STORAGE
In an airtight container.

LABELLING
The label states the number of moles of propylene oxide reacted per mole of stearyl alcohol (nominal value).

FUNCTIONALITY-RELATED CHARACTERISTICS
This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristic may be relevant for polyoxypropylene stearyl ether used as solvent or emollient in preparations for cutaneous application.

Viscosity
(see Identification).

Ph Eur

Polysorbate 20

Polyoxyethylene 20 Sorbitan Monolaurate
(Ph. Eur. monograph 0426)

Action and use
Non-ionic surfactants.

Ph Eur

DEFINITION
Mixture of partial esters of fatty acids, mainly lauric (dodecanoic) acid, with sorbitol and its anhydrides ethoxylated with approximately 20 moles of ethylene oxide for each mole of sorbitol and sorbitol anhydrides.

CHARACTERS
Appearance
Oily, yellow or brownish-yellow, clear or slightly opalescent liquid.

Solubility
Soluble in water, in anhydrous ethanol, in ethyl acetate and in methanol, practically insoluble in fatty oils and in liquid paraffin.

Relative density
About 1.10.

Viscosity
About 400 mPa·s at 25 °C.

IDENTIFICATION
First identification: A, D.
Second identification: B, C, D, E.

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *Ph. Eur. reference spectrum of polysorbate 20.*

B. Hydroxyl value (see Tests).

C. Saponification value (see Tests).

D. Composition of fatty acids (see Tests).

E. Dissolve 0.1 g in 5 mL of *methylene chloride R*. Add 0.1 g of *potassium thiocyanate R* and 0.1 g of *cobalt nitrate R*. Stir with a glass rod. The solution becomes blue.

TESTS

Acid value (*2.5.1*)
Maximum 2.0.

Dissolve 5.0 g in 50 mL of the prescribed solvent mixture.

Hydroxyl value (*2.5.3, Method A*)
96 to 108.

Peroxide value
Maximum 10.0.

Introduce 10.0 g into a 100 mL beaker and dissolve with 20 mL of *glacial acetic acid R*. Add 1 mL of *saturated potassium iodide solution R*, mix and allow to stand for 1 min. Add 50 mL of *carbon dioxide-free water R* and a magnetic stirring bar. Titrate with *0.01 M sodium thiosulfate*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration. If the result of the blank determination exceeds 0.1 mL of titration reagent, replace the reagents and repeat the determination.

Determine the peroxide value using the following expression:

$$\frac{(n_1 - n_2) \times M \times 1000}{m}$$

n_1 = volume of *0.01 M sodium thiosulfate* required for the substance to be examined, in millilitres;
n_2 = volume of *0.01 M sodium thiosulfate* required for the blank titration, in millilitres;
M = molarity of the sodium thiosulfate solution, in moles per litre;
m = mass of the substance to be examined, in grams.

Saponification value (*2.5.6*)
40 to 50, determined on 4.0 g.

Use 15.0 mL of 0.5 M alcoholic potassium hydroxide and dilute with 50 mL of *ethanol (96 per cent) R* before carrying out the titration. Heat under reflux for 60 min.

Composition of fatty acids (*2.4.22, Method C*)
Prepare reference solution (a) as indicated in Table 2.4.22.-2.

Column:
— *material*: fused silica;
— *size*: *l* = 30 m, Ø = 0.32 mm;
— *stationary phase*: *macrogol 20 000 R* (film thickness 0.5 µm).

Carrier gas *helium for chromatography R.*

Linear velocity 50 cm/s.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 14	80 → 220
	14 - 54	220
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 1 µL.

Composition of the fatty-acid fraction of the substance:
— *caproic acid*: maximum 1.0 per cent;
— *caprylic acid*: maximum 10.0 per cent;
— *capric acid*: maximum 10.0 per cent;
— *lauric acid*: 40.0 per cent to 60.0 per cent;
— *myristic acid*: 14.0 per cent to 25.0 per cent;
— *palmitic acid*: 7.0 per cent to 15.0 per cent;
— *stearic acid*: maximum 7.0 per cent;
— *oleic acid*: maximum 11.0 per cent;
— *linoleic acid*: maximum 3.0 per cent.

Ethylene oxide and dioxan (*2.4.25, Method A*)
Maximum 1 ppm of ethylene oxide and 10 ppm of dioxan.

Water (*2.5.12*)
Maximum 3.0 per cent, determined on 1.00 g.

Total ash (*2.4.16*)
Maximum 0.25 per cent, determined on 2.0 g.

STORAGE
In an airtight container, protected from light.

Ph Eur

Polysorbate 40

Polyoxyethylene 20 Sorbitan Monopalmitate
(Ph. Eur. monograph 1914)

Action and use
Non-ionic surfactants.

Ph Eur

DEFINITION
Mixture of partial esters of fatty acids, mainly *Palmitic acid (1904)*, with sorbitol and its anhydrides ethoxylated with approximately 20 moles of ethylene oxide for each mole of sorbitol and sorbitol anhydrides.

CHARACTERS

Appearance
Oily, viscous, yellowish or brownish-yellow liquid.

Solubility
Miscible with water, with anhydrous ethanol, with ethyl acetate and with methanol, practically insoluble in fatty oils and in liquid paraffin.

Relative density
About 1.10.

Viscosity
About 400 mPa·s at 30 °C.

IDENTIFICATION
First identification: A, D.
Second identification: B, C, D, E.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of polysorbate 40.*

B. Hydroxyl value (see Tests).

C. Saponification value (see Tests).

D. Composition of fatty acids (see Tests).

E. Dissolve 0.1 g in 5 mL of *methylene chloride R*. Add 0.1 g of *potassium thiocyanate R* and 0.1 g of *cobalt nitrate R*. Stir with a glass rod. The solution becomes blue.

TESTS

Acid value (*2.5.1*)
Maximum 2.0.

Dissolve 5.0 g in 50 mL of the prescribed solvent mixture.

Hydroxyl value (*2.5.3, Method A*)
89 to 105.

Peroxide value
Maximum 10.0.

Introduce 10.0 g into a 100 mL beaker and dissolve with 20 mL of *glacial acetic acid R*. Add 1 mL of *saturated potassium iodide solution R*, mix and allow to stand for 1 min. Add 50 mL of *carbon dioxide-free water R* and a magnetic stirring bar. Titrate with *0.01 M sodium thiosulfate*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration. If the result of the blank determination exceeds 0.1 mL of titration reagent, replace the reagents and repeat the determination.

Determine the peroxide value using the following expression:

$$\frac{(n_1 - n_2) \times M \times 1000}{m}$$

n_1 = volume of *0.01 M sodium thiosulfate* required for the substance to be examined, in millilitres;
n_2 = volume of *0.01 M sodium thiosulfate* required for the blank titration, in millilitres;
M = molarity of the sodium thiosulfate solution, in moles per litre;
m = mass of the substance to be examined, in grams.

Saponification value (*2.5.6*)
41 to 52, determined on 4.0 g.

Use 15.0 mL of 0.5 M alcoholic potassium hydroxide and dilute with 50 mL of *ethanol (96 per cent) R* before carrying out the titration. Heat under reflux for 60 min.

Composition of fatty acids (*2.4.22, Method C*)
Prepare reference solution (a) as indicated in Table 2.4.22.-1.

Column:
— *material*: fused silica;
— *size*: *l* = 30 m, Ø = 0.32 mm;
— *stationary phase*: *macrogol 20 000 R* (film thickness 0.5 µm).

Carrier gas *helium for chromatography R.*

Linear velocity 50 cm/s.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 14	80 → 220
	14 - 54	220
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 1 µL.

Composition of the fatty-acid fraction of the substance:
— *palmitic acid*: minimum 92.0 per cent.

Ethylene oxide and dioxan (*2.4.25, Method A*)
Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Water (*2.5.12*)
Maximum 3.0 per cent, determined on 1.00 g.

Total ash (*2.4.16*)
Maximum 0.25 per cent, determined on 2.0 g.

STORAGE
In an airtight container, protected from light.

Ph Eur

Polysorbate 60

Polyoxyethylene 20 Sorbitan Monostearate
(Ph. Eur. monograph 0427)

Action and use
Non-ionic surfactants.

Ph Eur

DEFINITION
Mixture of partial esters of fatty acids, mainly *Stearic acid 50 (1474)*, with sorbitol and its anhydrides ethoxylated with approximately 20 moles of ethylene oxide for each mole of sorbitol and sorbitol anhydrides.

CHARACTERS

Appearance
Yellowish-brown gelatinous mass which becomes a clear liquid at temperatures above 25 °C.

Solubility
Soluble in water, in anhydrous ethanol, in ethyl acetate and in methanol, practically insoluble in fatty oils and in liquid paraffin.

Relative density
About 1.10.

Viscosity
About 400 mPa·s at 30 °C.

IDENTIFICATION
First identification: A, D.

Second identification: B, C, D, E.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of polysorbate 60.*

B. Hydroxyl value (see Tests).

C. Saponification value (see Tests).

D. Composition of fatty acids (see Tests).

E. Dissolve 0.1 g in 5 mL of *methylene chloride R*. Add 0.1 g of *potassium thiocyanate R* and 0.1 g of *cobalt nitrate R*. Stir with a glass rod. The solution becomes blue.

TESTS

Acid value (*2.5.1*)
Maximum 2.0.

Dissolve 5.0 g in 50 mL of the prescribed solvent mixture.

Hydroxyl value (*2.5.3, Method A*)
81 to 96.

Peroxide value
Maximum 10.0.

Introduce 10.0 g into a 100 mL beaker and dissolve with 20 mL of *glacial acetic acid R*. Add 1 mL of *saturated*

potassium iodide solution R, mix and allow to stand for 1 min. Add 50 mL of *carbon dioxide-free water R* and a magnetic stirring bar. Titrate with *0.01 M sodium thiosulfate*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration. If the result of the blank determination exceeds 0.1 mL of titration reagent, replace the reagents and repeat the determination.

Determine the peroxide value using the following expression:

$$\frac{(n_1 - n_2) \times M \times 1000}{m}$$

n_1 = volume of *0.01 M sodium thiosulfate* required for the substance to be examined, in millilitres;
n_2 = volume of *0.01 M sodium thiosulfate* required for the blank titration, in millilitres;
M = molarity of the sodium thiosulfate solution, in moles per litre;
m = mass of the substance to be examined, in grams.

Saponification value (*2.5.6*)
45 to 55, determined on 4.0 g.

Use 15.0 mL of 0.5 M alcoholic potassium hydroxide and dilute with 50 mL of *ethanol (96 per cent) R* before carrying out the titration. Heat under reflux for 60 min.

Composition of fatty acids (*2.4.22, Method G*)
Prepare reference solution (a) as indicated in Table 2.4.22.-1.

Column:
— *material*: fused silica;
— *size*: *l* = 30 m, Ø = 0.32 mm;
— *stationary phase*: *macrogol 20 000 R* (film thickness 0.5 µm).

Carrier gas *helium for chromatography R.*

Linear velocity 50 cm/s.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 14	80 → 220
	14 - 54	220
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 1 µL.

Composition of the fatty-acid fraction of the substance:
— *stearic acid*: 40.0 per cent to 60.0 per cent;
— *sum of the contents of palmitic and stearic acids*: minimum 90.0 per cent.

Ethylene oxide and dioxan (*2.4.25, Method A*)
Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Water (*2.5.12*)
Maximum 3.0 per cent, determined on 1.00 g.

Total ash (*2.4.16*)
Maximum 0.25 per cent, determined on 2.0 g.

STORAGE
In an airtight container, protected from light.

Ph Eur

Polysorbate 80[1]

Polyoxyethylene 20 Sorbitan Mono-oleate
(Ph. Eur. monograph 0428)

Action and use
Non-ionic surfactant.

Ph Eur

DEFINITION
Mixture of partial esters of fatty acids, mainly *Oleic acid (0799)*, with sorbitol and its anhydrides ethoxylated with approximately 20 moles of ethylene oxide for each mole of sorbitol and sorbitol anhydrides.

♦CHARACTERS

Appearance
Oily, colourless or brownish-yellow, clear or slightly opalescent liquid.

Solubility
Dispersible in water, in anhydrous ethanol, in ethyl acetate and in methanol, practically insoluble in fatty oils and in liquid paraffin.

Relative density
About 1.10.

Viscosity
About 400 mPa·s at 25 °C.♦

IDENTIFICATION
First identification: ♦A♦, D.

◊Second identification: B, C, D, E.◊

♦A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of polysorbate 80.♦*

◊B. Hydroxyl value (see Tests).

C. Saponification value (see Tests).◊

D. Composition of fatty acids (see Tests).

◊E. Dissolve 0.1 g in 5 mL of *methylene chloride R*. Add 0.1 g of *cobalt nitrate R* and 0.1 g of *potassium thiocyanate R*. Stir with a glass rod. The solution becomes blue.◊

TESTS

Acid value (*2.5.1*)
Maximum 2.0.

Dissolve 5.0 g in 50 mL of the prescribed mixture of solvents.

Hydroxyl value (*2.5.3, Method A*)
65 to 80.

Peroxide value
Maximum 10.0.

Introduce 10.0 g into a 100 mL beaker and dissolve with 20 mL of *glacial acetic acid R*. Add 1 mL of *saturated potassium iodide solution R*, mix and allow to stand for 1 min. Add 50 mL of *carbon dioxide-free water R* and a magnetic stirring bar. Titrate with *0.01 M sodium thiosulfate*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

Determine the peroxide value using the following expression:

$$\frac{(n_1 - n_2) \times M \times 1000}{m}$$

[1] *This monograph has undergone pharmacopoeial harmonisation. See chapter 5.8 Pharmacopoeial harmonisation.*

n_1 = volume of *0.01 M sodium thiosulfate* required for the substance to be examined, in millilitres;
n_2 = volume of *0.01 M sodium thiosulfate* required for the blank titration, in millilitres;
M = molarity of the sodium thiosulfate solution, in moles per litre;
m = mass of the substance to be examined, in grams.

Saponification value (*2.5.6*)
45 to 55, determined on 4.0 g.

Use 30.0 mL of *0.5 M alcoholic potassium hydroxide*, heat under reflux for 60 min and add 50 mL of *anhydrous ethanol R* before carrying out the titration.

Composition of fatty acids (*2.4.22, Method C*)
Use the mixture of calibrating substances in Table 2.4.22.-3.

Column:
— *material*: fused silica;
— *size*: *l* = 30 m, Ø = 0.32 mm;
— *stationary phase*: *macrogol 20 000 R* (film thickness 0.5 µm).

Carrier gas *helium for chromatography R.*

Linear velocity 50 cm/s.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 14	80 → 220
	14 - 54	220
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 1 µL.

Composition of the fatty-acid fraction of the substance:
— *myristic acid*: maximum 5.0 per cent;
— *palmitic acid*: maximum 16.0 per cent;
— *palmitoleic acid*: maximum 8.0 per cent;
— *stearic acid*: maximum 6.0 per cent;
— *oleic acid*: minimum 58.0 per cent;
— *linoleic acid*: maximum 18.0 per cent;
— *linolenic acid*: maximum 4.0 per cent.

Ethylene oxide and dioxan
Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Head-space gas chromatography (*2.2.28*).

Ethylene oxide stock solution Dilute 0.5 mL of a commercially available solution of ethylene oxide in methylene chloride (50 mg/mL) to 50.0 mL with *water R*. [NOTE: the solution is stable for 3 months, if stored in vials with polytetrafluoroethylene-coated silicone membrane crimped caps at −20 °C]. Allow to reach room temperature. Dilute 1.0 mL of this solution to 250.0 mL with *water R*.

Dioxan stock solution Dilute 1.0 mL of *dioxan R* to 200.0 mL with *water R*. Dilute 1.0 mL of this solution to 100.0 mL with *water R*.

Acetaldehyde stock solution Weigh about 0.100 g of *acetaldehyde R* into a 100 mL volumetric flask and dilute to 100.0 mL with *water R*. Dilute 1.0 mL of this solution to 100.0 mL with *water R*.

Standard solution To 6.0 mL of ethylene oxide stock solution add 2.5 mL of dioxan stock solution and dilute to 25.0 mL with *water R*.

Test solution (a) Weigh 1.00 g of the substance to be examined into a 10 mL head-space vial. Add 2.0 mL of *water R*, seal the vial immediately with a polytetrafluoroethylene-coated silicon membrane and an aluminum cap. Mix carefully.

Test solution (b) Weigh 1.00 g of the substance to be examined into a 10 mL head-space vial. Add 2.0 mL of standard solution, seal the vial immediately with a polytetrafluoroethylene-coated silicon membrane and an aluminum cap. Mix carefully.

Reference solution Introduce 2.0 mL of acetaldehyde stock solution and 2.0 mL of ethylene oxide stock solution into a 10 mL head-space vial and seal the vial immediately with a polytetrafluoroethylene-coated silicon membrane and an aluminum cap. Mix carefully.

Column:
— *material*: fused silica;
— *size*: *l* = 50 m, Ø = 0.53 mm;
— *stationary phase*: *phenyl(5)methyl(95)polysiloxane R* (5 µm).

Carrier gas *helium for chromatography R.*

Flow rate 4.0 mL/min.

Split ratio 1:3.5.

Static head-space conditions that may be used:
— *equilibration temperature*: 80 °C;
— *equilibration time*: 30 min.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 18	70 → 250
	18 - 23	250
Injection port		85
Detector		250

Detection Flame ionisation.

Injection 1.0 mL of test solutions (a) and (b) and of the reference solution.

Relative retention With reference to ethylene oxide (retention time = about 6.5 min): acetaldehyde = about 0.9; dioxan = about 1.9.

System suitability Reference solution:
— *resolution*: minimum 2.0 between the peaks due to acetaldehyde and ethylene oxide.

Calculate the content of ethylene oxide in ppm using the following expression:

$$\frac{2\,C_{EO} \times A_a}{A_b - A_a}$$

C_{EO} = concentration of added ethylene oxide in test solution (b), in micrograms per millilitre;
A_a = peak area of ethylene oxide in the chromatogram obtained with test solution (a);
A_b = peak area of ethylene oxide in the chromatogram obtained with test solution (b).

Calculate the content of dioxan in ppm using the following expression:

$$\frac{2 \times 1.03 \times C_D \times A_{a'}}{A_{b'} - A_{a'}} \times 1000$$

C_D = concentration of added dioxan in test solution (b), in microlitres per millilitre;
1.03 = density of dioxan, in grams per millilitre;
$A_{a'}$ = peak area of dioxan in the chromatogram obtained with test solution (a);
$A_{b'}$ = peak area of dioxan in the chromatogram obtained with test solution (b).

Water (*2.5.12*)
Maximum 3.0 per cent, determined on 1.00 g.

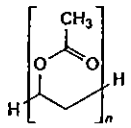
Total ash (*2.4.16*)
Maximum 0.25 per cent, determined on 2.0 g.

STORAGE
In an airtight container, protected from light.

Ph Eur

Poly(vinyl acetate)

(Ph. Eur. monograph 1962)



Ph Eur

DEFINITION
Poly(vinyl acetate) is a thermoplastic polymer obtained by polymerisation of vinyl acetate using a suitable starter, without solvent or with water or 2-propanol. The vast majority of the acetate moieties are attached to non-neighbouring carbon atoms of the chain.

The index *n* is about 100-17 000. The relative molecular mass lies between 10 000 and 1500 000. The viscosity is 4 mPa·s to 250 mPa·s. The ester value, which characterises the degree of hydrolysis, is 615 to 675.

CHARACTERS

Appearance
White or almost white powder or colourless granules or beads.

Solubility
Practically insoluble in water, freely soluble in ethyl acetate, soluble in ethanol (96 per cent). It is hygroscopic and swells in water.

It softens at temperatures above 40-50 °C.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs prepared as follows: dissolve about 200 mg in 5 mL of *acetone R*, place 100 µL on a *potassium bromide R* disc and dry to evaporate the solvent. Alternatively, the spectrum may be recorded directly by attenuated total reflectance (ATR).

Comparison *poly(vinyl acetate) CRS.*

B. Viscosity (see Tests).

C. Saponify (*2.5.6*) 0.500 g in a mixture of 25.0 mL of *0.5 M alcoholic potassium hydroxide* and 25.0 mL of *water R*. 0.15 mL of the solution obtained gives reaction (b) of acetates (*2.3.1*).

TESTS

Solution S
Suspend 50.0 g in 100 mL of *ethyl acetate R* in a borosilicate glass flask with a ground-glass neck. Heat under a reflux condenser with constant stirring for 30 min. Allow to cool. Filter through a sintered-glass filter (16) (*2.1.2*), wash the residue with 50.0 mL of *ethyl acetate R* and pour the filtrate into a 250 mL graduated flask. Dilute to 250 mL with *ethyl acetate R*.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method I*).

Viscosity (*2.2.49, Method A*)
85 per cent to 115 per cent of the value stated on the label.

Determine the viscosity immediately after preparation of solution S at 20 ± 0.1 °C by using a falling ball viscometer.

Acid value (*2.5.1*)
Maximum 2.0, determined on 5.0 g dissolved in 50 mL of *ethanol (96 per cent) R* by shaking for 3 h.

Ester value (*2.5.2*)
615 to 675.

Saponify (*2.5.6*) 0.500 g in a mixture of 25.0 mL of *0.5 M alcoholic potassium hydroxide* and 25.0 mL of *water R*.

Residual peroxides
Maximum 100 ppm, calculated as hydrogen peroxide.

Place 0.85 g in a borosilicate glass flask with a ground-glass neck. Add 10 mL of *ethyl acetate R* and heat under a reflux condenser with constant agitation. Allow to cool. Replace the air in the container with *oxygen-free nitrogen R* and add a solution of 1 mL of *glacial acetic acid R* and 0.5 g of *sodium iodide R* in 40 mL of *water R*. Shake thoroughly and allow to stand protected from light for 20 min. Titrate with *0.005 M sodium thiosulfate* until the yellow colour is discharged. Carry out a blank titration. The difference between the titration volumes is not greater than 1.0 mL.

Vinyl acetate
Head-space gas chromatography (*2.2.28*).

Test solution (a) Place 0.200 g of the substance to be examined in a 20 mL vial and add 1.0 mL of *dimethylformamide R*. Close the vial and secure the stopper. Shake, avoiding contact between the stopper and the liquid.

Test solution (b) Place 0.200 g of the substance to be examined in a 20 mL vial and add 1.0 mL of the reference solution. Close the vial and secure the stopper. Shake, avoiding contact between the stopper and the liquid.

Reference solution Place 15.0 mL of *dimethylformamide R* in a 20 mL vial, add 45 µL of *vinyl acetate R* and 50 µL of *butanal R* and dilute to volume with *dimethylformamide R*. Dilute 1.0 mL of the solution to 10.0 mL with *dimethylformamide R*.

Column:
— *material*: fused silica;
— *size*: *l* = 25 m, Ø = 0.32 mm;
— *stationary phase*: *vinyl(1)phenyl(5)methyl(94)polysiloxane R* (film thickness 0.32 µm).

Carrier gas *nitrogen for chromatography R.*

Flow rate 20 mL/min.

Static head-space conditions that may be used:
— *equilibration temperature*: 60 °C;
— *equilibration time*: 20 min;
— *transfer-line temperature*: 120 °C;
— *carrier gas*: *nitrogen for chromatography R.*

Temperature:
— *column*: 155 °C;
— *injection port*: 120 °C;
— *detector*: 180 °C.

Detection Flame ionisation.

Injection 1.6 mL of the gaseous phase of test solutions (a) and (b).

System suitability Test solution (b):
— *resolution*: minimum 2.0 between the peaks due to vinyl acetate and butanal;

— *signal-to-noise ratio*: minimum 5 for the peak due to vinyl acetate.

Calculate the percentage content of vinyl acetate using the following expression:

$$\frac{V \times S_1 \times 0.931}{(m_1 S_2 - m_2 S_1) \times 2000}$$

S_1	=	area (or height) of the peak due to vinyl acetate in the chromatogram obtained with test solution (a);
S_2	=	area (or height) of the peak due to vinyl acetate in the chromatogram obtained with test solution (b);
m_1	=	mass of the substance to be examined used to prepare test solution (a), in grams;
m_2	=	mass of the substance to be examined used to prepare test solution (b), in grams;
0.931	=	density of vinyl acetate, in grams per millilitre;
V	=	volume of vinyl acetate used to prepare the reference solution, in microlitres.

Limit:
— *vinyl acetate*: maximum 0.3 per cent.

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

STORAGE
In an airtight container.

LABELLING
The label states:
— the nominal relative molecular mass;
— the viscosity.

FUNCTIONALITY-RELATED CHARACTERISTICS
This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for poly(vinyl acetate) used as matrix former in prolonged-release dosage forms or film former.

Viscosity
(see Tests).

Solubility of a film
Place 3 mL of solution S on a glass plate and dry. Place the film obtained in 50 mL of *phosphate buffer solution pH 6.8 R* whilst stirring continuously. The film does not dissolve within 30 min.

Ph Eur

Poly(vinyl acetate) Dispersion 30 per cent

(Ph. Eur. monograph 2152)

Ph Eur

DEFINITION
Dispersion in water of poly(vinyl acetate) having a mean relative molecular mass of about 450 000. It may contain *Povidone (0685)* and a suitable surface-active agent, such as *Sodium laurilsulfate (0098)*, as stabilisers.

Content
25.0 per cent to 30.0 per cent of poly(vinyl acetate).

CHARACTERS
Appearance
Opaque, white or almost white, slightly viscous liquid.

Solubility
Miscible with water and with ethanol (96 per cent).
It is sensitive to spoilage by microbial contaminants.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Dry 1 mL *in vacuo*, dissolve the residue in *acetone R*, and spread 1 drop of the solution between 2 sodium chloride R plates; remove 1 plate and allow the solvent to evaporate.

Comparison Repeat the operation using *poly(vinyl acetate) dispersion 30 per cent CRS*.

B. Place 3 mL on a glass plate and allow to dry. A clear film is formed.

C. 50 mg gives the reaction of acetyl (*2.3.1*).

TESTS
Agglomerates
Filter 100.0 g through a tared stainless steel sieve (90). Rinse with *water R* until a clear filtrate is obtained and dry to constant mass at 100-105 °C. The mass of the residue is not greater than 0.5 g.

Vinyl acetate
Liquid chromatography (*2.2.29*).

Test solution Introduce 0.250 g into a 10 mL volumetric flask and add about 1 mL of *methanol R*. Sonicate. Add about 8 mL of *water R*. Sonicate and dilute to 10.0 mL with *water R*. Centrifuge for about 10 min and filter.

Reference solution (a) Dissolve 5.0 mg of *vinyl acetate CRS* in *methanol R* and dilute to 10.0 mL with the same solvent. Dilute 1.0 mL of the solution to 20.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

Reference solution (b) To 5 mg of *vinyl acetate R* and 5 mg of *1-vinylpyrrolidin-2-one R*, add 10 mL of *methanol R* and sonicate. Dilute to 50 mL with mobile phase A. Dilute 1 mL of the solution to 20 mL with mobile phase A.

A precolumn containing *end-capped octadecylsilyl silica gel for chromatography R* (5 µm) may be used if a matrix effect is observed.

Column:
— *size*: *l* = 0.25 m, Ø = 4.0 mm;
— *stationary phase*: *polar end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 30 °C.

Mobile phase:
— *mobile phase A*: *acetonitrile R1, methanol R2, water for chromatography R* (5:5:90 *V/V/V*);

— *mobile phase B*: *methanol R2, acetonitrile R1, water for chromatography R* (5:45:50 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	100	0
2 - 26	100 → 80	0 → 20
26 - 27	80 → 0	20 → 100
27 - 30	0 → 100	100 → 0

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 205 nm.

Injection 10 µL.

System suitability Reference solution (b):

— *resolution*: minimum 5.0 between the peaks due to vinyl acetate and 1-vinylpyrrolidin-2-one.

Limit:

— *vinyl acetate*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (100 ppm).

Povidone

Maximum 4.0 per cent.

Carry out the determination of nitrogen by sulfuric acid digestion (*2.5.9*), using 0.25 g. Calculate the percentage content of povidone using the following expression:

$$\frac{N}{0.126}$$

N = percentage content of nitrogen.

Acetic acid

Liquid chromatography (*2.2.29*).

Test solution Mix 0.200 g with *water R*. Sonicate for about 10 min and dilute to 10.0 mL with *water R*.

Reference solution Dissolve 30.0 mg of *acetic acid R* and 30 mg of *citric acid monohydrate R* in the mobile phase. Shake gently to dissolve and dilute to 100.0 mL with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *polar end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *0.005 M sulfuric acid.*

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 205 nm.

Injection 20 µL; after each injection, rinse the column with a mixture of equal volumes of *acetonitrile R1* and *0.005 M sulfuric acid.*

Retention time Acetic acid = about 6 min; citric acid = about 8 min.

System suitability Reference solution:

— *resolution*: minimum 2.0 between the peaks due to acetic acid and citric acid.

Limit:

— *acetic acid*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (1.5 per cent).

Residue on evaporation

28.5 per cent to 31.5 per cent, determined on 1.000 g at 110 °C for 5 h.

Sulfated ash

Maximum 0.5 per cent, determined on 1.0 g.

Heat a silica crucible to redness for 30 min, allow to cool in a desiccator and weigh. Evenly distribute 1.00 g of the preparation to be examined in the crucible and weigh. Dry at 100-105 °C for 1 h and ignite in a muffle furnace at 600 ± 25 °C, until the substance is thoroughly charred. Carry out the test for sulfated ash (*2.4.14*) on the residue obtained, starting with "Moisten the substance to be examined...".

Microbial contamination

TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*).

TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

ASSAY

Determine the saponification value (*2.5.6*) on 1.5 g and calculate the percentage content of poly(vinyl acetate) using the following expression:

$$I_s \times 0.1534$$

I_s = saponification value.

STORAGE

At a temperature of 5 °C to 30 °C. Handle the substance so as to minimise microbial contamination.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for poly(vinyl acetate) dispersion 30 per cent used in the manufacture of modified-release dosage forms and to mask taste.

Solubility of a film

Place the film obtained in identification test B in 50 mL of *phosphate buffer solution pH 6.8 R* whilst stirring continuously. The film does not dissolve within 30 min.

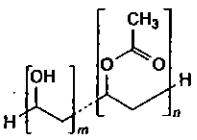
Apparent viscosity (*2.2.10*)

Maximum 100 mPa·s, determined using a rotating viscometer at 20 °C and a shear rate of 100 s^{-1}.

Ph Eur

Poly(vinyl alcohol)

(Ph. Eur. monograph 1961)



Ph Eur

DEFINITION

Poly(vinyl alcohol) is obtained by polymerisation of vinyl acetate, followed by partial or almost complete hydrolysis of poly(vinyl acetate) in the presence of catalytic amounts of alkali or mineral acids.

Poly(vinyl alcohol) polymers comply with the following indices:

$$0 \leq \frac{n}{m} \leq 0.35$$

The mean relative molecular mass lies between 20 000 and 150 000. The viscosity is 3 mPa·s to 70 mPa·s. The ester value, which characterises the degree of hydrolysis, is not greater than 280.

CHARACTERS

Appearance
Yellowish-white powder or translucent granules.

Solubility
Soluble in water, slightly soluble in ethanol, practically insoluble in acetone.

Various grades of poly(vinyl alcohol) are available. They differ in their degree of polymerisation and their degree of hydrolysis which determine the physical properties of the different grades. They are characterised by the viscosity and the ester value of the substance.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison poly(vinyl alcohol) CRS.

The intensities of the absorption bands at about 1720 cm^{-1} and 1260 cm^{-1} are inversely proportional to the degree of hydrolysis.

B. Viscosity (see Tests).

TESTS

Solution S
Heat on a water-bath 250 mL of *water R* in a borosilicate round-bottomed flask attached to a reflux condenser with stirrer, add 10.0 g of the substance to be examined (correcting for the loss on drying) and continue heating for 30 min with continuous stirring. Remove the flask from the water-bath and continue stirring until room temperature is reached.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

pH (*2.2.3*)
4.5 to 6.5 for solution S.

Viscosity (*2.2.49, Method A*)
85 per cent to 115 per cent of the value stated on the label.

Determine the viscosity using a falling ball viscometer immediately after preparation of solution S at 20 ± 0.1 °C.

Acid value
Maximum 3.0.

Add 1 mL of *phenolphthalein solution R* to 50 mL of solution S and titrate with *0.05 M potassium hydroxide* until the pink colour persists for 15 s. Calculate the acid value using the following expression:

$$\frac{2.805V}{2}$$

V = volume of *0.05 M potassium hydroxide* used, in millilitres.

Ester value (*2.5.2*)
90 per cent to 110 per cent of the value stated on the label.

Saponify (*2.5.6*) 1.00 g in a mixture of 25.0 mL of 0.5 M alcoholic potassium hydroxide and 25.0 mL of *water R*.

Loss on drying (*2.2.32*)
Maximum 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulfated ash (*2.4.14*)
Maximum 1.0 per cent, determined on 1.0 g.

LABELLING

The label states:
— the viscosity for a 40 g/L solution;
— the ester value.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for poly(vinyl alcohol) used as viscosity-increasing agent, binder or film former.

Viscosity
(see Tests).

Ester value
(see Tests).

Infrared absorption spectrophotometry
(see Identification A).

Ph Eur

Potassium Acetate

(Ph. Eur. monograph 1139)

$C_2H_3KO_2$ 98.1 *127-08-2*

Action and use
Used in solutions for dialysis.

Preparation
Potassium Acetate Sterile Concentrate

Ph Eur

DEFINITION

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder or colourless crystals, deliquescent.

Solubility
Very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

A. It gives reaction (a) of acetates (*2.3.1*).

B. It gives reaction (a) of potassium (*2.3.1*).

TESTS

Solution S
Dissolve 10.0 g in *distilled water R* and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)
7.5 to 9.0.

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 mL with the same solvent.

Reducing substances
Dilute 10 mL of solution S to 100 mL with *water R*.
Add 5 mL of *dilute sulfuric acid R* and 0.5 mL of a 0.32 g/L solution of *potassium permanganate R*. Mix and boil gently for 5 min. The solution remains pink.

Chlorides (*2.4.4*)
Maximum 200 ppm.

Dilute 2.5 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 200 ppm.

Dilute 7.5 mL of solution S to 15 mL with *distilled water R*.

Aluminium (*2.4.17*)
Maximum 1 ppm, if intended for use in the manufacture of peritoneal dialysis solutions, haemofiltration solutions or haemodialysis solutions.

Prescribed solution Dissolve 2.0 g in 50 mL of *water R* and add 5 mL of *acetate buffer solution pH 6.0 R*.

Reference solution Mix 1 mL of *aluminium standard solution (2 ppm Al) R*, 5 mL of *acetate buffer solution pH 6.0 R* and 49 mL of *water R*.

Blank solution Mix 5 mL of *acetate buffer solution pH 6.0 R* and 50 mL of *water R*.

Iron (*2.4.9*)
Maximum 20 ppm.

Dilute 5 mL of solution S to 10 mL with *water R*.

Sodium
Maximum 0.5 per cent.

Atomic emission spectrometry (*2.2.22, Method II*).

Test solution Dissolve 1.00 g in *water R* and dilute to 100.0 mL with the same solvent.

Reference solutions Prepare the reference solutions using *sodium standard solution (200 ppm Na) R*, diluted as necessary with *water R*.

Wavelength 589 nm.

Loss on drying (*2.2.32*)
Maximum 3.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY
Dissolve 80.0 mg in 20 mL of *anhydrous acetic acid R*.
Add 0.2 mL of *naphtholbenzein solution R*. Titrate with *0.1 M perchloric acid*. Carry out a blank titration.

1 mL of *0.1 M perchloric acid* is equivalent to 9.81 mg of $C_2H_3KO_2$.

STORAGE
In an airtight container.

Ph Eur

Potassium Bicarbonate

(Potassium Hydrogen Carbonate, Ph. Eur. monograph 1141)

$KHCO_3$ 100.1 *298-14-6*

Action and use
Excipient.

Ph Eur

DEFINITION

Content
99.0 per cent to 101.0 per cent.

CHARACTERS

Appearance
White or almost white, crystalline powder or colourless crystals.

Solubility
Freely soluble in water, practically insoluble in ethanol (96 per cent).

When heated in the dry state or in solution, it is gradually converted to potassium carbonate.

IDENTIFICATION
A. To 5 mL of solution S (see Tests) add 0.1 mL of *phenolphthalein solution R*. A pale pink colour is produced. Heat; gas is evolved and the colour becomes red.

B. It gives the reaction of carbonates and bicarbonates (*2.3.1*).

C. 1 mL of solution S gives reaction (b) of potassium (*2.3.1*).

TESTS

Solution S
Dissolve 5.0 g in 90 mL of *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Carbonates
The pH (*2.2.3*) of freshly prepared solution S is not greater than 8.6.

Chlorides (*2.4.4*)
Maximum 150 ppm.

Dilute 7 mL of solution S to 15 mL with *dilute nitric acid R*.

Sulfates (*2.4.13*)
Maximum 150 ppm.

Dilute 10 mL of solution S to 15 mL with *acetic acid R*. Prepare the standard using a mixture of 7.5 mL of *sulfate standard solution (10 ppm SO_4) R* and 7.5 mL of *distilled water R*.

Ammonium (*2.4.1*)
Maximum 20 ppm.

Dilute 10 mL of solution S to 15 mL with *water R*.

Calcium (*2.4.3*)
Maximum 100 ppm.

Dilute 10 mL of solution S to 15 mL with *acetic acid R*. Prepare the standard using a mixture of 5 mL of *calcium standard solution (10 ppm Ca) R* and 10 mL of *distilled water R*.

Iron (*2.4.9*)
Maximum 20 ppm, determined on solution S.

Sodium
Maximum 0.5 per cent.

Atomic emission spectrometry (*2.2.22, Method II*).

Test solution Dissolve 1.00 g in *water R* and dilute to 100.0 mL with the same solvent.

Reference solutions Prepare the reference solutions using *sodium standard solution (200 ppm Na) R*, diluted as necessary with *water R*.

Wavelength 589 nm.

ASSAY
Dissolve 0.800 g in 50 mL of *carbon dioxide-free water R*. Titrate with *1 M hydrochloric acid*, determining the end-point potentiometrically (*2.2.20*). Read the volume added at the 2nd point of inflection, or at the point of inflection if only 1 point is detected.

1 mL of *1 M hydrochloric acid* is equivalent to 0.1001 g of $KHCO_3$.

Ph Eur

Potassium Bromide

(Ph. Eur. monograph 0184)

KBr	119.0	*7758-02-3*

Ph Eur

DEFINITION

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder or colourless crystals.

Solubility
Freely soluble in water and in glycerol, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. It gives reaction (a) of bromides (*2.3.1*).

B. Solution S (see Tests) gives the reactions of potassium (*2.3.1*).

TESTS

Solution S
Dissolve 10.0 g in *carbon dioxide-free water R* and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity
To 10 mL of solution S add 0.1 mL of *bromothymol blue solution R1*. Not more than 0.5 mL of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Bromates
To 10 mL of solution S add 1 mL of *starch solution R*, 0.1 mL of a 100 g/L solution of *potassium iodide R* and 0.25 mL of *0.5 M sulfuric acid* and allow to stand protected from light for 5 min. No blue or violet colour develops.

Chlorides and sulfates
Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 0.400 g of the substance to be examined in 50 mL of *water for chromatography R* and dilute to 100.0 mL with the same solvent.

Test solution (b) Dilute 25.0 mL of test solution (a) to 50.0 mL with *water for chromatography R*.

Reference solution (a) To 25.0 mL of test solution (a) add 1.0 mL of *sulfate standard solution (10 ppm SO_4) R* and 12.0 mL of *chloride standard solution (50 ppm Cl) R* and dilute to 50.0 mL with *water for chromatography R*.

Reference solution (b) Dilute 10 mL of test solution (a) to 100 mL with *water for chromatography R*. To 2 mL of this solution add 8 mL of *chloride standard solution (50 ppm Cl) R* and dilute to 20 mL with *water for chromatography R*.

Blank solution *water for chromatography R*.

Column:
— *size*: *l* = 0.25 m, Ø = 2 mm;
— *stationary phase*: *strongly basic anion-exchange resin for chromatography R2* (13 µm).

Mobile phase Dissolve 0.600 g of *potassium hydroxide R* in *water for chromatography R* and dilute to 1000 mL with the same solvent.

Flow rate 0.4 mL/min.

Detection Conductivity detector equipped with a suitable ion suppressor.

Injection 50 µL of test solution (b), reference solutions (a) and (b) and the blank solution.

Run time 2.5 times the retention time of bromide.

Retention time Chloride = about 5 min; bromide = about 8 min; sulfate = about 16 min.

System suitability Reference solution (b):
— *resolution*: minimum 8.0 between the peaks due to chloride and bromide.

Calculation of percentage contents:
— for chlorides, use the concentration of chloride in reference solution (a); correct the area of the peak due to chloride in the chromatogram obtained with reference solution (a) by subtracting the area of the peak due to chloride in the chromatogram obtained with test solution (b);
— for sulfates, use the concentration of sulfate in reference solution (a); correct the area of the peak due to sulfate in the chromatogram obtained with reference solution (a) by subtracting the area of the peak due to sulfate in the chromatogram obtained with test solution (b).

Limits:
— *chlorides*: maximum 0.6 per cent;
— *sulfates*: maximum 0.01 per cent.

Iodides
To 5 mL of solution S add 0.15 mL of *ferric chloride solution R1* and 2 mL of *methylene chloride R*. Shake and allow to separate. The lower layer is colourless (*2.2.2, Method I*).

Iron (*2.4.9*)
Maximum 20 ppm.

Dilute 5 mL of solution S to 10 mL with *water R*.

Magnesium and alkaline-earth metals (*2.4.7*)
Maximum 200 ppm, calculated as Ca.

10.0 g complies with the test for magnesium and alkaline-earth metals. The volume of *0.01 M sodium edetate* used does not exceed 5.0 mL.

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

ASSAY

Dissolve 100.0 mg in *water R*, add 5 mL of *dilute nitric acid R* and dilute to 50 mL with *water R*. Titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M silver nitrate* is equivalent to 11.90 mg of KBr.

Calculate the percentage content of KBr using the following expression:

$$a - 3.357\ b$$

a = percentage content of KBr and KCl obtained in the assay and calculated as KBr;

b = percentage content of Cl obtained in the test for chlorides.

Ph Eur

Potassium Carbonate

(Ph. Eur. monograph 1557)

K_2CO_3 138.2 *584-08-7*

Action and use

Excipient.

Ph Eur

DEFINITION

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white granular powder, hygroscopic.

Solubility

Freely soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Dissolve 1 g in 10 mL of *water R*. The solution is strongly alkaline (*2.2.4*).

B. 2 mL of the solution prepared for identification test A gives the reaction of carbonates and bicarbonates (*2.3.1*).

C. 1 mL of the solution prepared for identification test A gives reaction (b) of potassium (*2.3.1*).

TESTS

Solution S

Dissolve 10.0 g in 25 mL of *distilled water R*. Slowly add 14 mL of *hydrochloric acid R*. When the effervescence has ceased, boil for a few minutes. Allow to cool and dilute to 50 mL with *distilled water R*.

Appearance of solution

Solution S is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Chlorides (*2.4.4*)

Maximum 100 ppm.

Dissolve 0.50 g in 10 mL of *water R*. Carefully add dropwise 1 mL of *nitric acid R*. Boil. Cool, add 5 mL of *dilute nitric acid R* and dilute to 15 mL with *water R*.

Sulfates (*2.4.13*)

Maximum 100 ppm.

Dilute 7.50 mL of solution S to 15 mL with *distilled water R*.

Calcium (*2.4.3*)

Maximum 100 ppm.

To 5 mL of solution S add 1 mL of *concentrated ammonia R*. Boil. Cool. Dilute to 15 mL with *distilled water R*.

Iron (*2.4.9*)

Maximum 10 ppm.

Dilute 5 mL of solution S to 10 mL with *water R*.

Loss on drying (*2.2.32*)

Maximum 5.0 per cent, determined on 0.300 g by drying in an oven at 120-125 °C for 5 h.

ASSAY

Dissolve 0.500 g in 50 mL of *carbon dioxide-free water R*. Carry out a potentiometric titration (*2.2.20*), using *1 M hydrochloric acid*. Read the volume added at the 2nd point of inflexion.

1 mL of *1 M hydrochloric acid* is equivalent to 69.1 mg of K_2CO_3.

STORAGE

In an airtight container.

Ph Eur

Potassium Chloride

(Ph. Eur. monograph 0185)

KCl 74.5 *7447-40-7*

Action and use

Used in prevention and treatment of potassium deficiency and electrolyte imbalance.

Preparations

Bumetanide and Potassium Prolonged-release Tablets

Oral Rehydration Salts

Magnesium Sulfate, Potassium Chloride and Sodium Chloride Infusion

Potassium Chloride and Glucose Infusion

Potassium Chloride and Sodium Chloride Infusion

Potassium Chloride, Sodium Chloride and Glucose Infusion

Potassium Chloride Oral Solution

Potassium Chloride Sterile Concentrate

Potassium Chloride Prolonged-release Tablets

Ph Eur

DEFINITION

Content

99.0 per cent to 101.0 per cent of KCl (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water, practically insoluble in anhydrous ethanol.

IDENTIFICATION

A. It gives reaction (a) of chlorides (*2.3.1*).

B. Solution S (see Tests) gives the reactions of potassium (*2.3.1*).

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity
To 50 mL of solution S add 0.1 mL of *bromothymol blue solution R1*. Not more than 0.5 mL of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Bromides
Maximum 0.1 per cent.

Dilute 1.0 mL of solution S to 50 mL with *water R*. To 5.0 mL of the solution add 2.0 mL of *phenol red solution R2* and 1.0 mL of *chloramine solution R1* and mix immediately. After exactly 2 min add 0.15 mL of *0.1 M sodium thiosulfate*, mix and dilute to 10.0 mL with *water R*. The absorbance (*2.2.25*) of the solution measured at 590 nm, using *water R* as the compensation liquid, is not greater than that of a standard prepared at the same time and in the same manner using 5 mL of a 3.0 mg/L solution of *potassium bromide R*.

Iodides
Moisten 5 g by the dropwise addition of a freshly prepared mixture of 0.15 mL of *sodium nitrite solution R*, 2 mL of *0.5 M sulfuric acid*, 25 mL of *iodide-free starch solution R* and 25 mL of *water R*. After 5 min, examine in daylight. The substance shows no blue colour.

Sulfates (*2.4.13*)
Maximum 300 ppm.

Dilute 5 mL of solution S to 15 mL with *distilled water R*.

Aluminium (*2.4.17*)
Maximum 1.0 ppm, if intended for use in the manufacture of haemodialysis solutions.

Prescribed solution Dissolve 4 g in 100 mL of *water R* and add 10 mL of *acetate buffer solution pH 6.0 R*.

Reference solution Mix 2 mL of *aluminium standard solution (2 ppm Al) R*, 10 mL of *acetate buffer solution pH 6.0 R* and 98 mL of *water R*.

Blank solution Mix 10 mL of *acetate buffer solution pH 6.0 R* and 100 mL of *water R*.

Iron (*2.4.9*)
Maximum 20 ppm.

Dilute 5 mL of solution S to 10 mL with *water R*.

Magnesium and alkaline-earth metals (*2.4.7*)
Maximum 200 ppm, calculated as Ca and determined on 10.0 g using 0.15 g of *mordant black 11 triturate R*. The volume of *0.01 M sodium edetate* used is not more than 5.0 mL.

Sodium
Maximum 0.1 per cent, if intended for use in the manufacture of parenteral preparations or haemodialysis solutions.

Atomic emission spectrometry (*2.2.22, Method I*).

Test solution Dissolve 1.00 g of the substance to be examined in *water R* and dilute to 100.0 mL with the same solvent.

Reference solutions Prepare the reference solutions by diluting as required a solution containing 200 µg of Na per millilitre, prepared as follows: dissolve in *water R* 0.5084 g of *sodium chloride R*, previously dried at 105 °C for 3 h, and dilute to 1000.0 mL with the same solvent.

Wavelength 589 nm.

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

ASSAY
Dissolve 60.0 mg in *water R*, add 5 mL of *dilute nitric acid R* and dilute to 50 mL with *water R*. Titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (*2.2.20*).

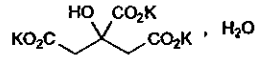
1 mL of *0.1 M silver nitrate* is equivalent to 7.45 mg of KCl.

LABELLING
The label states:
— where applicable, that the substance is suitable for use in the manufacture of parenteral preparations;
— where applicable, that the substance is suitable for use in the manufacture of haemodialysis solutions.

Ph Eur

Potassium Citrate

(Ph. Eur. monograph 0400)



$C_6H_5K_3O_7,H_2O$ 324.4 *6100-05-6*

Action and use
Alkalisation of urine.

Preparation
Potassium Citrate Mixture

Ph Eur

DEFINITION
Tripotassium 2-hydroxypropane-1,2,3-tricarboxylate monohydrate.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white, granular powder or transparent crystals, hygroscopic.

Solubility
Very soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION
A. To 1 mL of solution S (see Tests) add 4 mL of *water R*. The solution gives the reaction of citrates (*2.3.1*).

B. 0.5 mL of solution S gives reaction (b) of potassium (*2.3.1*).

TESTS
Solution S
Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity
To 10 mL of solution S add 0.1 mL of *phenolphthalein solution R*. Not more than 0.2 mL of *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Readily carbonisable substances
To 0.20 g of the powdered substance to be examined add 10 mL of *sulfuric acid R* and heat in a water-bath at 90 ± 1 °C for 60 min. Cool rapidly. The solution is not more intensely coloured than reference solution Y_2 or GY_2 (*2.2.2, Method II*).

Chlorides (*2.4.4*)
Maximum 50 ppm.

Dilute 10 mL of solution S to 15 mL with *water R*.

Oxalates
Maximum 300 ppm.

Dissolve 0.50 g in 4 mL of *water R*, add 3 mL of *hydrochloric acid R* and 1 g of *zinc R* in granules and heat on a water-bath for 1 min. Allow to stand for 2 min, decant the liquid into a test-tube containing 0.25 mL of a 10 g/L solution of *phenylhydrazine hydrochloride R* and heat to boiling. Cool rapidly, transfer to a graduated cylinder and add an equal volume of *hydrochloric acid R* and 0.25 mL of *potassium ferricyanide solution R*. Shake and allow to stand for 30 min. Any pink colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using 4 mL of a 0.05 g/L solution of *oxalic acid R*.

Sulfates (*2.4.13*)
Maximum 150 ppm.

To 10 mL of solution S add 2 mL of *hydrochloric acid R1* and dilute to 15 mL with *distilled water R*.

Sodium
Maximum 0.3 per cent.

Atomic emission spectrometry (*2.2.22, Method II*).

Test solution To 10 mL of solution S add 1 mL of *dilute hydrochloric acid R* and dilute to 100 mL with *distilled water R*.

Reference solutions Prepare the reference solutions using a solution of *sodium chloride R* containing 1 mg of Na per millilitre diluted as necessary with *distilled water R*.

Wavelength 589 nm.

Water (*2.5.12*)
4.0 per cent to 7.0 per cent, determined on 0.250 g. Use a mixture of 1 volume of *formamide R* and 2 volumes of *methanol R* as solvent. After adding the substance to be examined, stir for 15 min before titrating.

ASSAY
Dissolve 0.150 g in 20 mL of *anhydrous acetic acid R*, heating to about 50 °C. Allow to cool. Titrate with *0.1 M perchloric acid* using 0.25 mL of *naphtholbenzein solution R* as indicator until a green colour is obtained.

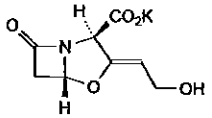
1 mL of *0.1 M perchloric acid* is equivalent to 10.21 mg of $C_6H_5K_3O_7$.

STORAGE
In an airtight container.

Ph Eur

Potassium Clavulanate

(Ph. Eur. monograph 1140)



$C_8H_8KNO_5$ 237.3 *61177-45-5*

Action and use
Beta-lactamase inhibitor, potentiation of the action of amoxicillin and ticarcillin.

Preparations
Co-amoxiclav Injection
Co-amoxiclav Oral Suspension
Co-amoxiclav Tablets
Co-amoxiclav Dispersible Tablets
Ticarcillin and Clavulanic Acid Infusion

Ph Eur

DEFINITION
Potassium (2*R*,3*Z*,5*R*)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylate, the potassium salt of a substance produced by the growth of certain strains of *Streptomyces clavuligerus* or obtained by any other means.

Content
96.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white, crystalline powder, hygroscopic.

Solubility
Freely soluble in water, slightly soluble in ethanol (96 per cent), very slightly soluble in acetone.

PRODUCTION
The methods of production, extraction and purification are such that clavam-2-carboxylate is eliminated or present at a level not exceeding 0.01 per cent.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of potassium clavulanate.

B. It gives reaction (b) of potassium (*2.3.1*).

TESTS
Solution S
Dissolve 0.400 g in *carbon dioxide-free water R* and dilute to 20.0 mL with the same solvent.

pH (*2.2.3*)
5.5 to 8.0.

Dilute 5 mL of solution S to 10 mL with *carbon dioxide-free water R*.

Specific optical rotation (*2.2.7*)
+ 53 to + 63 (anhydrous substance), determined on solution S.

Polymeric impurities and other impurities absorbing at 278 nm
Dissolve 50.0 mg in *0.1 M phosphate buffer solution pH 7.0 R* and dilute to 50.0 mL with the same buffer solution. Measure the absorbance immediately.

The absorbance (*2.2.25*) of the solution determined at 278 nm is not greater than 0.40.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 0.250 g of the substance to be examined in mobile phase A and dilute to 25.0 mL with mobile phase A.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A.

Reference solution (b) Dissolve 10 mg of *lithium clavulanate CRS* and 10 mg of *amoxicillin trihydrate CRS* in mobile phase A and dilute to 100 mL with mobile phase A.

Reference solution (c) Dissolve 2 mg of *potassium clavulanate impurity G CRS* in 20 mL of mobile phase A.

Column:
- *size*: *l* = 0.10 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase:
- *mobile phase A*: 7.8 g/L solution of *sodium dihydrogen phosphate R*, adjusted to pH 4.0 with *phosphoric acid R*;
- *mobile phase B*: a mixture of equal volumes of *methanol R1* and mobile phase A;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 4	100	0
4 - 15	100 → 50	0 → 50
15 - 18	50	50

Flow rate 1 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peak due to impurity G.

Relative retention With reference to clavulanate (retention time = about 3 min): impurity E = about 2.3; impurity G = about 3.6.

System suitability Reference solution (b):
- *resolution*: minimum 13 between the peaks due to clavulanate (1st peak) and amoxicillin (2nd peak).

Limits:
- *impurities E, G*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
- *any other impurity*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent);
- *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Aliphatic amines

Gas chromatography (*2.2.28*).

The method shown below can be used to determine the following aliphatic amines: 1,1-dimethylethylamine; tetramethylethylenediamine; 1,1,3,3-tetramethylbutylamine; *N,N'*-diisopropylethylenediamine; 2,2'-oxybis(*N,N*) dimethylethylamine.

Internal standard solution Dissolve 50 µL of *3-methylpentan-2-one R* in *water R* and dilute to 100.0 mL with the same solvent.

Test solution Weigh 1.00 g of the substance to be examined into a centrifuge tube. Add 5.0 mL of the internal standard solution, 5.0 mL of *dilute sodium hydroxide solution R*, 10.0 mL of *water R*, 5.0 mL of *2-methylpropanol R* and 5 g of *sodium chloride R*. Shake vigorously for 1 min. Centrifuge to separate the layers.

Reference solution Dissolve 80.0 mg of each of the following amines: *1,1-dimethylethylamine R*; *tetramethylethylenediamine R*; *1,1,3,3-tetramethylbutylamine R*; *N,N'-diisopropylethylenediamine R* and *2,2'-oxybis(N,N-dimethylethylamine) R* in *dilute hydrochloric acid R* and dilute to 200.0 mL with the same acid. Introduce 5.0 mL of this solution into a centrifuge tube. Add 5.0 mL of the internal standard solution, 10.0 mL of *dilute sodium hydroxide solution R*, 5.0 mL of *2-methylpropanol R* and 5 g of *sodium chloride R*. Shake vigorously for 1 min. Centrifuge to separate the layers.

Column:
- *material*: fused silica;
- *size*: *l* = 50 m, Ø = 0.53 mm;
- *stationary phase*: *phenyl(5)methyl(95)polysiloxane R* (film thickness 5 µm).

Carrier gas *helium for chromatography R*.

Flow rate 8 mL/min.

Split ratio 1:10.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 7	35
	7 - 10.8	35 → 150
	10.8 - 25.8	150
Injection port		200
Detector		250

Detection Flame ionisation.

Injection 1 µL of the upper layers obtained from the test solution and the reference solution.

Relative retention With reference to 3-methylpentan-2-one (retention time = about 11.4 min): impurity H = about 0.55; impurity J = about 1.07; impurity K = about 1.13; impurity L = about 1.33; impurity M = about 1.57.

Limit:
- *total of aliphatic amines*: maximum 0.2 per cent.

2-Ethylhexanoic acid (*2.4.28*)

Maximum 0.8 per cent.

Water (*2.5.12*)

Maximum 0.5 per cent, determined on 1.00 g.

Bacterial endotoxins (*2.6.14*)

Less than 0.03 IU/mg if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 50.0 mg of the substance to be examined in a 4.1 g/L solution of *sodium acetate R* previously

adjusted to pH 6.0 with *glacial acetic acid R*, and dilute to 50.0 mL with the same solution.

Reference solution (a) Dissolve 50.0 mg of *lithium clavulanate CRS* in a 4.1 g/L solution of *sodium acetate R* previously adjusted to pH 6.0 with *glacial acetic acid R* and dilute to 50.0 mL with the same solution.

Reference solution (b) Dissolve 10 mg of *amoxicillin trihydrate CRS* in 10 mL of reference solution (a).

Column:
— *size*: *l* = 0.3 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (10 µm).

Mobile phase Mix 5 volumes of *methanol R1* and 95 volumes of a 15 g/L solution of *sodium dihydrogen phosphate R* previously adjusted to pH 4.0 with *dilute phosphoric acid R*.

Flow rate 1 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 10 µL.

System suitability Reference solution (b):
— *resolution*: minimum 3.5 between the peaks due to clavulanate (1st peak) and amoxicillin (2nd peak).

1 mg of clavulanate ($C_8H_9NO_5$) is equivalent to 1.191 mg of $C_8H_8KNO_5$.

STORAGE

In an airtight container, at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-evident container.

IMPURITIES

Test for related substances

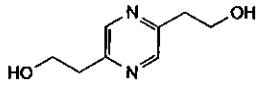
A, B, C, D, E, F, G.

Test for aliphatic amines

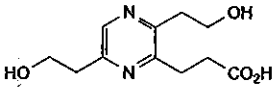
H, J, K, L, M.

Specified impurities *E, G, H, J, K, L, M.*

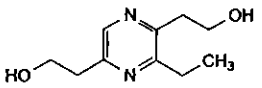
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, F.*



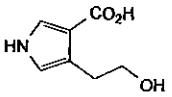
A. 2,2′-(pyrazine-2,5-diyl)diethanol,



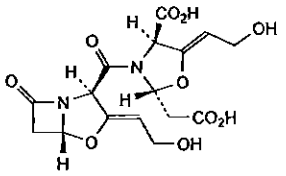
B. 3-[3,6-bis(2-hydroxyethyl)pyrazin-2-yl]propanoic acid,



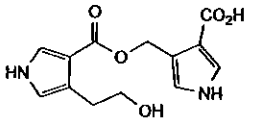
C. 2,2′-(3-ethylpyrazine-2,5-diyl)diethanol,



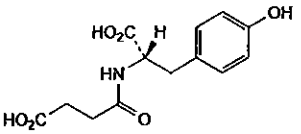
D. 4-(2-hydroxyethyl)-1*H*-pyrrole-3-carboxylic acid,



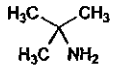
E. (2*R*,4*R*,5*Z*)-2-(carboxymethyl)-5-(2-hydroxyethylidene)-3-[[(2*R*,3*Z*,5*R*)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]oxazolidine-4-carboxylic acid,



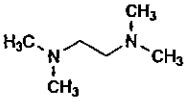
F. 4-[[[[4-(2-hydroxyethyl)-1*H*-pyrrol-3-yl]carbonyl]oxy]methyl]-1*H*-pyrrole-3-carboxylic acid,



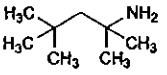
G. 4-[[(1*S*)-1-carboxy-2-(4-hydroxyphenyl)ethyl]amino]-4-oxobutanoic acid (*N*-(hydrogensuccinyl)tyrosine),



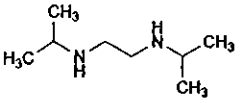
H. 2-methylpropan-2-amine (2-amino-2-methylpropane, *tert*-butylamine, 1,1-dimethylethylamine),



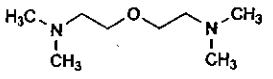
J. *N*,*N*,*N*′,*N*′-tetramethylethane-1,2-diamine (1,2-bis(dimethylamino)ethane, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine),



K. 2,4,4-trimethylpentan-2-amine (2-amino-2,4,4-trimethylpentane, 1,1,3,3-tetramethylbutylamine),



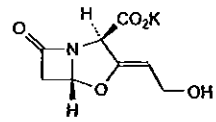
L. *N*,*N*′-diisopropylethane-1,2-diamine (*N*,*N*′-bis(1-methylethyl)ethane-1,2-diamine),



M. 2,2′-oxybis(*N*,*N*-dimethylethanamine) (bis[2-(dimethylamino)ethyl] ether, *N*,*N*,*N*′,*N*′-tetramethyl(oxydiethylene)diamine).

Diluted Potassium Clavulanate

(Ph. Eur. monograph 1653)



$C_8H_8KNO_5$ 237.3

Action and use
Beta-lactamase inhibitor, potentiation of the action of amoxicillin and ticarcillin.

Preparations
Co-amoxiclav Oral Suspension
Co-amoxiclav Tablets
Co-amoxiclav Dispersible Tablets

Ph Eur

DEFINITION

Dry mixture of *Potassium clavulanate (1140)* and *Cellulose, microcrystalline (0316)* or *Silica, colloidal anhydrous (0434)* or *Silica, colloidal hydrated (0738)*.

Content
91.2 per cent to 107.1 per cent of the content of potassium clavulanate stated on the label.

CHARACTERS

Appearance of diluted potassium clavulanate White or almost white powder, hygroscopic.

Solubility of potassium clavulanate Freely soluble in water, slightly soluble in ethanol (96 per cent), very slightly soluble in acetone.

The solubility of the diluted product depends on the diluent and its concentration.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

B. It gives reaction (b) of potassium *(2.3.1)*.

C. Depending on the diluent used, carry out the corresponding identification test (a) or (b).

(a) A quantity of the substance to be examined, corresponding to 20 mg of cellulose, when placed on a watch-glass and dispersed in 4 mL of *iodinated zinc chloride solution R*, becomes violet-blue.

(b) It gives the reaction of silicates *(2.3.1)*.

TESTS

pH *(2.2.3)*
4.8 to 8.0.

Suspend a quantity of the substance to be examined corresponding to 0.200 g of potassium clavulanate in 20 mL of *carbon dioxide-free water R*.

Polymeric impurities and other impurities absorbing at 278 nm
Disperse a quantity of the substance to be examined corresponding to 50.0 mg of potassium clavulanate in 10 mL of *0.1 M phosphate buffer solution pH 7.0 R*, dilute to 50.0 mL with the same buffer solution and filter. Measure the absorbance immediately.

The absorbance *(2.2.25)* of the solution determined at 278 nm is not greater than 0.40.

Related substances
Liquid chromatography *(2.2.29)*. *Prepare the solutions immediately before use.*

Test solution Disperse a quantity of the substance to be examined corresponding to 0.250 g of potassium clavulanate in 5 mL of mobile phase A, dilute to 25.0 mL with mobile phase A and filter.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A.

Reference solution (b) Dissolve 10 mg of *amoxicillin trihydrate CRS* in 1 mL of the test solution and dilute to 100 mL with mobile phase A.

Reference solution (c) 2 mg of *potassium clavulanate impurity G CRS* in 20 mL of mobile phase A.

Column:
— *size: l* = 0.10 m, Ø = 4.6 mm;
— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature:* 40 °C.

Mobile phase:
— *mobile phase A*: 7.8 g/L solution of *sodium dihydrogen phosphate R* adjusted to pH 4.0 with *dilute phosphoric acid R*;
— *mobile phase B*: a mixture of equal volumes of mobile phase A and *methanol R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 4	100	0
4 - 15	100 → 50	0 → 50
15 - 18	50	50

Flow rate 1 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peak due to impurity G.

Relative retention With reference to clavulanate (retention time = about 3 min): impurity E = about 2.3; impurity G = about 3.6.

System suitability Reference solution (b):
— *resolution*: minimum 13 between the peaks due to clavulanate (1st peak) and amoxicillin (2nd peak).

Limits:
— *impurities E, G*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
— *any other impurity*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent);
— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water *(2.5.12)*
Maximum 2.5 per cent, determined on 1.000 g.

ASSAY

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Disperse a quantity of the substance to be examined corresponding to 50.0 mg of potassium clavulanate in a 4.1 g/L solution of *sodium acetate R* previously adjusted to pH 6.0 with *glacial acetic acid R*, dilute to 50.0 mL with the same solution and filter.

Reference solution (a) Dissolve 50.0 mg of *lithium clavulanate CRS* in a 4.1 g/L solution of *sodium acetate R* previously adjusted to pH 6.0 with *glacial acetic acid R* and dilute to 50.0 mL with the same solution.

Reference solution (b) Dissolve 10 mg of *amoxicillin trihydrate CRS* in 10 mL of reference solution (a).

Column:
- *size*: *l* = 0.3 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (10 µm).

Mobile phase Mix 5 volumes of *methanol R1* and 95 volumes of a 15 g/L solution of *sodium dihydrogen phosphate R* previously adjusted to pH 4.0 with *dilute phosphoric acid R*.

Flow rate 1 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 10 µL.

System suitability Reference solution (b):
- *resolution*: minimum 3.5 between the peaks due to clavulanate (1st peak) and amoxicillin (2nd peak).

1 mg of $C_8H_9NO_5$ is equivalent to 1.191 mg of $C_8H_8KNO_5$.

STORAGE

In an airtight container.

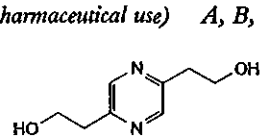
LABELLING

The label states the percentage content of potassium clavulanate and the diluent used to prepare the mixture.

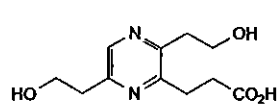
IMPURITIES

Specified impurities *E, G.*

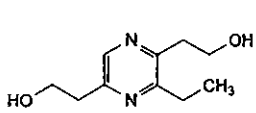
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, F.*



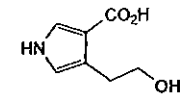
A. 2,2′-(pyrazine-2,5-diyl)diethanol,



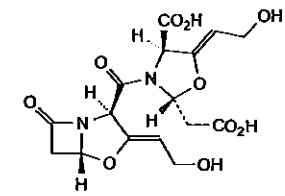
B. 3-[3,6-bis(2-hydroxyethyl)pyrazin-2-yl]propanoic acid,



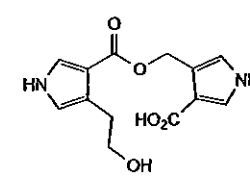
C. 2,2′-(3-ethylpyrazine-2,5-diyl)diethanol,



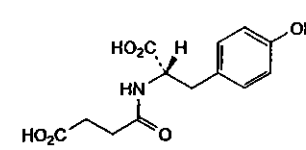
D. 4-(2-hydroxyethyl)-1*H*-pyrrole-3-carboxylic acid,



E. (2*R*,4*R*,5*Z*)-2-(carboxymethyl)-5-(2-hydroxyethylidene)-3-[[(2*R*,3*Z*,5*R*)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]oxazolidine-4-carboxylic acid,



F. 4-[[[[4-(2-hydroxyethyl)-1*H*-pyrrol-3-yl]carbonyl]oxy]methyl]-1*H*-pyrrole-3-carboxylic acid,



G. 4-[[(1*S*)-1-carboxy-2-(4-hydroxyphenyl)ethyl]amino]-4-oxobutanoic acid (*N*-(hydrogensuccinyl)tyrosine).

Ph Eur

Potassium Dihydrogen Phosphate

(Ph. Eur. monograph 0920)

KH_2PO_4 136.1 *7778-77-0*

Preparations

Potassium Dihydrogen Phosphate Sterile Concentrate

Potassium Dihydrogen Phosphate Oral Solution

Ph Eur

DEFINITION

Content

98.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Solution S (see Tests) is slightly acid (*2.2.4*).

B. Solution S gives reaction (b) of phosphates (*2.3.1*).

C. 0.5 mL of solution S gives reaction (b) of potassium (*2.3.1*).

TESTS

Solution S
Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)
4.2 to 4.5.

To 5 mL of solution S add 5 mL of *carbon dioxide-free water R*.

Reducing substances
To 5 mL of solution S add 5 mL of *dilute sulfuric acid R* and 0.25 mL of *0.02 M potassium permanganate*. Heat on a water-bath for 5 min. The colour of the permanganate is not completely discharged.

Chlorides (*2.4.4*)
Maximum 200 ppm.

Dilute 2.5 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 300 ppm.

To 5 mL of solution S add 0.5 mL of *hydrochloric acid R* and dilute to 15 mL with *distilled water R*.

Arsenic (*2.4.2, Method A*)
Maximum 2 ppm, determined on 0.5 g.

Iron (*2.4.9*)
Maximum 10 ppm, determined on solution S.

Sodium
Maximum 0.1 per cent, if intended for use in the manufacture of parenteral preparations.

Atomic emission spectrometry (*2.2.22, Method I*).

Test solution Dissolve 1.00 g of the substance to be examined in *water R* and dilute to 100.0 mL with the same solvent.

Reference solutions Prepare the reference solutions using the following solution, diluted as necessary with *water R*: dissolve 0.5084 g of *sodium chloride R*, previously dried at 100-105 °C for 3 h, in *water R* and dilute to 1000.0 mL with the same solvent (200 µg of Na per millilitre).

Wavelength 589 nm.

Loss on drying (*2.2.32*)
Maximum 2.0 per cent, determined on 1.000 g by drying in an oven at 125-130 °C.

ASSAY
Dissolve 1.000 g in 50 mL of *carbon dioxide-free water R*. Titrate with carbonate-free *1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

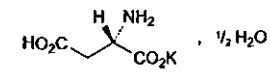
1 mL of *1 M sodium hydroxide* is equivalent to 0.1361 g of KH_2PO_4.

LABELLING
The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

Ph Eur

Potassium Hydrogen Aspartate Hemihydrate

(Ph. Eur. monograph 2076)



$C_4H_6KNO_4,½H_2O$ 180.2

Action and use
Excipient.

Ph Eur

DEFINITION
Potassium hydrogen (2*S*)-2-aminobutanedioate hemihydrate.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, powder or crystalline powder, or colourless crystals.

Solubility
Very soluble in water, practically insoluble in alcohol and in methylene chloride.

IDENTIFICATION
A. Specific optical rotation (see Tests).

B. Examine the chromatograms obtained in the test for ninhydrin-positive substances.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. It gives reaction (b) of potassium (*2.3.1*).

TESTS

Solution S
Dissolve 2.5 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)
6.0 to 7.5 for solution S.

Specific optical rotation (*2.2.7*)
+ 18.0 to + 20.5 (anhydrous substance).

Dissolve 0.50 g in a mixture of equal volumes of *hydrochloric acid R* and *water R* and dilute to 25.0 mL with the same mixture of solvents.

Ninhydrin-positive substances
Thin-layer chromatography (*2.2.27*).

Test solution (a) Solution S.

Test solution (b) Dilute 1.0 mL of solution S to 10.0 mL with *water R*.

Reference solution (a) Dissolve 25 mg of *potassium hydrogen aspartate hemihydrate CRS* in *water R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of test solution (b) to 20.0 mL with *water R*.

Reference solution (c) Dissolve 10 mg of *glutamic acid CRS* and 10 mg of the substance to be examined in *water R* and dilute to 25 mL with the same solvent.

Plate *TLC silica gel plate R*.

Mobile phase *glacial acetic acid R, water R, butanol R* (20:20:60 *V/V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *ninhydrin solution R* and heat at 100-105 °C for 15 min.

System suitability Reference solution (c):
— the chromatogram shows 2 clearly separated principal spots.

Limits Test solution (a):
— *any impurity*: any spot, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Chlorides (*2.4.4*)
Maximum 200 ppm.

To 10 mL of solution S add 5 mL of *water R*.

Sulfates (*2.4.13*)
Maximum 500 ppm.

To 12 mL of solution S add 3 mL of *distilled water R*.

Ammonium (*2.4.1, Method B*)
Maximum 200 ppm, determined on 50 mg.

Prepare the standard using 0.1 mL of *ammonium standard solution (100 ppm NH_4) R*.

Iron (*2.4.9*)
Maximum 30 ppm.

In a separating funnel, dissolve 0.33 g in 10 mL of *dilute hydrochloric acid R*. Shake with 3 quantities, each of 10 mL, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 mL of *water R* and shake for 3 min. The aqueous layer complies with the limit test for iron.

Water (*2.5.12*)
4.0 per cent to 6.0 per cent, determined on 0.200 g.

Dissolve the substance to be examined in 10 mL of *formamide R1* and add 10 mL of *anhydrous methanol R*.

ASSAY
Dissolve 70.0 mg in 5 mL of *anhydrous formic acid R*, add 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

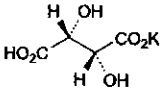
1 mL of *0.1 M perchloric acid* is equivalent to 8.56 mg of $C_4H_6KNO_4$.

Ph Eur

Potassium Hydrogen Tartrate

Cream of Tartar

(Ph. Eur. monograph 1984)



$C_4H_5KO_6$ 188.2 *868-14-4*

Action and use
Excipient.

Ph Eur

DEFINITION
Potassium (2*R*,3*R*)-3-carboxy-2,3-dihydroxypropanoate.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder or colourless crystals.

Solubility
Slightly soluble in water, practically insoluble in ethanol (96 per cent). It dissolves in dilute solutions of mineral acids and alkali hydroxides.

IDENTIFICATION
A. Specific optical rotation (see Tests).

B. Suspend 0.5 g in 50 mL of *water R* and boil until dissolution is complete. Allow to cool (solution A). To 5 mL of solution A, add 0.1 mL of *methyl red solution R*.
The solution is red.

C. Solution A gives reaction (a) of tartrates (*2.3.1*).

D. Solution A gives reaction (b) of potassium (*2.3.1*).

TESTS

Specific optical rotation (*2.2.7*)
+ 8.0 to + 9.2 (dried substance).

Dissolve 2.50 g in 20 mL of *1 M hydrochloric acid* with heating. Allow to cool. Dilute to 25.0 mL with *water R*.

Oxalic acid
Maximum 500 ppm.

Dissolve 0.43 g in 4 mL of *water R*. Add 3 mL of *hydrochloric acid R* and 1 g of *zinc R* in granules and boil for 1 min. Allow to stand for 2 min. Collect the liquid in a test-tube containing 0.25 mL of a 10 g/L solution of *phenylhydrazine hydrochloride R* and heat to boiling. Cool rapidly, transfer to a graduated cylinder and add an equal volume of *hydrochloric acid R* and 0.25 mL of a 50 g/L solution of *potassium ferricyanide R*. Shake and allow to stand for 30 min. Any pink colour in the solution is not more intense than that in a standard prepared at the same time in the same manner using a mixture of 1 mL of *water R* and 3 mL of a 0.1 g/L solution of *oxalic acid R*.

Chlorides (*2.4.4*)
Maximum 500 ppm.

Dissolve 1.0 g with heating in a mixture of 3 mL of *dilute nitric acid R* and 50 mL of *water R*. Dilute to 100 mL with *water R*. Dilute 10 mL of the solution to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 500 ppm.

Suspend 0.30 g in 3.0 mL of *dilute hydrochloric acid R* and dilute to 15 mL with *distilled water R*. Heat until dissolution is complete.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY
Dissolve 0.170 g in 100 mL of *water R* at 100 °C. Titrate the hot solution with *0.1 M sodium hydroxide*, using 0.3 mL of *phenolphthalein solution R* as indicator.

1 mL of *0.1 M sodium hydroxide* is equivalent to 18.82 mg of $C_4H_5KO_6$.

Ph Eur

Potassium Hydroxide

Caustic Potash

(Ph. Eur. monograph 0840)

KOH 56.11 *1310-58-3*

Preparations
Potassium Ascorbate Eye Drops
Potassium Hydroxide Solution

Ph Eur

DEFINITION

Content
85.0 per cent to 100.5 per cent of total alkali, calculated as KOH.

CHARACTERS

Appearance
White or almost white, crystalline, hard masses, supplied as sticks, pellets or irregularly shaped pieces, deliquescent, absorbing carbon dioxide.

Solubility
Very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

A. pH (*2.2.3*): minimum 10.5.

Dissolve 0.1 g in 10 mL of *water R* (solution A used for identification test B). Dilute 1 mL of this solution to 100 mL with *water R*.

B. 1 mL of solution A prepared in identification test A gives reaction (b) of potassium (*2.3.1*).

TESTS

Solution S1
Dissolve 2.5 g in 10 mL of *water R*. Carefully add 2 mL of *nitric acid R* while cooling, and dilute to 25 mL with *dilute nitric acid R*.

Solution S2
Dissolve 10 g in 15 mL of *distilled water R*. Carefully add 12 mL of *hydrochloric acid R* while cooling, and dilute to 50 mL with *dilute hydrochloric acid R*.

Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 5 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Carbonates
Maximum 2.0 per cent, calculated as K_2CO_3 as determined in the assay.

Chlorides (*2.4.4*)
Maximum 200 ppm.

Dilute 2.5 mL of solution S1 to 15 mL with *water R*.

Phosphates (*2.4.11*)
Maximum 100 ppm.

Dilute 1 mL of solution S1 to 100 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 200 ppm.

Dilute 5 mL of solution S2 to 20 mL with *distilled water R*.

Aluminium (*2.4.17*)
Maximum 0.2 ppm, if intended for use in the manufacture of haemodialysis solutions.

Prescribed solution Dissolve 20 g in 100 mL of *water R* and add 10 mL of *acetate buffer solution pH 6.0 R*.

Reference solution Mix 2 mL of *aluminium standard solution (2 ppm Al) R*, 10 mL of *acetate buffer solution pH 6.0 R* and 98 mL of *water R*.

Blank solution Mix 10 mL of *acetate buffer solution pH 6.0 R* and 100 mL of *water R*.

Iron (*2.4.9*)
Maximum 10 ppm.

Dilute 5 mL of solution S2 to 10 mL with *water R*.

Sodium
Maximum 1.0 per cent.

Atomic absorption spectrometry (*2.2.23, Method II*).

Test solution Dissolve 1.00 g in 50 mL of *water R*, add 5 mL of *sulfuric acid R* and dilute to 100.0 mL with *water R*. Dilute 1.0 mL of this solution to 10.0 mL with *water R*.

Reference solutions Prepare the reference solutions using *sodium standard solution (200 ppm Na) R*, diluting with *water R*.

Source Sodium hollow-cathode lamp.

Wavelength 589 nm.

Atomisation device Air-acetylene flame.

ASSAY
Dissolve 2.000 g in 25 mL of *carbon dioxide-free water R*. Add 25 mL of freshly prepared *barium chloride solution R1* and 0.3 mL of *phenolphthalein solution R*. Add slowly, while shaking, 25.0 mL of *1 M hydrochloric acid* and continue the titration with *1 M hydrochloric acid* until the colour changes from pink to colourless. Add 0.3 mL of *bromophenol blue solution R* and continue the titration with *1 M hydrochloric acid* until the colour changes from violet-blue to yellow.

1 mL of *1 M hydrochloric acid* used in the 2nd part of the titration is equivalent to 69.11 mg of K_2CO_3.

1 mL of *1 M hydrochloric acid* used in the combined titrations is equivalent to 56.11 mg of total alkali, calculated as KOH.

STORAGE
In an airtight, non-metallic container.

LABELLING
The label states, where applicable, that the substance is suitable for use in the manufacture of haemodialysis solutions.

Ph Eur

Potassium Hydroxyquinoline Sulfate

Potassium Hydroxyquinoline Sulphate

Action and use
Used in treatment of acne.

Preparation
Potassium Hydroxyquinoline Sulfate and Benzoyl Peroxide Cream.

DEFINITION
Potassium Hydroxyquinoline Sulfate is an equimolecular mixture of quinolin-8-ol sulfate monohydrate, $(C_9H_7NO)_2$, H_2SO_4,H_2O, and potassium sulfate, K_2SO_4. It contains not less than 50.6% and not more than 52.6% of quinolin-8-ol, C_9H_7NO, and not less than 29.5% and not more than 32.5% of K_2SO_4, calculated with reference to the anhydrous substance.

CHARACTERISTICS

A pale yellow, microcrystalline powder. It partly liquefies between 172° and 184°.

Freely soluble in *water*; insoluble in *ether*. On extraction with hot *absolute ethanol* a residue of potassium sulfate and a solution of quinolin-8-ol sulfate are obtained.

IDENTIFICATION

A. To 5 mL of a 5% w/v solution add drop wise, with shaking, 5M *sodium hydroxide* until a heavy precipitate is produced. Filter, wash with *water* and dry at a pressure not exceeding 0.7 kPa for 3 hours. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of quinolin-8-ol *(RS 310)*.

B. To 5 mL of a 5% w/v solution add 0.5 mL of *iron(III) chloride solution R1*. A dark green colour is produced.

C. Yields reaction A characteristic of *potassium salts*, Appendix VI.

D. Yields reaction A characteristic of *sulfates*, Appendix VI.

TESTS

Water

Not more than 5.0% w/w, Appendix IX C. Use 0.5 g.

ASSAY

For quinolin-8-ol

Dissolve 0.35 g in 50 mL of *water* and 20 mL of *hydrochloric acid*, add 50 mL of *0.05M bromine VS*, stopper the flask and shake for 15 minutes. Allow to stand for 15 minutes, add 80 mL of *water* and 10 mL of *dilute potassium iodide solution* and titrate with 0.1M *sodium thiosulfate VS* using *starch mucilage*, added towards the end of the titration, as indicator. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of bromine required. Each mL of *0.05M bromine VS* is equivalent to 3.629 mg of C_9H_7NO.

For potassium sulfate

Prepare a solution of suitable concentration with *water*. Carry out the method for *atomic emission spectrophotometry*, Appendix II D, measuring at 766.5 nm and using *potassium standard solution (600 ppm K)*, suitably diluted with *water*, to prepare the standard solutions. Each mg of potassium is equivalent to 2.2284 mg of K_2SO_4.

Potassium Iodate

KIO_3 214.0 *7758-05-6*

Action and use

Iodine supplement for emergency use.

Preparation

Potassium Iodate Tablets

DEFINITION

Potassium Iodate contains not less than 99.0% and not more than 101.0% of KIO_3, calculated with reference to the dried substance.

CHARACTERISTICS

A white crystalline powder.

Slowly soluble in water; insoluble in ethanol (96%).

Dissolve 10 g of the substance being examined in sufficient water to produce 200 mL (solution S1).

Add 25 mL of *hydrochloric acid* to 6 g of the substance being examined, evaporate to dryness and repeat. Heat until iodine is removed. Dissolve the residue in 2.5 mL of a 25% v/v solution of *hydrochloric acid* and dilute to 50 mL with *water* (solution S2).

IDENTIFICATION

A. 1 mL of solution S1 yields reaction B characteristic of *potassium salts*, Appendix VI.

B. Dissolve 0.1 g in 5 mL of *water*. Add 1 mL of *silver nitrate solution* followed by 1 mL of *sulfur dioxide solution*. A yellow precipitate is produced immediately.

TESTS

Acidity or alkalinity

pH of solution S1, 5.0 to 8.0, Appendix V L.

Clarity and colour of solution

Solution S1 is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method II.

Chloride, chlorate, bromide, bromate

Dilute 5 mL of solution S1 to 15 mL with *water*, add 20 mL of *sulfur dioxide solution* and heat on a water bath for 30 minutes. Heat to boiling, cool, add 10 mL of 18M *ammonia* and 20 mL of *silver nitrate solution R2* and dilute to 70 mL with *water*. Filter, transfer 35 mL of the filtrate to a *Nessler cylinder* and acidify with 6 mL of *nitric acid*. After 5 minutes, any opalescence, when viewed vertically, is not greater than that produced by treating 5 mL of a 0.00165% w/v solution of *sodium chloride* at the same time and in the same manner (0.02%).

Iodide

Add 1 mL of 1.8M *sulfuric acid* to 25 mL of solution S1 and shake with 1 mL of *chloroform*. Any violet colour produced is not more intense than that of a solution prepared at the same time and in the same manner but using 5 mL of solution S1 and 2 mL of *iodide standard solution (10 ppm I)* (20 ppm).

Sulfate

Add 1 mL of a 25% w/v solution of *barium chloride* to 1.5 mL of *sulfate standard solution (10 ppm SO_4)*, shake and allow to stand for 1 minute. Add 12.5 mL of solution S2 diluted to 15 mL with *distilled water* and 0.5 mL of 5M *acetic acid* and allow to stand for 5 minutes. Any opalescence produced is not more intense than that of a standard prepared in the same manner but using 7.5 mL of *sulfate standard solution (10 ppm SO_4)* diluted to 15 mL with *distilled water* in place of the solution being examined (50 ppm).

Loss on drying

When dried at 130° for 1 hour, loses not more than 0.5% of its weight. Use 1 g.

ASSAY

To 1.5 g add sufficient *water* to produce 250 mL. To 25 mL of the resulting solution in an iodine flask add 3 g of *potassium iodide*, 100 mL of *water* and 10 mL of *hydrochloric acid*. Close the flask and stand in the dark for 5 minutes. Titrate the solution with 0.1M *sodium thiosulfate VS* to a light straw colour and then complete the titration to a colourless end point using *starch mucilage* as indicator. Each mL of 0.1M *sodium thiosulfate VS* is equivalent to 3.567 mg of KIO_3.

Potassium Iodide

(Ph. Eur. monograph 0186)

KI 166.0 *7681-11-0*

Action and use
Antithyroid.

Ph Eur

DEFINITION
Content
99.0 per cent to 100.5 per cent (dried substance).

CHARACTERS
Appearance
White or almost white powder or colourless crystals.

Solubility
Very soluble in water, freely soluble in glycerol, soluble in ethanol (96 per cent).

IDENTIFICATION
A. Solution S (see Tests) gives the reactions of iodides (*2.3.1*).

B. Solution S gives the reactions of potassium (*2.3.1*).

TESTS
Solution S
Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Alkalinity
To 12.5 mL of solution S add 0.1 mL of *bromothymol blue solution R1*. Not more than 0.5 mL of *0.01 M hydrochloric acid* is required to change the colour of the indicator.

Iodates
To 10 mL of solution S add 0.25 mL of *iodide-free starch solution R* and 0.2 mL of *dilute sulfuric acid R* and allow to stand protected from light for 2 min. No blue colour develops.

Sulfates (*2.4.13*)
Maximum 150 ppm.

Dilute 10 mL of solution S to 15 mL with *distilled water R*.

Thiosulfates
To 10 mL of solution S add 0.1 mL of *starch solution R* and 0.1 mL of 0.005 M iodine. A blue colour is produced.

Iron (*2.4.9*)
Maximum 20 ppm.

Dilute 5 mL of solution S to 10 mL with *water R*.

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.00 g of previously powdered substance by drying in an oven at 105 °C for 3 h.

ASSAY
Dissolve 1.500 g in *water R* and dilute to 100.0 mL with the same solvent. To 20.0 mL of the solution add 40 mL of *hydrochloric acid R* and titrate with *0.05 M potassium iodate* until the colour changes from red to yellow. Add 5 mL of *chloroform R* and continue the titration, shaking vigorously, until the chloroform layer is decolourised.

1 mL of *0.05 M potassium iodate* is equivalent to 16.60 mg of KI.

STORAGE
Protected from light.

Ph Eur

Potassium Metabisulfite

Potassium Metabisulphite

(Ph. Eur. monograph 2075)

$K_2S_2O_5$ 222.3 *16731-55-8*

Action and use
Preservative.

Ph Eur

DEFINITION
Potassium metabisulfite (potassium disulfite).

Content
95.0 per cent to 101.0 per cent.

CHARACTERS
Appearance
White or almost white powder, or colourless crystals.

Solubility
Freely soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION
A. pH (see Tests).

B. To 5 mL of solution S (see Tests), add 0.5 mL of *0.05 M iodine*. The mixture is colourless and gives reaction (a) of sulfates (*2.3.1*).

C. Solution S gives reaction (a) of potassium (*2.3.1*).

TESTS
Solution S
Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method I*).

pH (*2.2.3*)
3.0 to 4.5 for solution S.

Thiosulfates
To 2.00 g add 25 mL of a 42.5 g/L solution of *sodium hydroxide R* and 75 mL of *water R*. Shake until dissolved and add 10 mL of *formaldehyde R* and 10 mL of *acetic acid R*. After 5 min, titrate with *0.05 M iodine* using 1 mL of *starch solution R*. Carry out a blank titration. The difference between the volumes consumed in the 2 titrations is not more than 0.15 mL.

Iron
Maximum 10 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Dilute 20 mL of solution S to 50 mL with *water R*.

Reference solutions Prepare the reference solutions using *iron standard solution (20 ppm Fe) R*, diluted as necessary with *water R*.

Source Iron hollow-cathode lamp.

Wavelength 248.3 nm.

Atomisation device Air-acetylene flame.

Selenium
Maximum 10 ppm.

To 3.0 g add 10 mL of *formaldehyde R*. Carefully add 2 mL of *hydrochloric acid R* in small portions. Heat on a water-bath for 20 min. Any pink colour in the solution is not more intense than that of a reference solution prepared at the same time in the same manner using 1.0 g of the substance to be

examined to which 0.2 mL of *selenium standard solution (100 ppm Se) R* has been added.

Zinc
Maximum 25 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Dilute 20 mL of solution S to 50 mL with *water R*.

Reference solutions Prepare the reference solutions using *zinc standard solution (100 ppm Zn) R*, diluted as necessary with *water R*.

Source Zinc hollow-cathode lamp.

Wavelength 213.9 nm.

Atomisation device Air-acetylene flame.

ASSAY
In a 500 mL conical flask containing 50.0 mL of *0.05 M iodine* introduce 0.150 g and add 5 mL of *hydrochloric acid R*. Titrate the excess of iodine with *0.1 M sodium thiosulfate* using 0.1 mL of *starch solution R*, added towards the end of the titration, as indicator.

1 mL of *0.05 M iodine* is equivalent to 5.558 mg of $K_2S_2O_5$.

STORAGE
In an airtight container, protected from light.

Ph Eur

Potassium Nitrate

(Ph. Eur. monograph 1465)

KNO_3 101.1 *7757-79-1*

Ph Eur

DEFINITION
Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder or colourless crystals.

Solubility
Freely soluble in water, very soluble in boiling water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION
A. It gives the reaction of nitrates (*2.3.1*).

B. Solution S (see Tests) gives the reactions of potassium (*2.3.1*).

TESTS
Solution S
Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity
To 10 mL of solution S add 0.05 mL of *bromothymol blue solution R1*. Not more than 0.5 mL of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Reducible substances
To 10 mL of solution S, add 0.5 mL of *dilute sulfuric acid R* and 2 mL of *zinc iodide and starch solution R*. The solution does not become blue within 2 min.

Chlorides (*2.4.4*)
Maximum 20 ppm, if intended for ophthalmic use.

Dissolve 2.5 g in *water R* and dilute to 15 mL with the same solvent.

Sulfates (*2.4.13*)
Maximum 150 ppm.

Dilute 10 mL of solution S to 15 mL with *distilled water R*.

Ammonium (*2.4.1*)
Maximum 100 ppm, determined in 1 mL of solution S; maximum 50 ppm if intended for ophthalmic use.

Calcium (*2.4.3*)
Maximum 100 ppm; maximum 50 ppm if intended for ophthalmic use.

Dilute 10 mL of solution S to 15 mL with *distilled water R*.

Iron (*2.4.9*)
Maximum 20 ppm; maximum 10 ppm if intended for ophthalmic use.

Dilute 5 mL of solution S to 10 mL with *water R*.

Sodium
Maximum 0.1 per cent.

Atomic emission spectrometry (*2.2.22, Method II*).

Test solution Dissolve 1.00 g in *water R* and dilute to 100.0 mL with the same solvent.

Reference solutions Prepare the reference solutions using *sodium standard solution (200 ppm Na) R*, diluting with *water R*.

Wavelength 589 nm.

Loss on drying
(*2.2.32*)Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY
Prepare a chromatography column 0.3 m long and 10 mm in internal diameter and filled with 10 g of *strongly acidic ion-exchange resin R* covered with *carbon dioxide-free water R*. Maintain a 1 cm layer of liquid above the resin at all times. Allow 100 mL of *dilute hydrochloric acid R* to run through the column at a flow rate of about 5 mL/min. Wash the column (with the tap completely open) with *carbon dioxide-free water R* until neutral to *blue litmus paper R*. Dissolve 0.200 g of the substance to be examined in 2 mL of *carbon dioxide-free water R* in a beaker and transfer it to the column reservoir, allow the solution to run through the column at a flow rate of about 3 mL/min and collect the eluate. Wash the beaker with 10 mL of *carbon dioxide-free water R* and transfer this solution at the same flow rate to the column before it runs dry. Finally wash the column with 200 mL of *carbon dioxide-free water R* (with the tap completely open) until neutral to *blue litmus paper R*. Titrate the combined eluate and washings with *0.1 M sodium hydroxide*, using 1 mL of *phenolphthalein solution R* as indicator.

1 mL of *0.1 M sodium hydroxide* is equivalent to 10.11 mg of KNO_3.

LABELLING
The label states, where applicable, that the substance is suitable for ophthalmic use.

Ph Eur

Potassium Perchlorate

(Ph. Eur. monograph 1987)

$KClO_4$ 138.6 *7778-74-7*

Action and use
Diagnostic aid; treatment of hyperthyroidism.

Ph Eur

DEFINITION
Content
99.0 per cent to 102.0 per cent.

CHARACTERS
Appearance
White or almost white, crystalline powder or colourless crystals.

Solubility
Sparingly soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION
A. Dissolve 0.1 g in 5 mL of *water R*. Add 5 mL of *indigo carmine solution R* and heat to boiling. The colour of the solution does not disappear.

B. Chlorates and chlorides (see Tests).

C. Heat 10 mg over a flame for 2 min. Dissolve the residue in 2 mL of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

D. Dissolve 50 mg with heating in 5 mL of *water R*. Allow to cool to room temperature. The solution gives reaction (a) of potassium (*2.3.1*).

TESTS
Solution S
Suspend 5.0 g in 90 mL of *distilled water R* and heat to boiling. Allow to cool. Filter. Dilute the filtrate to 100 mL with *carbon dioxide-free water R*.

Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 0.20 g in *water R* and dilute to 20 mL with the same solvent.

Acidity or alkalinity
To 5 mL of solution S add 5 mL of *water R* and 0.1 mL of *phenolphthalein solution R*. Not more than 0.25 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator. To 5 mL of solution S, add 5 mL of *water R* and 0.1 mL of *bromocresol green solution R*. Not more than 0.25 mL of *0.01 M hydrochloric acid* is required to change the colour of the indicator.

Chlorates and chlorides (*2.4.4*)
Maximum 100 ppm (calculated as chlorides).

To 5 mL of solution S, add 5 mL of *water R* and heat to boiling. Add 1 mL of *nitric acid R* and 0.1 g of *sodium nitrite R*. Allow to cool to room temperature. Dilute to 15 mL with *water R*. The solution complies with the limit test for chlorides. Prepare the standard using 5 mL of *chloride standard solution (5 ppm Cl) R* and 10 mL of *water R*, and adding only 1 mL of *dilute nitric acid R*.

Sulfates (*2.4.13*)
Maximum 100 ppm, determined on solution S.

Prepare the standard using a mixture of 7.5 mL of *sulfate standard solution (10 ppm SO_4) R* and 7.5 mL of *water R*.

Calcium (*2.4.3*)
Maximum 100 ppm, determined on solution S.

Prepare the standard using a mixture of 7.5 mL of *calcium standard solution (10 ppm Ca) R*, 1 mL of *dilute acetic acid R* and 7.5 mL of *distilled water R*.

ASSAY
Prepare a chromatography column 0.3 m long and 10 mm in internal diameter and filled with 10 g of *strongly acidic ion-exchange resin R* covered with *carbon dioxide-free water R*. Maintain a 1 cm layer of liquid above the resin throughout the determination. Allow 100 mL of *dilute hydrochloric acid R* to run through the column at a flow rate of about 5 mL/min. Wash the column (with the tap completely open) with *carbon dioxide-free water R* until the eluate is neutral to *blue litmus paper R*. Dissolve 0.100 g of the substance to be examined in 10 mL of *carbon dioxide-free water R* in a beaker and transfer it to the column reservoir, allow the solution to run through the column at a flow rate of about 3 mL/min and collect the eluate. Wash the beaker 3 times with 10 mL of *carbon dioxide-free water R* and transfer this solution at the same flow rate to the column before it runs dry. Finally, wash the column with 200 mL of *carbon dioxide-free water R* (with the tap completely open) until the eluate is neutral to *blue litmus paper R*. Titrate the combined eluate and washings with *0.1 M sodium hydroxide*, using 1 mL of *phenolphthalein solution R* as indicator.

1 mL of *0.1 M sodium hydroxide* is equivalent to 13.86 mg of $KClO_4$.

Ph Eur

Potassium Permanganate

(Ph. Eur. monograph 0121)

$KMnO_4$ 158.0 *7722-64-7*

Action and use
Antiseptic.

Ph Eur

DEFINITION
Content
99.0 per cent to 100.5 per cent.

CHARACTERS
Appearance
Dark purple or brownish-black, granular powder or dark purple or almost black crystals, usually having a metallic lustre.

Solubility
Soluble in cold water, freely soluble in boiling water.

It decomposes on contact with certain organic substances.

IDENTIFICATION
A. Dissolve about 50 mg in 5 mL of *water R* and add 1 mL of *ethanol (96 per cent) R* and 0.3 mL of *dilute sodium hydroxide solution R*. A green colour develops. Heat to boiling. A dark brown precipitate is formed.

B. Filter the mixture obtained in identification test A. The filtrate gives reaction (b) of potassium (*2.3.1*).

TESTS
Solution S
Dissolve 0.75 g in 25 mL of *distilled water R*, add 3 mL of *ethanol (96 per cent) R* and boil for 2-3 min. Cool, dilute to 30 mL with *distilled water R* and filter.

Appearance of solution
Solution S is colourless (*2.2.2, Method II*).

Substances insoluble in water
Maximum 1.0 per cent.

Dissolve 0.5 g in 50 mL of *water R* and heat to boiling. Filter through a tared sintered-glass filter (16) (*2.1.2*). Wash with *water R* until the filtrate is colourless and collect the residue on the filter. The residue, dried in an oven at 100-105 °C, weighs a maximum of 5 mg.

Chlorides (*2.4.4*)
Maximum 200 ppm.

Dilute 10 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 500 ppm.

Dilute 12 mL of solution S to 15 mL with *distilled water R*.

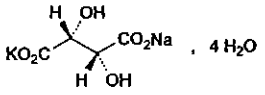
ASSAY
Dissolve 0.300 g in *water R* and dilute to 100.0 mL with the same solvent. To 20.0 mL of the solution add 20 mL of *water R*, 1 g of *potassium iodide R* and 10 mL of *dilute hydrochloric acid R*. Titrate the liberated iodine with *0.1 M sodium thiosulfate*, using 1 mL of *starch solution R* as indicator.

1 mL of *0.1 M sodium thiosulfate* is equivalent to 3.160 mg of $KMnO_4$.

Ph Eur

Potassium Sodium Tartrate Tetrahydrate

(Ph. Eur. monograph 1986)



$C_4H_4KNaO_6,4H_2O$ 282.2 *6381-59-5*

Action and use
Excipient.

Ph Eur

DEFINITION
Potassium sodium (+)-(2*R*,3*R*)-2,3-dihydroxybutanedioate tetrahydrate.

Content
98.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white, crystalline powder or colourless, transparent crystals.

Solubility
Very soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION
A. Specific optical rotation (see Tests).
B. It gives reaction (b) of tartrates (*2.3.1*).
C. It gives reaction (b) of potassium (*2.3.1*).
D. It gives reaction (a) of sodium (*2.3.1*).

TESTS
Solution S
Dissolve 5.000 g in *carbon dioxide-free water R*, prepared from *distilled water R*, and dilute to 100.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity
To 5 mL of solution S, add 0.1 mL of *phenolphthalein solution R*. Not more than 0.5 mL of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Specific optical rotation (*2.2.7*)
+ 28.0 to + 30.0 (anhydrous substance), determined on solution S.

Chlorides (*2.4.4*)
Maximum 100 ppm.

Dilute 10 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 50 ppm.

Dissolve 1.0 g in *distilled water R* and dilute to 15 mL with the same solvent. Prepare the reference solution with a mixture of 5 mL of *sulfate standard solution (10 ppm SO_4) R* and 10 mL of *distilled water R*.

Ammonium (*2.4.1*)
Maximum 40 ppm, determined on 5 mL of solution S.

Barium and oxalates
To 5 mL of solution S, add 3 mL of *calcium sulfate solution R*. Allow to stand for 5 min. Any opalescence in the solution is not more intense than that in a mixture of 3 mL of *calcium sulfate solution R* and 5 mL of *distilled water R*.

Calcium (*2.4.3*)
Maximum 200 ppm.

Dilute 10 mL of solution S to 15 mL with *distilled water R*.

Water (*2.5.12*)
24.0 per cent to 26.5 per cent, determined on 50.0 mg. Use 50 mL of *anhydrous methanol R*. Titrate slowly.

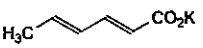
ASSAY
To 0.100 g of finely powdered substance add 40 mL of *anhydrous acetic acid R* and 20 mL of *acetic anhydride R*. Titrate slowly with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 10.51 mg of $C_4H_4KNaO_6$.

Ph Eur

Potassium Sorbate

(Ph. Eur. monograph 0618)



$C_6H_7KO_2$ 150.2 *590-00-1*

Action and use
Antimicrobial preservative.

Ph Eur

DEFINITION
Potassium (*E*,*E*)-hexa-2,4-dienoate.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white powder or granules.

Solubility
Very soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION
First identification: B, D.
Second identification: A, C, D.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 50.0 mg in *water R* and dilute to 250.0 mL with the same solvent. Dilute 2.0 mL of this solution to 200.0 mL with *0.1 M hydrochloric acid.*

Spectral range 230-350 nm.

Absorption maximum At 264 nm.

Specific absorbance at the absorption maximum 1650 to 1900.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *potassium sorbate CRS.*

C. Dissolve 1.0 g in 50 mL of *water R*, add 10 mL of *dilute hydrochloric acid R* and shake. Filter the crystalline precipitate, wash with *water R* and dry *in vacuo* over *sulfuric acid R* for 4 h. The residue obtained melts (*2.2.14*) at 132 °C to 136 °C.

D. Dissolve 0.2 g in 2 mL of *water R* and add 2 mL of *dilute acetic acid R*. Filter. The solution gives reaction (b) of potassium (*2.3.1*).

TESTS

Solution S
Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5 (*2.2.2, Method II*).

Acidity or alkalinity
To 20 mL of solution S add 0.1 mL of *phenolphthalein solution R*. Not more than 0.25 mL of *0.1 M sodium hydroxide* or *0.1 M hydrochloric acid* is required to change the colour of the indicator.

Aldehydes
Maximum 0.15 per cent, expressed as C_2H_4O.

Dissolve 1.0 g in a mixture of 30 mL of *water R* and 50 mL of *2-propanol R*, adjust to pH 4 with *1 M hydrochloric acid* and dilute to 100 mL with *water R*. To 10 mL of the solution add 1 mL of *decolorised fuchsin solution R* and allow to stand for 30 min. Any colour in the solution is not more intense than that in a standard prepared at the same time by adding 1 mL of *decolorised fuchsin solution R* to a mixture of 1.5 mL of *acetaldehyde standard solution (100 ppm C_2H_4O) R*, 4 mL of *2-propanol R* and 4.5 mL of *water R*.

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

ASSAY
Dissolve 0.120 g in 20 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* using 0.1 mL of *crystal violet solution R* as indicator until the colour changes from violet to bluish-green.

1 mL of *0.1 M perchloric acid* is equivalent to 15.02 mg of $C_6H_7KO_2$.

STORAGE
Protected from light.

Ph Eur

Potassium Sulfate

Potassium Sulphate
(Ph. Eur. monograph 1622)

K_2SO_4 174.3 *7778-80-5*

Ph Eur

DEFINITION

Content
98.5 per cent to 101.0 per cent of K_2SO_4 (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder or colourless crystals.

Solubility
Soluble in water, practically insoluble in ethanol.

IDENTIFICATION
A. It gives reaction (a) of sulfates (*2.3.1*).
B. It gives reaction (a) of potassium (*2.3.1*).

TESTS

Solution S
Dissolve 10.0 g in 90 mL of *carbon dioxide-free water R* prepared from *distilled water R*, heating gently. Allow to cool and dilute to 100 mL with *carbon dioxide-free water R* prepared from *distilled water R*.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity
To 10 mL of solution S add 0.1 mL of *bromothymol blue solution R1*. Not more than 0.5 mL of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Chlorides (*2.4.4*)
Maximum 40 ppm.
Dilute 12.5 mL of solution S to 15 mL with *water R*.

Calcium (*2.4.3*)
Maximum 200 ppm.
Dilute 5 mL of solution S to 15 mL with *distilled water R*.

Iron (*2.4.9*)
Maximum 10 ppm, determined on 10 mL of solution S.

Magnesium
Maximum 20 ppm.
To 5 mL of solution S add 5 mL of *water R*, 1 mL of *glycerol (85 per cent) R*, 0.15 mL of *titan yellow solution R*, 0.25 mL of *ammonium oxalate solution R* and 5 mL of *dilute sodium hydroxide solution R* and shake. Any pink colour in the test solution is not more intense than that in a standard prepared at the same time and in the same manner using a mixture of 1 mL of *magnesium standard solution (10 ppm Mg) R* and 9 mL of *water R*.

Sodium
Maximum 0.10 per cent.
Atomic emission spectrometry (*2.2.22, Method I*).

Test solution Dissolve 1.00 g of the substance to be examined in *water R* and dilute to 100.0 mL with the same solvent.

Reference solutions Dissolve in *water R* 0.50 g of *sodium chloride R*, previously dried at 100-105 °C for 3 h, and dilute to 1000.0 mL with the same solvent (200 µg of Na per millilitre). Dilute as required.

Wavelength 589 nm.

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 130 °C for 4 h.

ASSAY
Pack 20 g of *strongly acidic ion-exchange resin R* into a glass column, at least 20 cm long and 20 mm in internal diameter, and cover it with *water R*. After 5 min, wash the resin with *water R* until the pH of the eluate is about 6 to 7 using a *pH indicator strip R*. Keep the resin covered with *water R*. Dissolve 0.600 g of the substance to be examined in 10 mL of *water R* in a beaker. Load the solution onto the column and pass through the resin at a flow rate of about 4 mL/min until the resin is just covered with the solution. Using about 200 mL of *water R*, rinse the beaker and pass the rinsings through the column at the same flow rate. Check that the pH of the last eluate is about 6 to 7 using a *pH indicator strip R*.

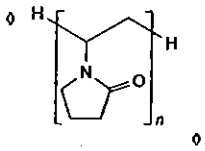
Titrate the eluate with *1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *1 M sodium hydroxide* is equivalent to 87.15 mg of K_2SO_4.

Ph Eur

Povidone[1]

(Ph. Eur. monograph 0685)



◊$C_{6n}H_{9n+2}N_nO_n$◊ *9003-39-8*

Action and use
Disinfectant.

Preparations
Povidone-Iodine Eye Drops
Povidone-Iodine Mouthwash
Povidone-Iodine Solution

Ph Eur

DEFINITION
α-Hydro-ω-hydropoly[1-(2-oxopyrrolidin-1-yl)ethylene].
It consists of linear polymers of 1-ethenylpyrrolidin-2-one.

Content
11.5 per cent to 12.8 per cent of nitrogen (N; A_r 14.01) (anhydrous substance).

[1] *This monograph has undergone pharmacopoeial harmonisation. See chapter 5.8 Pharmacopoeial harmonisation.*

The different types of povidone are characterised by their viscosity in solution expressed as a nominal *K*-value. The nominal *K*-value is 10 to 120.

♦CHARACTERS
Appearance
White or yellowish-white, hygroscopic powder or flakes.

Solubility
Freely soluble in water, in ethanol (96 per cent) and in methanol, very slightly soluble in acetone.♦

IDENTIFICATION
First identification: A, D.

◊*Second identification: B, C, D.*◊

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Dry the substances beforehand at 105 °C for 6 h.

Comparison *povidone CRS.*

◊B. To 1 mL of solution S1 (see Tests) add 0.2 mL of *dimethylaminobenzaldehyde solution R1* and 0.1 mL of *sulfuric acid R*. A pink colour is produced.

C. To 0.1 mL of solution S1 add 5 mL of *water R* and 0.2 mL of *0.05 M iodine*. A red colour is produced.◊

D. To 0.5 g add 10 mL of *water R* and shake. The substance dissolves.

TESTS
Solution S
Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20.0 mL with the same solvent. Add the substance to be examined to the water in small portions, stirring using a magnetic stirrer.

Solution S1
Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent. Add the substance to be examined to the water in small portions, stirring using a magnetic stirrer.

♦Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_6, BY_6 or R_6 (*2.2.2, Method II*).♦

pH (*2.2.3*)
3.0 to 5.0 for solution S, for povidone having a nominal *K*-value of not more than 30; 4.0 to 7.0 for solution S, for povidone having a nominal *K*-value of more than 30.

Viscosity, expressed as *K*-value
For povidone having a nominal value of 18 or less, use a 50.0 g/L solution. For povidone having a nominal value of more than 18 and not more than 95, use a 10.0 g/L solution. For povidone having a nominal value of more than 95, use a 1.0 g/L solution. Allow to stand for 1 h and determine the viscosity (*2.2.9*) of the solution at 25 °C, ◊using a size no. 1 viscometer with a minimum flow time of 100 s◊. Calculate the *K*-value using the following expression:

$$\frac{1.5 \log \nu_{rel} - 1}{0.15 + 0.003c} + \frac{\sqrt{300c \log \nu_{rel} + (c + 1.5c \log \nu_{rel})^2}}{0.15c + 0.003c^2}$$

c = concentration of the substance to be examined (anhydrous substance), in grams per 100 mL;
ν_{rel} = kinematic viscosity of the solution relative to that of *water R*.

The *K*-value of povidone having a nominal *K*-value of 15 or less is 85.0 per cent to 115.0 per cent of the nominal value.

The *K*-value of povidone having a nominal *K*-value of more than 15 is 90.0 per cent to 108.0 per cent of the nominal value.

Aldehydes

Maximum 500 ppm, expressed as acetaldehyde.

Test solution Dissolve a quantity of the substance to be examined equivalent to 1.0 g of anhydrous substance in *phosphate buffer solution pH 9.0 R* and dilute to 100.0 mL with the same solvent. Stopper the flask tightly and heat at 60 °C for 1 h. Allow to cool to room temperature.

Reference solution Dissolve 0.140 g of *acetaldehyde ammonia trimer trihydrate R* in *water R* and dilute to 200.0 mL with the same solvent. Dilute 1.0 mL of the solution to 100.0 mL with *phosphate buffer solution pH 9.0 R*.

Into 3 identical spectrophotometric cells with a path length of 1 cm, introduce separately 0.5 mL of the test solution, 0.5 mL of the reference solution and 0.5 mL of *water R* (blank). To each cell add 2.5 mL of *phosphate buffer solution pH 9.0 R* and 0.2 mL of *nicotinamide-adenine dinucleotide solution R*. Mix and stopper tightly. Allow to stand at 22 ± 2 °C for 2-3 min and measure the absorbance (*2.2.25*) of each solution at 340 nm, using *water R* as the compensation liquid. To each cell add 0.05 mL of *aldehyde dehydrogenase solution R*, mix and stopper tightly. Allow to stand at 22 ± 2 °C for 5 min. Measure the absorbance of each solution at 340 nm using *water R* as the compensation liquid.

Calculate the content of aldehydes using the following expression:

$$\frac{(A_{t2} - A_{t1}) - (A_{b2} - A_{b1})}{(A_{s2} - A_{s1}) - (A_{b2} - A_{b1})} \times \frac{100\,000 \times C}{m}$$

A_{t1} = absorbance of the test solution before the addition of aldehyde dehydrogenase;
A_{t2} = absorbance of the test solution after the addition of aldehyde dehydrogenase;
A_{s1} = absorbance of the reference solution before the addition of aldehyde dehydrogenase;
A_{s2} = absorbance of the reference solution after the addition of aldehyde dehydrogenase;
A_{b1} = absorbance of the blank before the addition of aldehyde dehydrogenase;
A_{b2} = absorbance of the blank after the addition of aldehyde dehydrogenase;
m = mass of the substance to be examined (anhydrous substance) in the test solution, in grams;
C = concentration of acetaldehyde in the reference solution, calculated from the mass of *acetaldehyde ammonia trimer trihydrate R* with the factor 0.72, in milligrams per millilitre.

Peroxides

Maximum 400 ppm, expressed as H_2O_2.

Dissolve a quantity of the substance to be examined equivalent to 4.0 g of the anhydrous substance in *water R* and dilute to 100.0 mL with the same solvent (stock solution). To 25.0 mL of the stock solution add 2.0 mL of *titanium trichloride-sulfuric acid reagent R*. Allow to stand for 30 min. The absorbance (*2.2.25*) of the solution, measured at 405 nm using a mixture of 25.0 mL of the stock solution and 2.0 mL of a 13 per cent *V/V* solution of *sulfuric acid R* as the compensation liquid, is not greater than 0.35.

Formic acid

Liquid chromatography (*2.2.29*).

Test solution Dissolve a quantity of the substance to be examined equivalent to 2.0 g of the anhydrous substance in *water R* and dilute to 100.0 mL with the same solvent (stock solution). Transfer a suspension of *strongly acidic ion-exchange resin R* for column chromatography in *water R* to a column of about 0.8 cm in internal diameter to give a packing of about 20 mm in length and keep the strongly acidic ion-exchange resin layer constantly immersed in *water R*. Pour 5 mL of *water R* and adjust the flow rate to about 1 mL/min. When the level of the water comes down to near the top of the strongly acidic ion-exchange resin layer, introduce the stock solution into the column. Discard the first 2 mL of the eluate, then collect 1.5 mL of the solution and use this solution as the test solution.

Reference solution Dissolve 0.100 g of *anhydrous formic acid R* in *water R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of the solution to 100.0 mL with *water R*.

Column:
— *size*: l = 0.30 m, Ø = 7.8 mm;
— *stationary phase*: *strongly acidic ion-exchange resin R* for column chromatography (9 µm);
— *temperature*: 35 °C.

Mobile phase Dilute 1 mL of *perchloric acid R* to 700 mL with *water for chromatography R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 50 µL.

Retention time Formic acid = about 8 min.

System suitability Reference solution:
— *repeatability*: maximum relative standard deviation of 2.0 per cent determined on 6 injections;
— *number of theoretical plates*: minimum 1000;
— *symmetry factor*: 0.5 to 1.5.

Calculate the percentage content of formic acid using the following expression:

$$\frac{A_1}{A_2} \times \frac{M}{m}$$

A_1 = area of the peak due to formic acid in the chromatogram obtained with the test solution;
A_2 = area of the peak due to formic acid in the chromatogram obtained with the reference solution;
m = mass of the substance to be examined (anhydrous substance) in the test solution, in grams;
M = mass of *anhydrous formic acid R* in the reference solution, in grams.

Limit:
— *formic acid*: maximum 0.5 per cent.

Hydrazine

Thin-layer chromatography (*2.2.27*). *Use freshly prepared solutions.*

Test solution Dissolve a quantity of the substance to be examined equivalent to 2.5 g of the anhydrous substance in 25 mL of *water R*. Add 0.5 mL of a 50 g/L solution of *salicylaldehyde R* in *methanol R*, mix and heat in a water-bath at 60 °C for 15 min. Allow to cool, add 2.0 mL of *toluene R*, shake vigorously for 2 min and centrifuge. Use the upper layer.

Reference solution Dissolve 90 mg of *salicylaldehyde azine R* in *toluene R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of the solution to 100.0 mL with *toluene R*.

Plate *TLC silanised silica gel F_{254} plate R*.

Mobile phase *water R, methanol R* (1:2 *V/V*).

Application 10 µL.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 365 nm.

Retardation factor Salicylaldehyde azine = about 0.3.

Limit:
— *hydrazine*: any spot due to salicylaldehyde azine is not more intense than the spot in the chromatogram obtained with the reference solution (1 ppm).

Impurity A
Liquid chromatography (*2.2.29*).

Test solution Dissolve a quantity of the substance to be examined equivalent to 0.250 g of the anhydrous substance in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *1-vinylpyrrolidin-2-one R* (impurity A) in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 100.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve 10 mg of *1-vinylpyrrolidin-2-one R* (impurity A) and 0.5 g of *vinyl acetate R* in *methanol R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of the solution to 100.0 mL with the mobile phase.

Precolumn:
— *size*: *l* = 0.010 m, Ø = 4.0 mm;
— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 40 °C.

Mobile phase *acetonitrile for chromatography R, water for chromatography R* (10:90 *V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 235 nm.

Injection 20 µL; ◊after each injection of the test solution, wait for about 2 min and wash the precolumn by passing the mobile phase through the column backwards for 30 min at the same flow rate as applied in the test.

Relative retention With reference to vinyl acetate (retention time = about 14 min): impurity A = about 0.6.◊

System suitability:
— *resolution*: minimum 2.0 between the peaks due to impurity A and vinyl acetate in the chromatogram obtained with reference solution (b);
— *repeatability*: maximum relative standard deviation of 2.0 per cent determined on 6 injections of reference solution (a).

Calculate the content of impurity A in parts per million using the following expression:

$$\frac{A_1}{A_2} \times \frac{2.5}{m}$$

A_1 = area of the peak due to impurity A in the chromatogram obtained with the test solution;
A_2 = area of the peak due to impurity A in the chromatogram obtained with reference solution (a);
m = mass of the substance to be examined (anhydrous substance) in the test solution, in grams.

Limit:
— *impurity A*: maximum 10 ppm.

Impurity B
Liquid chromatography (*2.2.29*).

Test solution Dissolve a quantity of the substance to be examined equivalent to 0.500 g of the anhydrous substance in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution Dissolve 0.150 g of *2-pyrrolidone R* (impurity B) in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 2.0 mL of the solution to 100.0 mL with the mobile phase.

Precolumn:
— *size*: *l* = 0.010 m, Ø = 4.0 mm;
— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 40 °C.

Mobile phase *methanol R2, water for chromatography R* (5:95 *V/V*).

Flow rate 0.8 mL/min.

Detection Spectrophotometer at 205 nm.

Injection 50 µL; ◊after each injection of the test solution, wait for about 2 min and wash the precolumn by passing the mobile phase through the column backwards for about 30 min at the same flow rate as applied in the test.◊

Retention time Impurity B = about 7 min.

System suitability Reference solution:
— *repeatability*: maximum relative standard deviation of 2.0 per cent determined on 6 injections;
— *number of theoretical plates*: minimum 5000.

Calculate the percentage content of impurity B using the following expression:

$$\frac{A_1}{A_2} \times \frac{0.3}{m}$$

A_1 = area of the peak due to impurity B in the chromatogram obtained with the test solution;
A_2 = area of the peak due to impurity B in the chromatogram obtained with the reference solution;
m = mass of the substance to be examined (anhydrous substance) in the test solution, in grams.

Limit:
— *impurity B*: maximum 3.0 per cent.

Water (*2.5.12*)
Maximum 5.0 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Place 0.100 g of the substance to be examined (*m* mg) in a combustion flask and add 5 g of a mixture of 1 g of *copper sulfate pentahydrate R*, 1 g of *titanium dioxide R* and 33 g of *dipotassium sulfate R*, and 3 glass beads. Wash any adhering particles from the neck into the flask with a small quantity of *water R*. Add 7 mL of *sulfuric acid R*, allowing it to run down the insides of the flask. Heat the flask gradually until the solution has a clear, yellowish-green colour, and the inside wall of the flask is free from any carbonised material, and then heat for a further 45 min. After cooling, add cautiously 20 mL of *water R*, and connect the flask to the distillation apparatus, which has been previously washed by passing steam through it. To the absorption flask add 30 mL of a 40 g/L solution of *boric acid R*, 3 drops of *bromocresol green-methyl red solution R* and sufficient water to immerse the lower end of the condenser tube. Add 30 mL of *strong sodium hydroxide solution R* through the funnel, rinse the funnel

cautiously with 10 mL of *water R*, immediately close the clamp on the rubber tube, then start distillation with steam to obtain 80-100 mL of distillate. Remove the absorption flask from the lower end of the condenser tube, rinsing the end part with a small quantity of *water R*, and titrate the distillate with *0.025 M sulfuric acid* until the colour of the solution changes from green through pale greyish blue to pale greyish reddish-purple. Carry out a blank determination.

1 mL of *0.025 M sulfuric acid* is equivalent to 0.700 mg of N.

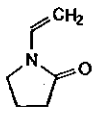
♦STORAGE

In an airtight container.♦

LABELLING

The label states the nominal *K*-value.

IMPURITIES



A. 1-ethenylpyrrolidin-2-one (1-vinylpyrrolidin-2-one),

B. pyrrolidin-2-one (2-pyrrolidone).

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for povidone used as solubiliser and stabiliser in liquid dosage forms.

Viscosity (*2.2.9*)

Determine the dynamic viscosity using a capillary viscometer on a 10 per cent solution (dried substance) at 25 °C. Typical values are shown in Table 0685.-1.

Molecular mass

(see Viscosity, expressed as *K*-value). Typical values are shown in Table 0685.-1.

The following characteristic may be relevant for povidone used as binder in tablets and granules.

Molecular mass

(see Viscosity, expressed as *K*-value). Typical values are shown in Table 0685.-1.

Table 0685.-1. – *Typical viscosity ranges and ranges for viscosity, expressed as K-value*

	Viscosity range (mPa·s)	Molecular mass: viscosity, expressed as *K*-value
Povidone K 12	1.3-2.3	11-14
Povidone K 17	1.5-3.5	16-18
Povidone K 25	3.5-5.5	24-27
Povidone K 30	5.5-8.5	28-32
Povidone K 90	300-700	85-95

Ph Eur

Iodinated Povidone

(Ph. Eur. monograph 1142)

Action and use

Antiseptic.

Preparations

Povidone-Iodine Eye Drops

Povidone-Iodine Mouthwash

Povidone-Iodine Solution

Ph Eur

DEFINITION

Complex of iodine and povidone.

Content

9.0 per cent to 12.0 per cent of available iodine (dried substance).

PRODUCTION

It is produced using povidone that complies with the monograph on *Povidone (0685)*, except that the povidone used may contain not more than 2.0 per cent of formic acid and not more than 8.0 per cent of water.

CHARACTERS

Appearance

Yellowish-brown or reddish-brown, amorphous powder.

Solubility

Soluble in water and in ethanol (96 per cent), practically insoluble in acetone.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison iodinated povidone CRS.

B. Dissolve 10 mg in 10 mL of *water R* and add 1 mL of *starch solution R*. An intense blue colour is produced.

TESTS

pH (*2.2.3*)

1.5 to 5.0.

Dissolve 1.0 g in 10 mL of *carbon dioxide-free water R*.

Iodide

Maximum 6.0 per cent (dried substance).

Dissolve 0.500 g in 100 mL of *water R*. Add *sodium metabisulfite R* until the colour of the iodine has disappeared. Add 25.0 mL of *0.1 M silver nitrate*, 10 mL of *nitric acid R* and 5 mL of *ferric ammonium sulfate solution R2*. Titrate with *0.1 M ammonium thiocyanate*. Carry out a blank titration.

1 mL of *0.1 M silver nitrate* is equivalent to 12.69 mg of total iodine. From the percentage of total iodine, calculated with reference to the dried substance, subtract the percentage of available iodine as determined in the assay to obtain the percentage of iodide.

Loss on drying (*2.2.32*)
Maximum 8.0 per cent, determined on 0.500 g by drying in an oven at 105 °C for 3 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Transfer 1.000 g into a ground-glass-stoppered flask containing 150 mL of *water R* and stir for 1 h. Add 0.1 mL of *dilute acetic acid R* and titrate with *0.1 M sodium thiosulfate* using *starch solution R* as indicator.

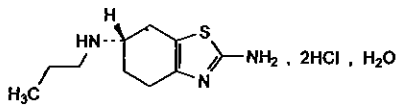
1 mL of *0.1 M sodium thiosulfate* is equivalent to 12.69 mg of available iodine.

STORAGE
Protected from light.

Ph Eur

Pramipexole Dihydrochloride Monohydrate

(Ph. Eur. monograph 2416)



$C_{10}H_{19}Cl_2N_3S,H_2O$ 302.3 *191217-81-9*

Action and use
Dopamine receptor agonist; treatment of Parkinson's disease.

Preparations
Pramipexole Tablets

Pramipexole Prolonged-release Tablets

Ph Eur

DEFINITION
(6*S*)-6-*N*-Propyl-4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-diamine dihydrochloride monohydrate.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water, soluble in methanol, sparingly soluble or slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION
Carry out either tests B, C, D or tests A, B, D.

A. Specific optical rotation (*2.2.7*): −69.5 to −67.0 (anhydrous substance).

Dissolve 0.250 g in *methanol R* and dilute to 25.0 mL with the same solvent.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *pramipexole dihydrochloride monohydrate CRS.*

C. Enantiomeric purity (see Tests).

D. It gives reaction (a) of chlorides (*2.3.1*).

TESTS
Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Dissolve 0.1 g in *water R* and dilute to 10 mL with the same solvent.

pH (*2.2.3*)
2.8 to 3.4.

Dissolve 0.4 g in *carbon dioxide-free water R* and dilute to 20 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Buffer solution Dissolve 5 g of *sodium octanesulfonate monohydrate R* and 9.1 g of *potassium dihydrogen phosphate R* in 900 mL of *water for chromatography R*. Adjust to pH 3.0 with *phosphoric acid R* and dilute to 1000 mL with *water for chromatography R*.

Solvent mixture *acetonitrile R*, buffer solution (20:80 *V/V*).

Test solution Dissolve 75 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 7.5 mg of *pramipexole for system suitability CRS* (containing impurities A, B and C) in 5 mL of the solvent mixture.

Column:
— *size*: *l* = 0.125 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: buffer solution;
— *mobile phase B*: *acetonitrile R*, buffer solution (50:50 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	60 → 20	40 → 80

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 264 nm.

Injection 5 µL.

Identification of impurities Use the chromatogram supplied with *pramipexole for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B and C.

Relative retention With reference to pramipexole (retention time = about 6 min): impurity A = about 0.7; impurity B = about 1.5; impurity C = about 1.7.

System suitability Reference solution (b):
— *resolution*: minimum 6.0 between the peaks due to impurity A and pramipexole.

Limits:
— *impurities A, B, C*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Enantiomeric purity
Liquid chromatography (*2.2.29*).

Test solution Dissolve 6 mg of the substance to be examined in 5 mL of *anhydrous ethanol R* and dilute to 20.0 mL with the mobile phase.

Reference solution (a) Dissolve 2 mg of *pramipexole impurity D CRS* in the mobile phase and dilute to 10 mL with the mobile phase. To 1 mL of this solution add 1 mL of the test solution and dilute to 20 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 20.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *amylose derivative of silica gel for chiral separation R*.

Mobile phase *diethylamine R, anhydrous ethanol R, hexane R* (0.1:15:85 *V/V/V*).

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 75 µL.

Run time 1.5 times the retention time of pramipexole.

Relative retention With reference to pramipexole (retention time = about 11 min): impurity D = about 0.5.

System suitability:
— *resolution*: minimum 5.0 between the peaks due to impurity D and pramipexole in the chromatogram obtained with reference solution (a);
— *symmetry factor*: maximum 2.4 for the peak due to pramipexole in the chromatogram obtained with reference solution (b).

Limit:
— *impurity D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent).

Water (*2.5.12*)
5.0 per cent to 7.0 per cent, determined on 0.500 g.

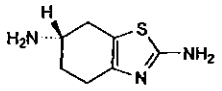
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.120 g in 150 mL of *water R*. Add 10 mL of *dilute nitric acid R2* and titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (*2.2.20*).

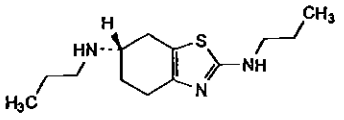
1 mL of *0.1 M silver nitrate* is equivalent to 14.213 mg of $C_{10}H_{19}Cl_2N_3S$.

IMPURITIES
Specified impurities *A, B, C, D.*

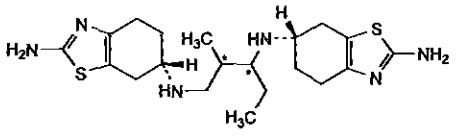
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *E.*



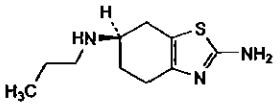
A. (6*S*)-4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-diamine,



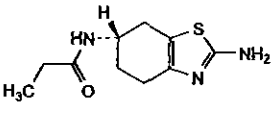
B. (6*S*)-*N*,*N*′-dipropyl-4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-diamine,



C. mixture of diastereoisomers of (6*S*)-6-*N*-[3-[[(6*S*)-2-amino-4,5,6,7-tetrahydro-1,3-benzothiazol-6-yl]amino]-1-ethyl-2-methylpropyl]-4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-diamine,



D. (6*R*)-6-*N*-propyl-4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-diamine,

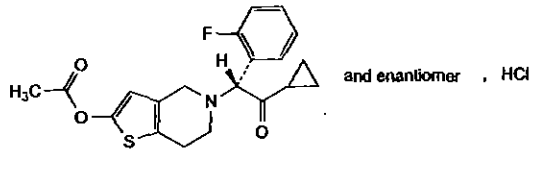


E. *N*-[(6*S*)-2-amino-4,5,6,7-tetrahydro-1,3-benzothiazol-6-yl] propanamide.

Ph Eur

Prasugrel Hydrochloride

(Ph. Eur. monograph 3040)



$C_{20}H_{21}ClFNO_3S$ 409.9 *389574-19-0*

Action and use
Inhibitor of ADP-mediated platelet aggregation.

Ph Eur

DEFINITION
5-[(1*RS*)-2-Cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridin-2-yl acetate hydrochloride.

Content
97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, slightly hygroscopic powder.

Solubility

Slightly soluble in water, freely soluble in methanol, slightly soluble in acetonitrile, practically insoluble in heptane.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *prasugrel hydrochloride CRS.*

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Solvent mixture *water R, acetonitrile R* (30:70 *V/V*).

Test solution (a) Dissolve 25.0 mg of the substance to be examined in the solvent mixture and dilute to 20.0 mL with the solvent mixture.

Test solution (b) Dilute 2.0 mL of test solution (a) to 25.0 mL with the solvent mixture.

Reference solution (a) Dissolve 25.0 mg of *prasugrel hydrochloride CRS* in the solvent mixture and dilute to 20.0 mL with the solvent mixture. Dilute 2.0 mL of the solution to 25.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 2.5 mg of *prasugrel for system suitability CRS* (containing impurities B and E) in the solvent mixture and dilute to 2 mL with the solvent mixture.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 45 °C.

Mobile phase Mix 35 volumes of *acetonitrile R1* and 65 volumes of a 1.36 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 2.8 with *phosphoric acid R*.

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 210 nm.

Autosampler Set at 5 °C.

Injection 10 µL of test solution (a) and reference solutions (b) and (c).

Run time 3.6 times the retention time of prasugrel.

Identification of impurities Use the chromatogram supplied with *prasugrel for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities B and E.

Relative retention With reference to prasugrel (retention time = about 11 min): impurity B = about 1.1; impurity E = about 2.3.

System suitability Reference solution (c):
- *resolution*: minimum 1.5 between the peaks due to prasugrel and impurity B.

Calculation of percentage contents:
- for each impurity, use the concentration of prasugrel hydrochloride in reference solution (b).

Limits:
- *impurity B*: maximum 0.3 per cent;
- *impurity E*: maximum 0.2 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 1.0 per cent;
- *reporting threshold*: 0.05 per cent.

Water (*2.5.32*)

Maximum 0.5 per cent, determined on 0.100 g by direct sample introduction.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Detection Spectrophotometer at 260 nm.

Injection Test solution (b) and reference solution (a).

Calculate the percentage content of $C_{20}H_{21}ClFNO_3S$ taking into account the assigned content of *prasugrel hydrochloride CRS*.

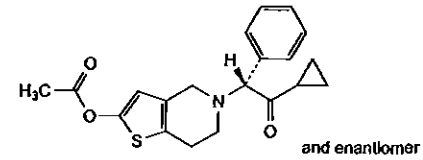
STORAGE

In an airtight container.

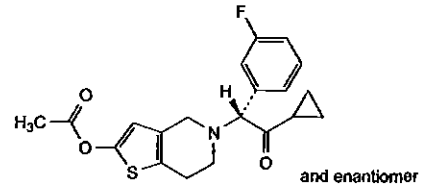
IMPURITIES

Specified impurities *B, E.*

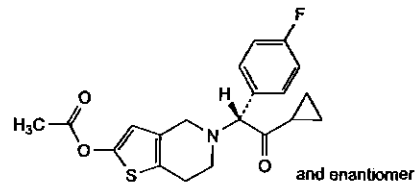
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, C, D, F, G.*



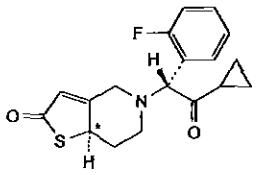
A. 5-[(1*RS*)-2-cyclopropyl-2-oxo-1-phenylethyl]-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridin-2-yl acetate,



B. 5-[(1*RS*)-2-cyclopropyl-1-(3-fluorophenyl)-2-oxoethyl]-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridin-2-yl acetate,

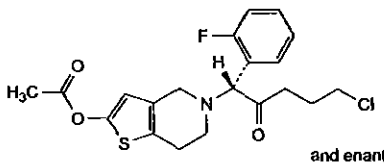


C. 5-[(1*RS*)-2-cyclopropyl-1-(4-fluorophenyl)-2-oxoethyl]-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridin-2-yl acetate,



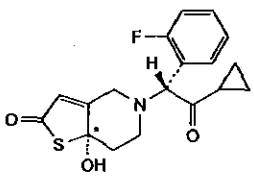
Its epimer at C* and their enantiomers

D. mixture of (7a*R*)-5-[(1*RS*)-2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-5,6,7,7a-tetrahydrothieno[3,2-*c*]pyridin-2(4*H*)-one and (7a*S*)-5-[(1*RS*)-2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-5,6,7,7a-tetrahydrothieno[3,2-*c*]pyridin-2(4*H*)-one,



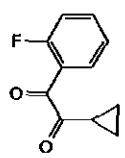
and enantiomer

E. 5-[(1*RS*)-5-chloro-1-(2-fluorophenyl)-2-oxopentyl]-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridin-2-yl acetate,



its epimer at C* and their enantiomers

F. mixture of (7a*R*)-5-[(1*RS*)-2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-7a-hydroxy-5,6,7,7a-tetrahydrothieno[3,2-*c*]pyridin-2(4*H*)-one and (7a*S*)-5-[(1*RS*)-2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-7a-hydroxy-5,6,7,7a-tetrahydrothieno[3,2-*c*]pyridin-2(4*H*)-one,



G. 1-cyclopropyl-2-(2-fluorophenyl)ethane-1,2-dione.

Ph Eur

Pravastatin Sodium

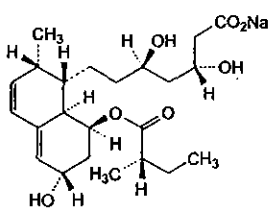
(Ph. Eur. monograph 2059)

$C_{23}H_{35}NaO_7$ 446.5 *81131-70-6*

Action and use
HMG Co-A reductase inhibitor; lipid-regulating drug.

Preparation
Pravastatin Tablets

Ph Eur



DEFINITION

Sodium (3*R*,5*R*)-3,5-dihydroxy-7-[(1*S*,2*S*,6*S*,8*S*,8a*R*)-6-hydroxy-2-methyl-8-[[(2*S*)-2-methylbutanoyl]oxy]-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]heptanoate.

Content
97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or yellowish-white powder or crystalline powder, hygroscopic.

Solubility
Freely soluble in water and in methanol, soluble in anhydrous ethanol.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of pravastatin sodium.*

C. 1 mL of solution S (see Tests) gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S
Dissolve 1.00 g in *carbon dioxide-free water R* and dilute to 20.0 mL with the same solvent.

Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Dilute 2.0 mL of solution S to 10.0 mL with *water R*.

pH (*2.2.3*)
7.2 to 9.0 for solution S.

Specific optical rotation (*2.2.7*)
+ 153 to + 159 (anhydrous substance).

Dilute 2.0 mL of solution S to 20.0 mL with *water R*.

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture *methanol R, water R* (9:11 *V/V*).

Test solution (a) Dissolve 0.1000 g of the substance to be examined in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Test solution (b) Dilute 10.0 mL of test solution (a) to 100.0 mL with the solvent mixture.

Reference solution (a) Dissolve the contents of a vial of *pravastatin impurity A CRS* in 1.0 mL of test solution (b).

Reference solution (b) Dilute 2.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 12.4 mg of *pravastatin 1,1,3,3-tetramethylbutylamine CRS* in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 25 °C.

Mobile phase *glacial acetic acid R, triethylamine R, methanol R, water R* (1:1:450:550 *V/V/V/V*).

Flow rate 1.3 mL/min.

Detection Spectrophotometer at 238 nm.

Injection 10 µL of test solution (a) and reference solutions (a) and (b).

Run time 2.5 times the retention time of pravastatin.

Relative retention With reference to pravastatin (retention time = about 21 min): impurity F = about 0.1; impurity B = about 0.2; impurity E = about 0.3; impurity G = about 0.4; impurity A = about 0.6; impurity D = about 1.9; impurity C = about 2.1.

System suitability Reference solution (a):
— *resolution*: minimum 7.0 between the peaks due to impurity A and pravastatin.

Limits:
— *impurity A*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
— *impurities B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
— *impurities F, G*: for each impurity, not more than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.6 per cent);
— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Ethanol (*2.4.24, System A*)
Maximum 3.0 per cent.

Water (*2.5.12*)
Maximum 4.0 per cent, determined on 0.500 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (c).

Calculate the percentage content of $C_{23}H_{35}NaO_7$ using the chromatogram obtained with reference solution (c) and the declared content of pravastatin in *pravastatin 1,1,3,3-tetramethylbutylamine CRS*.

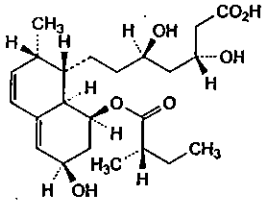
1 mg of pravastatin is equivalent to 1.052 mg of pravastatin sodium.

STORAGE

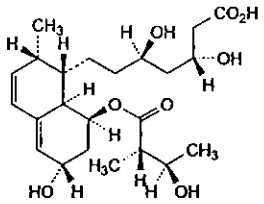
In an airtight container.

IMPURITIES

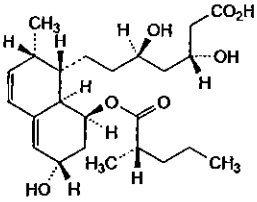
Specified impurities *A, B, C, D, E, F, G.*



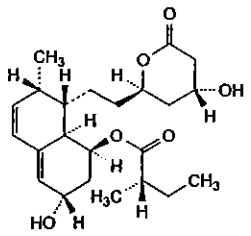
A. (3*R*,5*R*)-3,5-dihydroxy-7-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-6-hydroxy-2-methyl-8-[[(2*S*)-2-methylbutanoyl]oxy]-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]heptanoic acid (6′-epipravastatin),



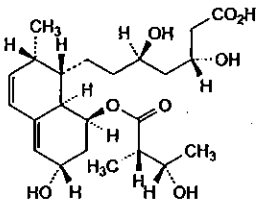
B. (3*R*,5*R*)-3,5-dihydroxy-7-[(1*S*,2*S*,6*S*,8*S*,8a*R*)-6-hydroxy-8-[[(2*S*,3*R*)3-hydroxy-2-methylbutanoyl]oxy]-2-methyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]heptanoic acid (3′′-(*R*)-hydroxypravastatin),



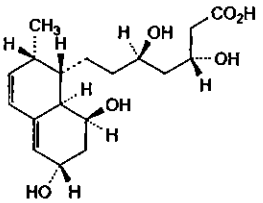
C. (3*R*,5*R*)-3,5-dihydroxy-7-[(1*S*,2*S*,6*S*,8*S*,8a*R*)-6-hydroxy2-methyl-8-[[(2*S*)-2-methylpentanoyl]oxy]-1,2,6,7,8,8ahexahydronaphthalen-1-yl]heptanoic acid,



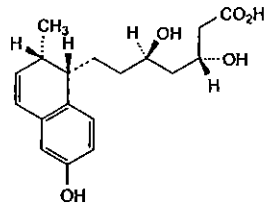
D. (1*S*,3*S*,7*S*,8*S*,8a*R*)-3-hydroxy-8-[2-[(2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl]-7-methyl-1,2,3,7,8,8ahexahydronaphthalen-1-yl (2*S*)-2-methylbutanoate (pravastatin lactone),



E. (3*R*,5*R*)-3,5-dihydroxy-7-[(1*S*,2*S*,6*S*,8*S*,8a*R*)-6-hydroxy-8-[[(2*S*,3*S*)3-hydroxy-2-methylbutanoyl]oxy]-2-methyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]heptanoic acid (3′′-(*S*)-hydroxypravastatin),



F. (3*R*,5*R*)-7-[(1*S*,2*S*,6*S*,8*S*,8a*R*)-6,8-dihydroxy-2-methyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]-3,5-dihydroxyheptanoic acid,

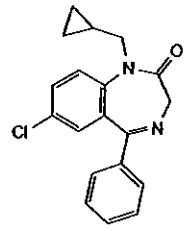


G. (3*R*,5*R*)-3,5-dihydroxy-7-[(1*S*,2*S*)-6-hydroxy-2-methyl-1,2-dihydronaphthalen-1-yl]heptanoic acid.

Ph Eur

Prazepam

(Ph. Eur. monograph 1466)



$C_{19}H_{17}ClN_2O$ 324.8 *2955-38-6*

Action and use
Anxiolytic.

Ph Eur

DEFINITION
7-Chloro-1-(cyclopropylmethyl)-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in anhydrous ethanol.

mp
About 145 °C.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *prazepam CRS.*

TESTS
Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R1, water for chromatography R* (50:50 *V/V*).

Test solution Dissolve 50 mg of the substance to be examined in 25 mL of *acetonitrile R1* and dilute to 50.0 mL with *water for chromatography R.*

Reference solution (a) Dissolve 5 mg of *aminochlorobenzophenone R* (impurity C) in *acetonitrile R1* and dilute to 50.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the test solution.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Column:
— *size:* *l* = 0.10 m, Ø = 4.6 mm;
— *stationary phase: end-capped extra-dense bonded octadecylsilyl silica gel for chromatography R* (1.8 µm);
— *temperature:* 55 °C.

Mobile phase:
— *mobile phase A: methanol R1, water for chromatography R* (5:95 *V/V*);
— *mobile phase B: methanol R1, acetonitrile R1* (5:95 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 0.5	55	45
0.5 - 6.5	55 → 15	45 → 85
6.5 - 8.5	15	85

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 235 nm.

Injection 2 µL.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity C.

Relative retention With reference to prazepam (retention time = about 3 min): impurity C = about 0.9.

System suitability Reference solution (a):
— *resolution:* minimum 2.5 between the peaks due to impurity C and prazepam.

Calculation of percentage contents:
— for each impurity, use the concentration of prazepam in reference solution (b).

Limits:
— *unspecified impurities:* for each impurity, maximum 0.10 per cent;
— *total:* maximum 0.2 per cent;
— *reporting threshold:* 0.05 per cent.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

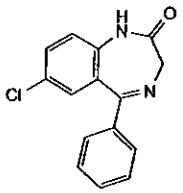
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.250 g in 25 mL of *anhydrous acetic acid R.* Titrate with *0.1 M perchloric acid,* determining the end-point potentiometrically (*2.2.20*).

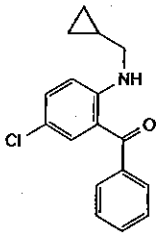
1 mL of *0.1 M perchloric acid* is equivalent to 32.48 mg of $C_{19}H_{17}ClN_2O$.

STORAGE
Protected from light.

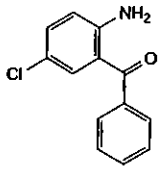
IMPURITIES
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D.*



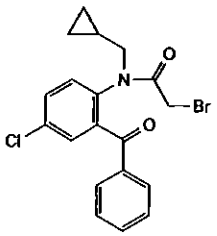
A. 7-chloro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (nordazepam),



B. [5-chloro-2-[(cyclopropylmethyl)amino]phenyl] phenylmethanone,



C. (2-amino-5-chlorophenyl)phenylmethanone (aminochlorobenzophenone),

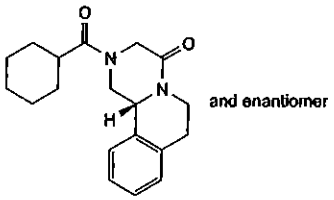


D. *N*-(2-benzoyl-4-chlorophenyl)-2-bromo-*N*-(cyclopropylmethyl)acetamide.

Ph Eur

Praziquantel

(Ph. Eur. monograph 0855)



$C_{19}H_{24}N_2O_2$ 312.4 *55268-74-1*

Action and use
Anthelminthic.

Ph Eur

DEFINITION
(11b*RS*)-2-(Cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4*H*-pyrazino[2,1-*a*]isoquinolin-4-one.

Content
97.5 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Very slightly soluble in water, freely soluble in ethanol (96 per cent) and in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *praziquantel CRS.*

If the spectra obtained show differences, dissolve 50 mg of the substance to be examined and 50 mg of the reference substance separately in 2 mL of *methanol R*. Evaporate and dry the residue at 60 °C at a pressure not exceeding 0.7 kPa. Record new spectra using the residues.

TESTS

Related substances
Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 40.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Test solution (b) Dilute 5.0 mL of test solution (a) to 100.0 mL with the mobile phase.

Reference solution (a) Dissolve 40.0 mg of *praziquantel CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve 2 mg of *praziquantel for system suitability CRS* (containing impurities A and B) in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (c) Dilute 1.0 mL of test solution (a) to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:
— *size: l* = 0.25 m, Ø = 4.0 mm;
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R1, water for chromatography R* (45:55 *V/V*).

Flow rate 1 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µL of test solution (a) and reference solutions (b) and (c).

Run time 4 times the retention time of praziquantel.

Identification of impurities Use the chromatogram supplied with *praziquantel for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and B.

Relative retention With reference to praziquantel (retention time = about 10 min): impurity A = about 0.6; impurity B = about 2.2.

System suitability Reference solution (b):
— *resolution*: minimum 3.0 between the peaks due to impurity A and praziquantel.

Limits:
- — *correction factor*: for the calculation of content, multiply the peak area of impurity B by 1.4;
- — *impurities A, B*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);
- — *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);
- — *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);
- — *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 50 °C at a pressure not exceeding 0.7 kPa for 2 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

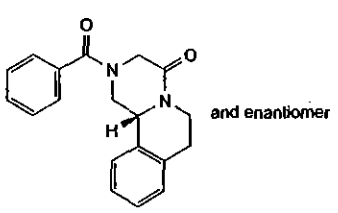
Injection Test solution (b) and reference solution (a).

Calculate the percentage content of $C_{19}H_{24}N_2O_2$ taking into account the assigned content of *praziquantel CRS*.

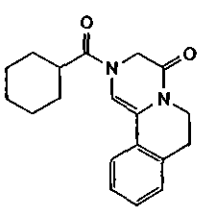
STORAGE
Protected from light.

IMPURITIES
Specified impurities *A, B.*

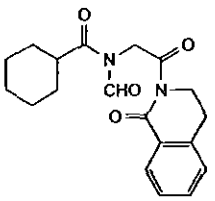
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C.*



A. (11b*RS*)-2-benzoyl-1,2,3,6,7,11b-hexahydro-4*H*-pyrazino [2,1-*a*]isoquinolin-4-one,



B. 2-(cyclohexylcarbonyl)-2,3,6,7-tetrahydro-4*H*-pyrazino [2,1-*a*]isoquinolin-4-one,

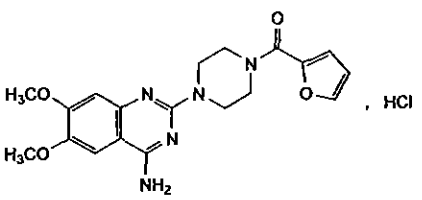


C. *N*-formyl-*N*-[2-oxo-2-(1-oxo-3,4-dihydroisoquinolin-2 (1*H*)-yl)ethyl]cyclohexanecarboxamide.

Ph Eur

Prazosin Hydrochloride

(Ph. Eur. monograph 0856)



$C_{19}H_{22}ClN_5O_4$ 419.9 *19237-84-4*

Action and use
Alpha1-adrenoceptor antagonist.

Preparation
Prazosin Tablets

Ph Eur

DEFINITION
[4-(4-Amino-6,7-dimethoxyquinazolin-2-yl)piperazin-1-yl] (furan-2-yl)methanone hydrochloride.

Content
98.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white powder.

Solubility
Very slightly soluble in water, slightly soluble in ethanol (96 per cent) and in methanol, practically insoluble in acetone.

IDENTIFICATION
First identification: A, C.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *prazosin hydrochloride CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in a mixture of 1 volume of *diethylamine R*, 10 volumes of *methanol R* and 10 volumes of *methylene chloride R* and dilute to 10 mL with the same mixture of solvents.

Reference solution Dissolve 10 mg of *prazosin hydrochloride CRS* in a mixture of 1 volume of *diethylamine R*, 10 volumes of *methanol R* and 10 volumes of *methylene chloride R* and dilute to 10 mL with the same mixture of solvents.

Plate *TLC silica gel GF_{254} plate R.*

Mobile phase *diethylamine R, ethyl acetate R* (5:95 *V/V*).

Application 10 µL.

Development Over 2/3 of the plate.

Drying In a current of warm air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. Dissolve about 2 mg in 2 mL of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dilute 1 mL of the test solution to 10 mL with the mobile phase. Use 1 mL of this solution to dissolve the contents of a vial of *prazosin impurity A CRS*.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 50 volumes of *methanol R* and 50 volumes of a solution containing 3.5 g/L of *sodium pentanesulfonate R* and 3.6 g/L of *tetramethylammonium hydroxide R* previously adjusted to pH 5.0 with *glacial acetic acid R*.

Flow rate 1 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL.

Run time 4 times the retention time of prazosin.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity A.

Relative retention With reference to prazosin (retention time = about 8 min): impurity A = about 0.8.

System suitability Reference solution (b):
- *resolution*: minimum 3.0 between the peaks due to impurity A and prazosin.

Calculation of percentage contents:
- for each impurity, use the concentration of prazosin hydrochloride in reference solution (a).

Limits:
- *impurity A*: maximum 0.2 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.5 per cent;
- *reporting threshold*: 0.05 per cent.

Iron

Maximum 100 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution To 1.0 g add dropwise about 1.5 mL of *nitric acid R*. After fuming has subsided, evaporate on a water-bath and ignite by gradually raising the temperature from 150 °C to 1000 ± 50 °C, maintaining the final temperature for 1 h. Cool, dissolve the residue in 20 mL of *dilute hydrochloric acid R*, evaporate to about 5 mL and dilute to 25.0 mL with *dilute hydrochloric acid R*.

Reference solutions Prepare the reference solutions using *iron standard solution (8 ppm Fe) R*, diluted as necessary with *water R*.

Source Iron hollow-cathode lamp.

Wavelength 248 nm.

Flame Air-acetylene.

Water (*2.5.12*)

Maximum 0.5 per cent, determined on 1.00 g using a mixture of equal volumes of *methanol R* and *methylene chloride R* as solvent.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

In order to avoid overheating in the reaction medium, mix thoroughly throughout the titration and stop the titration immediately after the end-point has been reached.

Dissolve 0.350 g in a mixture of 20 mL of *anhydrous formic acid R* and 30 mL of *acetic anhydride R*. Titrate quickly with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 41.99 mg of $C_{19}H_{22}ClN_5O_4$.

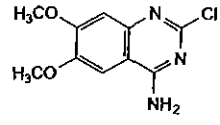
STORAGE

Protected from light.

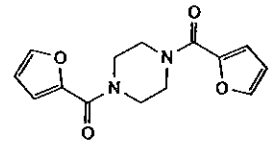
IMPURITIES

Specified impurities *A*.

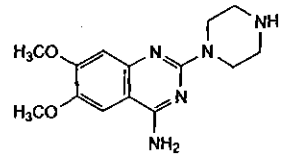
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D, E*.



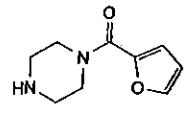
A. 2-chloro-6,7-dimethoxyquinazolin-4-amine,



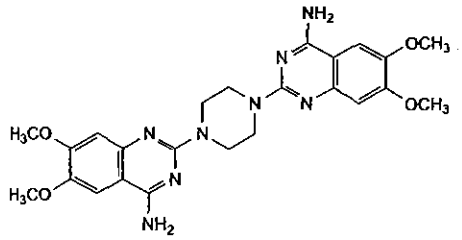
B. (piperazin-1,4-diyl)bis[(furan-2-yl)methanone],



C. 6,7-dimethoxy-2-(piperazin-1-yl)quinazolin-4-amine,



D. (furan-2-yl)(piperazin-1-yl)methanone,

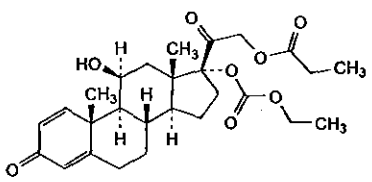


E. 2,2′-(piperazin-1,4-diyl)bis(6,7-dimethoxyquinazolin-4-amine).

Ph Eur

Prednicarbate

(Ph. Eur. monograph 1467)



$C_{27}H_{36}O_8$ 488.6 *73771-04-7*

Action and use
Glucocorticoid.

Ph Eur

DEFINITION
11β-Hydroxy-3,20-dioxopregna-1,4-diene-17,21-diyl 17-(ethyl carbonate) 21-propanoate.

Content
97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, freely soluble in acetone and in ethanol (96 per cent), sparingly soluble in propylene glycol.

It shows polymorphism (*5.9*).

IDENTIFICATION
First identification: A.

Second identification: B.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *prednicarbate CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *ethanol (96 per cent) R*, evaporate to dryness on a water-bath and record new spectra using the residues.

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution Dissolve 10 mg of *prednicarbate CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *methanol R, methylene chloride R* (10:90 *V/V*).

Application 5 µL; the volume may be adapted based on the type of plate used.

Development Over 3/4 of the plate.

Drying In air.

Detection Spray with a solution prepared as follows: dissolve 0.25 g of *2,4-dihydroxybenzaldehyde R* in *glacial acetic acid R*, dilute to 50 mL with the same solvent and add a mixture of 12.5 mL of *sulfuric acid R* and 37.5 mL of *glacial acetic acid R*; heat the plate at 90 °C for 35 min or until the spots appear, allow to cool and examine in daylight and in ultraviolet light at 365 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS
Specific optical rotation (*2.2.7*)
+ 60 to + 66 (dried substance).

Dissolve 0.250 g in *ethanol (96 per cent) R* and dilute to 25.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 30.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 3 mg of *prednicarbate for system suitability A CRS* (containing impurities B, C, D, E and F) in the mobile phase and dilute to 5 mL with the mobile phase.

Reference solution (b) Dissolve 3 mg of *prednicarbate for peak identification CRS* (containing impurity G) in the mobile phase and dilute to 5 mL with the mobile phase.

Reference solution (c) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (d) Dissolve 30.0 mg of *prednicarbate CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase.

Column:
— *size: l* = 0.125 m, Ø = 4 mm;
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile for chromatography R, water for chromatography R* (50:60 *V/V*).

Flow rate 0.7 mL/min.

Detection Spectrophotometer at 243 nm.

Injection 20 µL of the test solution and reference solutions (a), (b) and (c).

Run time 2.6 times the retention time of prednicarbate.

Identification of impurities Use the chromatogram supplied with *prednicarbate for system suitability A CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities B, C, D, E and F; use the chromatogram supplied with *prednicarbate for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity G.

Relative retention With reference to prednicarbate (retention time = about 20 min): impurity B = about 0.25; impurity C = about 0.35; impurity D = about 0.39; impurity E = about 0.6; impurity F = about 1.2; impurity G = about 2.4.

System suitability Reference solution (a):
— *resolution*: minimum 3.0 between the peaks due to prednicarbate and impurity F; minimum 1.5 between the peaks due to impurities C and D.

Calculation of percentage contents:
— for each impurity, use the concentration of prednicarbate in reference solution (c).

Limits:
— *impurity F*: maximum 0.8 per cent;
— *impurity C*: maximum 0.5 per cent;
— *impurity E*: maximum 0.3 per cent;
— *impurities B, D*: for each impurity, maximum 0.2 per cent;
— *impurity G*: maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 2.0 per cent;
— *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (d).

Calculate the percentage content of $C_{27}H_{36}O_8$ taking into account the assigned content of *prednicarbate CRS*.

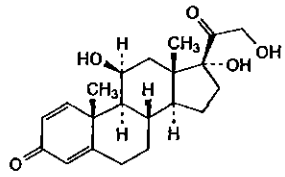
STORAGE

Protected from light.

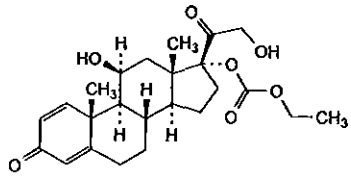
IMPURITIES

Specified impurities *B, C, D, E, F, G.*

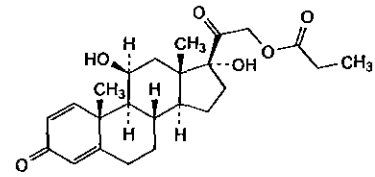
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A.*



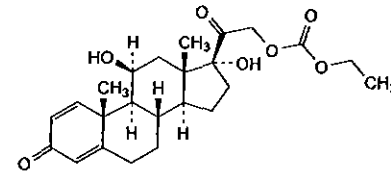
A. 11β,17,21-trihydroxypregna-1,4-diene-3,20-dione (prednisolone),



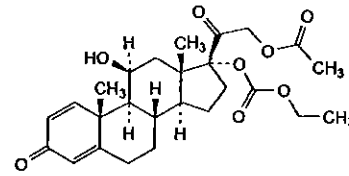
B. ethyl 11β,21-dihydroxy-3,20-dioxopregna-1,4-dien-17-yl carbonate (prednisolone 17-ethylcarbonate),



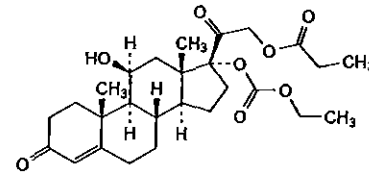
C. 11β,17-dihydroxy-3,20-dioxopregna-1,4-dien-21-yl propanoate (prednisolone 21-propanoate),



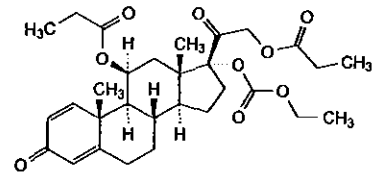
D. ethyl 11β,17-dihydroxy-3,20-dioxopregna-1,4-dien-21-yl carbonate (prednisolone 21-ethylcarbonate),



E. 11β-hydroxy-3,20-dioxopregna-1,4-diene-17,21-diyl 21-acetate 17-(ethyl carbonate) (prednisolone 21-acetate 17-ethylcarbonate),



F. 11β-hydroxy-3,20-dioxopregn-4-ene-17,21-diyl 17-(ethyl carbonate) 21-propanoate (1,2-dihydroprednicarbate),

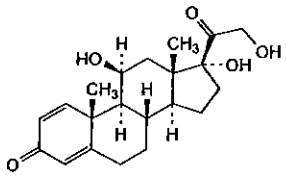


G. 3,20-dioxo-11β-pregna-1,4-diene-11,17,21-triyl 17-(ethyl carbonate) 11,21-dipropanoate (prednicarbate 11-propanoate).

Ph Eur

Prednisolone

(Ph. Eur. monograph 0353)



$C_{21}H_{28}O_5$ 360.5 *50-24-8*

Action and use
Glucocorticoid.

Preparations
Prednisolone Tablets
Prednisolone Gastro-resistant Tablets

Ph Eur

DEFINITION
11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione.

Content
96.5 per cent to 102.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline, hygroscopic powder.

Solubility
Very slightly soluble in water, soluble in ethanol (96 per cent) and in methanol, sparingly soluble in acetone, slightly soluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION
First identification: A, B.

Second identification: C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *prednisolone CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *acetone R*, evaporate to dryness on a water-bath and record new spectra using the residues.

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (d).

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution Dissolve 10 mg of *prednisolone CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *methanol R, methylene chloride R* (10:90 *V/V*).

Application 5 µL.

Development Over 3/4 of the plate.

Drying In air.

Detection Spray with a solution prepared as follows: dissolve 0.25 g of *2,4-dihydroxybenzaldehyde R* in *glacial acetic acid R*, dilute to 50 mL with the same solvent and add a mixture of 12.5 mL of *sulfuric acid R* and 37.5 mL of *glacial acetic acid R*; heat at 90 °C for 35 min or until the spots appear, allow to cool and examine in daylight and in ultraviolet light at 365 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS
Specific optical rotation (*2.2.7*)
+ 113 to + 119 (dried substance).

Dissolve 0.250 g in *ethanol (96 per cent) R* and dilute to 25.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Solvent mixture *acetonitrile R, water R* (40:60 *V/V*).

Test solution (a) Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 20.0 mL with the solvent mixture.

Test solution (b) Dissolve 25.0 mg of the substance to be examined in the solvent mixture and dilute to 20.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 10.0 mL with the solvent mixture.

Reference solution (a) Dissolve 5 mg of *prednisolone for system suitability CRS* (containing impurities A, B and C) in the solvent mixture and dilute to 10 mL with the solvent mixture.

Reference solution (b) Dissolve 5 mg of *prednisolone for peak identification CRS* (containing impurities F and J) in the solvent mixture and dilute to 10 mL with the solvent mixture.

Reference solution (c) Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (d) Dissolve 25.0 mg of *prednisolone CRS* in the solvent mixture and dilute to 20.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 10.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography compatible with 100 per cent aqueous mobile phases R* (3 µm);
— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: *water for chromatography R*;
— *mobile phase B*: *acetonitrile R, methanol R* (50:50 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 14	60	40
14 - 20	60 → 20	40 → 80
20 - 25	20	80

Flow rate 1 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µL of test solution (a) and reference solutions (a), (b) and (c).

Identification of impurities Use the chromatogram supplied with *prednisolone for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B and C; use the

chromatogram supplied with *prednisolone for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities F and J.

Relative retention With reference to prednisolone (retention time = about 12 min): impurity F = about 0.7; impurity B = about 0.9; impurity A = about 1.05; impurity J = about 1.5; impurity C = about 1.7.

System suitability Reference solution (a):

— *peak-to-valley ratio*: minimum 3, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to prednisolone.

Limits:

— *impurity A*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent);

— *impurity F*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *impurities B, C, J*: for each impurity, not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);

— *total*: not more than 15 times the area of the principal peak in the chromatogram obtained with reference solution (c) (1.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Loss on drying *(2.2.32)*

Maximum 1.0 per cent, determined on 0.500 g by drying in an oven at 105 °C.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (d).

Calculate the percentage content of $C_{21}H_{28}O_5$ taking into account the assigned content of *prednisolone CRS*.

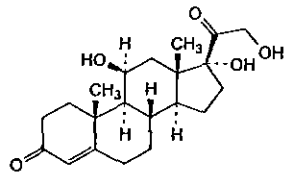
STORAGE

In an airtight container, protected from light.

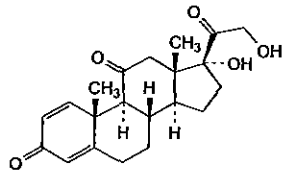
IMPURITIES

Specified impurities *A, B, C, F, J.*

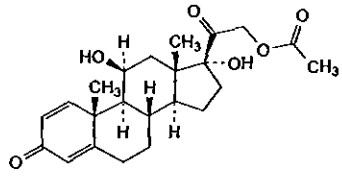
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *D, E, G, H, I.*



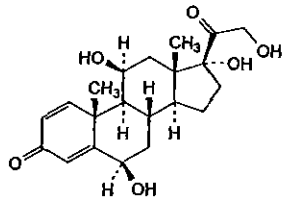
A. 11β,17,21-trihydroxypregn-4-ene-3,20-dione (hydrocortisone),



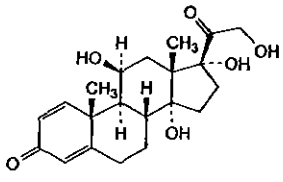
B. 17,21-dihydroxypregna-1,4-diene-3,11,20-trione (prednisone),



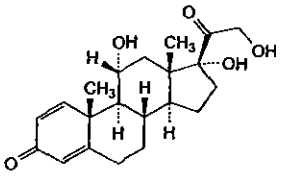
C. 11β,17-dihydroxy-3,20-dioxopregna-1,4-dien-21-yl acetate (prednisolone acetate),



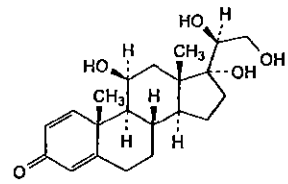
D. 6β,11β,17,21-tetrahydroxypregna-1,4-diene-3,20-dione (6β-hydroxyprednisolone),



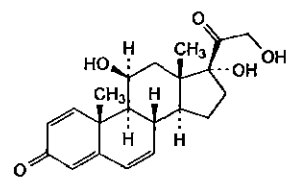
E. 11β,14α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione (14α-hydroxyprednisolone),



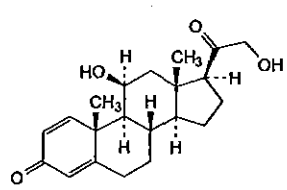
F. 11α,17,21-trihydroxypregna-1,4-diene-3,20-dione (11-*epi*-prednisolone),



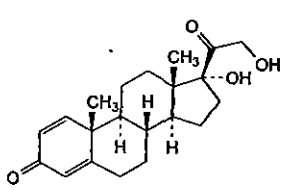
G. 11β,17,20β,21-tetrahydroxypregna-1,4-dien-3-one (20β-hydroxyprednisolone),



H. 11β,17,21-trihydroxypregna-1,4,6-triene-3,20-dione (Δ^6-prednisolone),



I. 11β,21-dihydroxypregna-1,4-diene-3,20-dione (17-deoxyprednisolone),

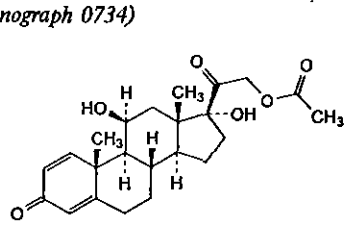


J. 17,21-dihydroxypregna-1,4-diene-3,20-dione (11-deoxyprednisolone).

Ph Eur

Prednisolone Acetate

(Ph. Eur. monograph 0734)



$C_{23}H_{30}O_6$ 402.5 *52-21-1*

Action and use
Glucocorticoid.

Preparation
Prednisolone Acetate Injection

Ph Eur

DEFINITION
11β,17-Dihydroxy-3,20-dioxopregna-1,4-dien-21-yl acetate.

Content
97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, slightly soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION
First identification: A, B.
Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *prednisolone acetate CRS.*

B. Thin-layer chromatography (*2.2.27*).
Test solution Dissolve 10 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.
Reference solution Dissolve 10 mg of *prednisolone acetate CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase.
Plate *TLC silica gel F_{254} plate R.*
Mobile phase *methanol R, methylene chloride R* (10:90 *V/V*).
Application 5 µL.
Development Over 3/4 of the plate.
Drying In air.
Detection Spray with a solution prepared as follows: dissolve 0.25 g of *2,4-dihydroxybenzaldehyde R* in *glacial acetic acid R*, dilute to 50 mL with the same solvent and add a mixture of 12.5 mL of *sulfuric acid R* and 37.5 mL of *glacial acetic acid R*; heat at 90 °C for 35 min or until the spots appear, allow to cool and examine in daylight and in ultraviolet light at 365 nm.
Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. Add about 2 mg to 2 mL of *sulfuric acid R* and shake to dissolve. Within 5 min, an intense red colour develops. When examined in ultraviolet light at 365 nm, a reddish-brown fluorescence is seen. Add the solution to 10 mL of *water R* and mix. The colour fades and there is greenish-yellow fluorescence in ultraviolet light at 365 nm.

TESTS

Specific optical rotation (*2.2.7*)
+ 128 to + 137 (dried substance).
Dissolve 70.0 mg in *methanol R2* and dilute to 20.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*
Buffer solution pH 4 Mix 1 volume of *dilute hydrochloric acid R*, 5 volumes of a 68.1 g/L solution of *sodium acetate R*, 15 volumes of a 37.3 g/L solution of *potassium chloride R* and 79 volumes of *water R*.
Solvent mixture Mix equal volumes of *acetonitrile R* and buffer solution pH 4.
Test solution Dissolve 25.0 mg of the substance to be examined in *methanol R* and dilute to 10.0 mL with the same solvent.
Reference solution (a) Dissolve 2 mg of *prednisolone acetate CRS* and 2 mg of *hydrocortisone acetate CRS* (impurity A) in the solvent mixture and dilute to 100 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 2.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 5 mg of *prednisolone acetate for peak identification CRS* (containing impurities A, B and C) in the solvent mixture and dilute to 50 mL with the solvent mixture.

Column:
- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature:* 40 °C.

Mobile phase *acetonitrile R, water for chromatography R* (35:65 *V/V*).

Flow rate 1 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL.

Run time 2.5 times the retention time of prednisolone acetate.

Identification of impurities Use the chromatogram supplied with *prednisolone acetate for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B and C.

Relative retention With reference to prednisolone acetate (retention time = about 17 min): impurity B = about 0.4; impurity A = about 1.1; impurity C = about 2.0.

System suitability Reference solution (a):
- *resolution:* minimum 2.0 between the peaks due to prednisolone acetate and impurity A.

Limits:
- *impurities A, B:* for each impurity, not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
- *impurity C:* not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *unspecified impurities:* for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- *total:* not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);
- *disregard limit:* 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying *(2.2.32)*
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.100 g in *ethanol (96 per cent) R* and dilute to 100.0 mL with the same solvent. Dilute 2.0 mL of the solution to 100.0 mL with *ethanol (96 per cent) R*. Measure the absorbance *(2.2.25)* at the absorption maximum at 243 nm.

Calculate the content of $C_{23}H_{30}O_6$ taking the specific absorbance to be 370.

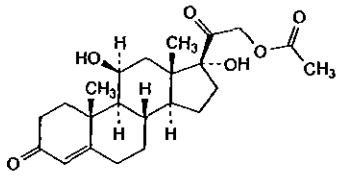
STORAGE

Protected from light.

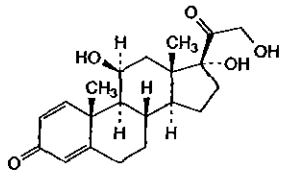
IMPURITIES

Specified impurities *A, B, C.*

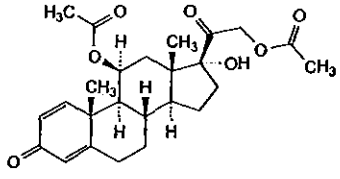
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *D, E.*



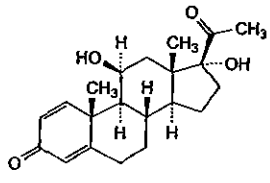
A. 11β,17-dihydroxy-3,20-dioxopregn-4-en-21-yl acetate (hydrocortisone acetate),



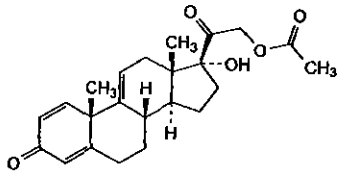
B. 11β,17,21-trihydroxypregna-1,4-diene-3,20-dione (prednisolone),



C. 17-hydroxy-3,20-dioxopregna-1,4-diene-11β,21-diyl diacetate (prednisolone 11,21-diacetate),



D. 11β,17-dihydroxypregna-1,4-diene-3,20-dione,

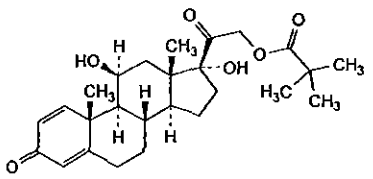


E. 17-hydroxy-3,20-dioxopregna-1,4,9(11)-trien-21-yl acetate.

Ph Eur

Prednisolone Pivalate

(Ph. Eur. monograph 0736)



$C_{26}H_{36}O_6$ 444.6 *1107-99-9*

Action and use
Glucocorticoid.

Ph Eur

DEFINITION

11β,17-Dihydroxy-3,20-dioxopregna-1,4-dien-21-yl 2,2-dimethylpropanoate.

Content
97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, slightly soluble in ethanol (96 per cent), soluble in methylene chloride.

mp
About 229 °C, with decomposition.

IDENTIFICATION

First identification: B, C.

Second identification: A, C, D.

A. Dissolve 10.0 mg in *anhydrous ethanol R* and dilute to 100.0 mL with the same solvent. Place 2.0 mL of this solution in a ground-glass-stoppered tube, add 10.0 mL of *phenylhydrazine-sulfuric acid solution R*, mix and heat in a water-bath at 60 °C for 20 min. Cool immediately. The absorbance (*2.2.25*) at the absorption maximum at 415 nm is 0.20 to 0.30.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *prednisolone pivalate CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *ethanol (96 per cent) R*, evaporate to dryness on a water-bath and record new spectra using the residues.

C. Thin-layer chromatography (*2.2.27*).

Solvent mixture *methanol R, methylene chloride R* (1:9 *V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 10 mL with the solvent mixture.

Reference solution (a) Dissolve 10 mg of *prednisolone pivalate CRS* in the solvent mixture and dilute to 10 mL with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *prednisolone acetate CRS* in the solvent mixture and dilute to 10 mL with the solvent mixture. Dilute 5 mL of this solution to 10 mL with reference solution (a).

Plate *TLC silica gel F_{254} plate R.*

Mobile phase Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R.*

Application 5 µL.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with *alcoholic solution of sulfuric acid R*, heat at 120 °C for 10 min or until the spots appear, and allow to cool; examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability Reference solution (b):
— the chromatogram shows 2 clearly separated spots.

D. To 2 mL of *sulfuric acid R*, add about 2 mg and shake to dissolve. Within 5 min, an intense red colour develops. When examined in ultraviolet light at 365 nm, a reddish-brown fluorescence is seen. Add this solution to 10 mL of *water R* and mix. The colour fades and there is greenish-yellow fluorescence in ultraviolet light at 365 nm.

TESTS

Specific optical rotation (*2.2.7*)
+ 104 to + 112 (dried substance).

Dissolve 0.250 g in *dioxan R* and dilute to 25.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 62.5 mg of the substance to be examined in 2 mL of a mixture of 1 volume of *water R* and 4 volumes of *tetrahydrofuran R* and dilute to 25.0 mL with the mobile phase.

Reference solution (a) Dissolve 25 mg of *prednisolone acetate CRS*, 25 mg of *cortisone acetate CRS* and 25 mg of *prednisolone pivalate CRS* in 2 mL of a mixture of 1 volume of *water R* and 4 volumes of *tetrahydrofuran R* and dilute to 25.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 25.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 50.0 mL with the mobile phase.

Column:
— *size: l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Carefully mix 19 mL of *butyl acetate R1* with 37 mL of *tetrahydrofuran R* and 213 mL of *ethylene glycol monomethyl ether R*, then add with 231 mL of *water R*; mix, allow to equilibrate for 1 h and filter through a 0.45 µm filter.

Flow rate 1 mL/min.

Detection Spectrophotometer at 254 nm.

Equilibration With the mobile phase for about 30 min.

Injection 20 µL.

Run time 1.5 times the retention time of prednisolone pivalate.

Retention time Prednisolone acetate = about 3.5 min; cortisone acetate = about 4.5 min; prednisolone pivalate = about 13 min.

System suitability Reference solution (a):
— *resolution*: minimum 2.5 between the peaks due to prednisolone acetate and cortisone acetate; if necessary, adjust the concentration of water in the mobile phase.

Limits:
— *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent), and not more than one such peak has an area greater than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
— *total*: not more than 1.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent);
— *disregard limit*: 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY
Dissolve 0.100 g in *ethanol (96 per cent) R* and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of this solution to 250.0 mL with *ethanol (96 per cent) R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 243 nm.

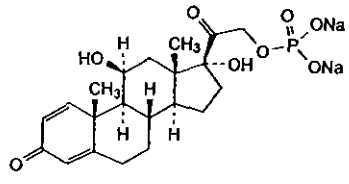
Calculate the content of $C_{26}H_{36}O_6$ taking the specific absorbance to be 337.

STORAGE
Protected from light.

Ph Eur

Prednisolone Sodium Phosphate

(Ph. Eur. monograph 0735)



$C_{21}H_{27}Na_2O_8P$ 484.4 *125-02-0*

Action and use
Glucocorticoid.

Preparations
Prednisolone Enema
Prednisolone Gastro-resistant Tablets
Prednisolone Sodium Phosphate Ear Drops
Prednisolone Soluble Tablets
Prednisolone Sodium Phosphate Oral Solution

Ph Eur

DEFINITION
11β,17-Dihydroxy-3,20-dioxopregna-1,4-dien-21-yl disodium phosphate.

Content
96.0 per cent to 103.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white, hygroscopic, crystalline powder.

Solubility
Freely soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION
First identification: B, C.

Second identification: A, C, D, E.

A. Dissolve 10.0 mg in 5 mL of *water R* and dilute to 100.0 mL with *anhydrous ethanol R*. Place 2.0 mL of this solution in a ground-glass-stoppered tube, add 10.0 mL of *phenylhydrazine-sulfuric acid solution R*, mix and heat in a water-bath at 60 °C for 20 min. Cool immediately. The absorbance (*2.2.25*) at the absorption maximum at 415 nm is 0.10 to 0.20.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *prednisolone sodium phosphate CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *ethanol (96 per cent) R*, evaporate to dryness on a water-bath and record new spectra using the residues.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dissolve 10 mg of *prednisolone sodium phosphate CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 10 mg of *dexamethasone sodium phosphate CRS* in *methanol R* and dilute to 10 mL with the same solvent. Dilute 5 mL of this solution to 10 mL with reference solution (a).

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *glacial acetic acid R, water R, butanol R* (20:20:60 *V/V/V*).

Application 5 µL.

Development Over a path of 15 cm.

Drying In air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with *alcoholic solution of sulfuric acid R*, heat at 120 °C for 10 min or until the spots appear, and allow to cool; examine in daylight and in ultraviolet light at 365 nm.

Results B The principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability Reference solution (b):
— the chromatogram shows 2 spots which may, however, not be completely separated.

D. To 2 mL of *sulfuric acid R* add about 2 mg and shake to dissolve. Within 5 min, an intense red colour develops. When

examined in ultraviolet light at 365 nm, a reddish-brown fluorescence is seen. Add this solution to 10 mL of *water R* and mix. The colour fades and there is a greenish-yellow fluorescence in ultraviolet light at 365 nm.

E. To about 40 mg add 2 mL of *sulfuric acid R* and heat gently until white fumes are evolved. Add *nitric acid R* dropwise, continue the heating until the solution is almost colourless, and cool. Add 2 mL of *water R*, heat until white fumes are again evolved, cool, add 10 mL of *water R* and neutralise to *red litmus paper R* with *dilute ammonia R1*. The solution gives reaction (a) of sodium (*2.3.1*) and reaction (b) of phosphates (*2.3.1*).

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_7 (*2.2.2, Method II*).

pH (*2.2.3*)

7.5 to 9.0 for solution S.

Specific optical rotation (*2.2.7*)

+ 94 to + 100 (anhydrous substance).

Dissolve 0.250 g in *water R* and dilute to 25.0 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 62.5 mg of the substance to be examined in the mobile phase and dilute to 25.0 mL with the mobile phase.

Reference solution (a) Dissolve 25 mg of *prednisolone sodium phosphate CRS* and 25 mg of *prednisolone CRS* in the mobile phase and dilute to 25.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 25.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 50.0 mL with the mobile phase.

Column:
- *size: l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Into a 250 mL conical flask weigh 1.360 g of *potassium dihydrogen phosphate R* and 0.600 g of *hexylamine R*, mix, allow to stand for 10 min, then dissolve in 185 mL of *water R*; add 65 mL of *acetonitrile R*, mix, and filter through a 0.45 µm filter.

Flow rate 1 mL/min.

Detection Spectrophotometer at 254 nm.

Equilibration With the mobile phase for about 30 min.

Injection 20 µL.

Run time 3 times the retention time of prednisolone sodium phosphate.

Retention time Prednisolone sodium phosphate = about 6.5 min; prednisolone = about 8.5 min.

System suitability Reference solution (a):
- *resolution*: minimum 4.5 between the peaks due to prednisolone sodium phosphate and prednisolone; if necessary, increase the concentration of *acetonitrile R* or *water R* in the mobile phase.

Limits:
- *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent), and not more than 1 such peak has an area greater than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);
- *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3 per cent);
- *disregard limit*: 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Inorganic phosphate

Maximum 1 per cent.

Dissolve 50 mg in *water R* and dilute to 100 mL with the same solvent. To 10 mL of this solution add 5 mL of *molybdovanadic reagent R*, mix, and allow to stand for 5 min. Any yellow colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using 10 mL of *phosphate standard solution (5 ppm PO_4) R*.

Water (*2.5.12*)

Maximum 8.0 per cent, determined on 0.200 g.

ASSAY

Dissolve 0.100 g in *water R* and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of this solution to 250.0 mL with *water R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 247 nm.

Calculate the content of $C_{21}H_{27}Na_2O_8P$ taking the specific absorbance to be 312.

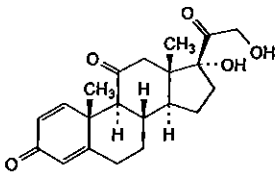
STORAGE

Protected from light.

Ph Eur

Prednisone

(Ph. Eur. monograph 0354)



$C_{21}H_{26}O_5$ 358.4 *53-03-2*

Action and use

Glucocorticoid.

Ph Eur

DEFINITION

17,21-Dihydroxypregna-1,4-diene-3,11,20-trione.

Content

97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, slightly soluble in ethanol (96 per cent) and in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification: A, C.

Second identification: B, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *prednisone CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *acetone R*, evaporate to dryness on a water-bath and record new spectra using the residues.

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution Dissolve 10 mg of *prednisone CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *methanol R, methylene chloride R* (10:90 *V/V*).

Application 5 µL.

Development Over 3/4 of the plate.

Drying In air.

Detection Spray with a solution prepared as follows: dissolve 0.25 g of *2,4-dihydroxybenzaldehyde R* in *glacial acetic acid R*, dilute to 50 mL with the same solvent and add a mixture of 12.5 mL of *sulfuric acid R* and 37.5 mL of *glacial acetic acid R*; heat at 90 °C for 35 min or until the spots appear, allow to cool and examine in daylight and in ultraviolet light at 365 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (d).

D. Add about 2 mg to 2 mL of *sulfuric acid R* and shake to dissolve. Within 5 min, a yellow colour develops with a blue fluorescence in ultraviolet light at 365 nm. Add this solution to 10 mL of *water R* and mix. The colour fades but the blue fluorescence in ultraviolet light does not disappear.

TESTS

Specific optical rotation (*2.2.7*)

+ 183 to + 191 (dried substance).

Dissolve 0.125 g in *ethanol (96 per cent) R* and dilute to 25.0 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*). *Carry out the test protected from light. Prepare the solutions immediately before use.*

Solvent mixture *acetonitrile R, water R* (50:50 *V/V*).

Test solution (a) Dissolve 20.0 mg of the substance to be examined in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

Test solution (b) Dilute 5.0 mL of test solution (a) to 20.0 mL with the solvent mixture.

Reference solution (a) Dissolve 10 mg of *prednisone impurity B CRS* in the solvent mixture and dilute to 25 mL with the solvent mixture. Dilute 1 mL of the solution to 50 mL with the solvent mixture.

Reference solution (b) Dissolve 4 mg of *prednisone for peak identification CRS* (containing impurities A, D and E) in the solvent mixture, add 1 mL of reference solution (a) and dilute to 5 mL with the solvent mixture.

Reference solution (c) Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (d) Dissolve 20.0 mg of *prednisone CRS* in the solvent mixture and dilute to 25.0 mL with the solvent mixture. Dilute 5.0 mL of the solution to 20.0 mL with the solvent mixture.

Column:

— *size*: l = 0.15 m, Ø = 3.0 mm;

— *stationary phase*: *end-capped ethylene-bridged polar-embedded octadecylsilyl silica gel for chromatography (hybrid material) R* (2.5 µm);

— *temperature*: 45 °C.

Mobile phase:

— *mobile phase A*: 0.68 g/L solution of *potassium dihydrogen phosphate R*, adjusted to pH 2.0 with *phosphoric acid R*;

— *mobile phase B*: *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	83	17
2 - 25	83 → 80	17 → 20
25 - 28	80 → 65	20 → 35
28 - 33	65	35
33 - 43	65 → 20	35 → 80

Flow rate 0.6 mL/min.

Detection Spectrophotometer at 244 nm.

Injection 5 µL of test solution (a) and reference solutions (a), (b) and (c).

Identification of impurities Use the chromatogram supplied with *prednisone for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, D and E; use the chromatogram obtained with reference solution (a) to identify the peak due to impurity B.

Relative retention With reference to prednisone (retention time = about 18 min): impurity B = about 1.06; impurity A = about 1.12; impurity D = about 1.6; impurity E = about 1.9.

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to impurity B and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to prednisone.

Calculation of percentage contents:

— for each impurity, use the concentration of prednisone in reference solution (c).

Limits:

— *impurity A*: maximum 0.5 per cent;

— *impurity E*: maximum 0.2 per cent;

— *impurity D*: maximum 0.15 per cent;

— *unspecified impurities*: for each impurity, maximum 0.10 per cent;

— *total*: maximum 1.0 per cent;

— *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 0.500 g by drying in an oven at 105 °C.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (d).

Calculate the percentage content of $C_{21}H_{26}O_5$ taking into account the assigned content of *prednisone CRS*.

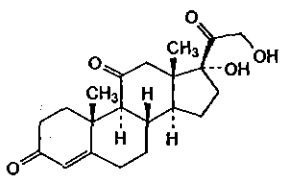
STORAGE

Protected from light.

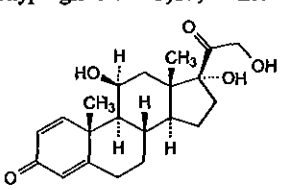
IMPURITIES

Specified impurities *A, D, E.*

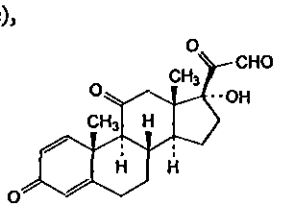
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, F, G, J, K, L.*



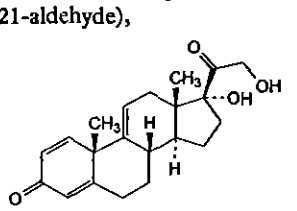
A. 17,21-dihydroxypregn-4-ene-3,11,20-trione (cortisone),



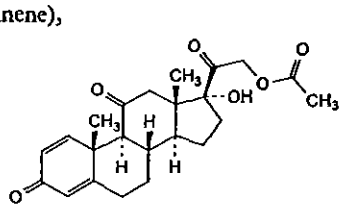
B. 11β,17,21-trihydroxypregna-1,4-diene-3,20-dione (prednisolone),



C. 17-hydroxy-3,11,20-trioxopregna-1,4-dien-21-al (prednisone-21-aldehyde),



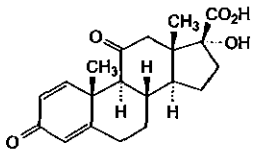
D. 17,21-dihydroxypregna-1,4,9(11)-triene-3,20-dione (deltacortinene),



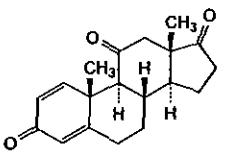
E. 17-hydroxy-3,11,20-trioxopregna-1,4-dien-21-yl acetate (prednisone acetate),

F. unknown structure,

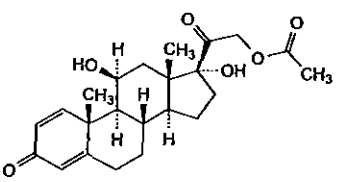
G. unknown structure,



J. 17α-hydroxy-3,11-dioxoandrosta-1,4-diene-17β-carboxylic acid,



K. androsta-1,4-diene-3,11,17-trione,

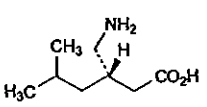


L. 11β,17-dihydroxy-3,20-dioxopregna-1,4-dien-21-yl acetate (prednisolone acetate).

Ph Eur

Pregabalin

(Ph. Eur. monograph 2777)



$C_8H_{17}NO_2$ 159.2 *148553-50-8*

Action and use
Antiepileptic.

Preparations
Pregabalin Capsules
Pregabalin Oral Solution

Ph Eur

DEFINITION

(3*S*)-3-(Aminomethyl)-5-methylhexanoic acid.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Sparingly soluble in water, very slightly soluble in methanol, practically insoluble in heptane.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *pregabalin CRS.*

B. Examine the chromatograms obtained in the test for enantiomeric purity.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the

principal peak in the chromatogram obtained with the reference solution.

TESTS

Enantiomeric purity

Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution Dissolve 20 mg of the substance to be examined in *water R* and dilute to 10.0 mL with the same solvent. Derivatise the solution as described under Derivatisation.

Reference solution Dissolve 2 mg of *pregabalin impurity B CRS* in *water R* and dilute to 20.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with *water R*. To 20 mg of *pregabalin CRS*, add 1.0 mL of this solution and dilute to 10.0 mL with *water R*. Derivatise this solution as described under Derivatisation.

Derivatisation Transfer 500 µL of the solution to a reaction vial. Add 500 µL of a 5 g/L solution of *1-fluoro-2,4-dinitrophenyl-5-L-alaninamide R* in *acetonitrile R*. Add 50 µL of an 84 g/L solution of *sodium hydrogen carbonate R*. Seal the vial, mix and derivatise by maintaining the vial at 40 °C for 1 h in a heating/stirring module. Stop the reaction by adding about 50 µL of a 103 g/L solution of *hydrochloric acid R*. Mix thoroughly. To 200 µL of the derivatised solution add 800 µL of the mobile phase.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 30 °C.

Mobile phase *acetonitrile R*, 1 per cent *V/V* solution of *triethylamine R* previously adjusted to pH 3.0 with *phosphoric acid R* (38:62 *V/V*).

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 340 nm.

Injection 20 µL.

Run time 2.5 times the retention time of the pregabalin derivative.

Relative retention With reference to the pregabalin derivative (retention time = about 10 min): impurity B derivative = about 1.3.

System suitability Reference solution:
- *resolution*: minimum 4.4 between the peaks due to the pregabalin derivative and impurity B derivative.

Limit:
- *impurity B*: maximum 0.15 per cent.

Related substances

A. Polar impurities eluting before pregabalin. Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dissolve 0.100 g of *pregabalin CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (c) Dissolve 5 mg of *mandelic acid R* (impurity C) in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 0.5 mL of the solution to 5.0 mL with the test solution.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography compatible with 100 per cent aqueous mobile phases R* (5 µm);
- *temperature*: 30 °C.

Mobile phase *methanol R2*, 3.40 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 6.3 with *concentrated ammonia R* (15:85 *V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µL of the test solution and reference solutions (b) and (c).

Run time 1.3 times the retention time of pregabalin.

Relative retention With reference to pregabalin (retention time = about 10 min): impurity C = about 0.6.

System suitability Reference solution (c):
- *resolution*: minimum 5.0 between the peaks due to impurity C and pregabalin.

Calculation of percentage contents:
- for each impurity, use the concentration of pregabalin in reference solution (b).

Limits:
- *unspecified impurities eluting before pregabalin*: for each impurity, maximum 0.10 per cent;
- *reporting threshold*: 0.05 per cent.

B. Non-polar impurities eluting after pregabalin. Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 2.5 mg of *pregabalin impurity D CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase (solution A). Dissolve the contents of a vial of *pregabalin impurity A CRS* in the mobile phase, add 1.0 mL of solution A and dilute to 50.0 mL with the mobile phase.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography compatible with 100 per cent aqueous mobile phases R* (5 µm);
- *temperature*: 30 °C.

Mobile phase 3.40 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 6.3 with *concentrated ammonia R*, *methanol R2* (45:55 *V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µL.

Run time 4 times the retention time of pregabalin.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and D.

Relative retention With reference to pregabalin (retention time = about 4 min): impurity A = about 2.4; impurity D = about 3.0.

System suitability Reference solution (b):
- *resolution*: minimum 3.5 between the peaks due to impurities A and D.

Calculation of percentage contents:
- for impurities A and D, use the concentration of each impurity in reference solution (b);
- for impurities other than A and D, use the concentration of pregabalin in reference solution (a).

Limits:
- *impurity A*: maximum 0.15 per cent;
- *unspecified impurities eluting after pregabalin*: for each impurity, maximum 0.10 per cent;
- *reporting threshold*: 0.05 per cent.

Limit:
- *total for tests A and B*: maximum 0.5 per cent.

Water (*2.5.12*)
Maximum 0.5 per cent, determined on 0.130 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Liquid chromatography (*2.2.29*) as described in test A for related substances with the following modification.

Injection Test solution and reference solution (a).

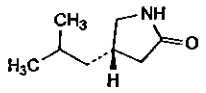
Calculate the percentage content of $C_8H_{17}NO_2$ taking into account the assigned content of *pregabalin CRS*.

IMPURITIES
Test A for related substances: C.

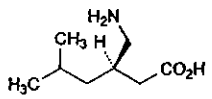
Test B for related substances: A, D.

Specified impurities *A, B.*

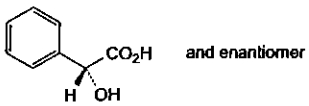
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C, D.*



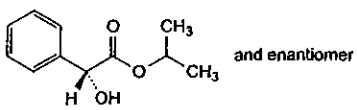
A. (4*S*)-4-(2-methylpropyl)pyrrolidin-2-one,



B. (3*R*)-3-(aminomethyl)-5-methylhexanoic acid (pregabalin enantiomer),



C. (2*RS*)-2-hydroxy-2-phenylacetic acid (mandelic acid),

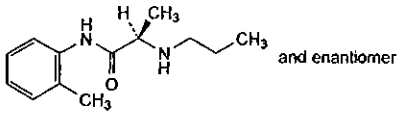


D. 1-methylethyl (2*RS*)-2-hydroxy-2-phenylacetate.

Ph Eur

Prilocaine

(Ph. Eur. monograph 1362)



$C_{13}H_{20}N_2O$ 220.3 *721-50-6*

Action and use
Local anaesthetic.

Ph Eur

DEFINITION
(2*RS*)-*N*-(2-Methylphenyl)-2-(propylamino)propanamide.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Slightly soluble in water, very soluble in acetone and in ethanol (96 per cent).

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Preparation Prepare a film between 2 plates of *sodium chloride R* by heating at 40-45 °C until the substance has melted.

Comparison *prilocaine CRS.*

TESTS
Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 2.50 g in 15 mL of *dilute hydrochloric acid R* and dilute to 50.0 mL with *water R*.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 25 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dissolve 2.5 mg of the substance to be examined and 3 mg of *prilocaine impurity E CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 10.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (c) Dissolve 33.5 mg of *prilocaine impurity B CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (d) Dissolve 15 mg of *prilocaine for peak identification CRS* (containing impurity G) in the mobile phase and dilute to 5.0 mL with the mobile phase.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase: end-capped extra-dense bonded octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 26 volumes of *acetonitrile for chromatography R* and 74 volumes of a solution prepared as follows: dissolve 0.180 g of *sodium dihydrogen phosphate monohydrate R* and 2.89 g of *disodium hydrogen phosphate dihydrate R* in 1000 mL of *water for chromatography R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 240 nm.

Injection 20 µL.

Run time Twice the retention time of prilocaine.

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peak due to impurity B; use the chromatogram obtained with reference solution (a) to identify the peak due to impurity E; use the chromatogram obtained with reference solution (d) to identify the peak due to impurity G.

Relative retention With reference to prilocaine (retention time = about 25 min): impurity B = about 0.3; impurity G = about 0.8; impurity E = about 1.2.

System suitability Reference solution (a):

— *resolution*: minimum 3.0 between the peaks due to prilocaine and impurity E.

Limits:

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (100 ppm);

— *impurity G*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.12*)
Maximum 0.5 per cent, determined on 1.000 g.

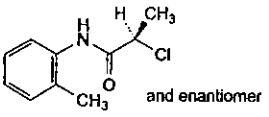
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.180 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 22.03 mg of $C_{13}H_{20}N_2O$.

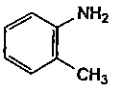
IMPURITIES
Specified impurities *B, G.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, C, D, E, F.*

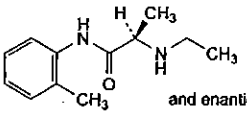


and enantiomer

A. (2*RS*)-2-chloro-*N*-(2-methylphenyl)propanamide,

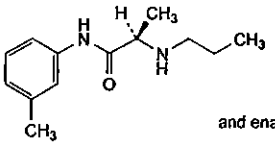


B. 2-methylbenzenamine (*o*-toluidine),



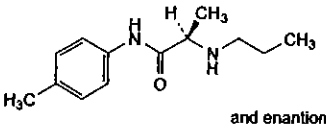
and enantiomer

C. (2*RS*)-2-(ethylamino)-*N*-(2-methylphenyl)propanamide,



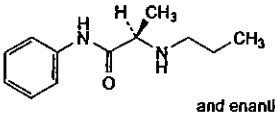
and enantiomer

D. (2*RS*)-*N*-(3-methylphenyl)-2-(propylamino)propanamide,



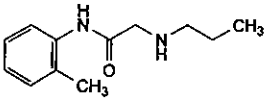
and enantiomer

E. (2*RS*)-*N*-(4-methylphenyl)-2-(propylamino)propanamide,



and enantiomer

F. (2*RS*)-*N*-phenyl-2-(propylamino)propanamide,

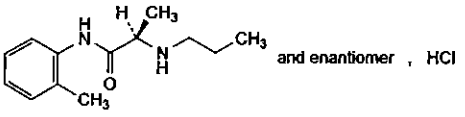


G. *N*-(2-methylphenyl)-2-(propylamino)acetamide.

Ph Eur

Prilocaine Hydrochloride

(Ph. Eur. monograph 1363)



and enantiomer , HCl

$C_{13}H_{21}ClN_2O$ 256.8 *1786-81-8*

Action and use
Local anaesthetic.

Preparation
Prilocaine Injection

Ph Eur

DEFINITION

(2*RS*)-*N*-(2-Methylphenyl)-2-(propylamino)propanamide hydrochloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water and in ethanol (96 per cent), very slightly soluble in acetone.

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 168 °C to 171 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *prilocaine hydrochloride CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 20.0 mg of the substance to be examined in *ethanol (96 per cent) R* and dilute to 5 mL with the same solvent.

Reference solution (a) Dissolve 20.0 mg of *prilocaine hydrochloride CRS* in *ethanol (96 per cent) R* and dilute to 5 mL with the same solvent.

Reference solution (b) Dissolve 20.0 mg of *lidocaine hydrochloride CRS* and 20.0 mg of *prilocaine hydrochloride CRS* in *ethanol (96 per cent) R* and dilute to 5 mL with the same solvent.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *concentrated ammonia R, methanol R, 1,1-dimethylethyl methyl ether R* (1:5:100 *V/V/V*).

Application 10 µL.

Development Over a path of 12 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 50.0 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

Dilute 4 mL of solution S to 10 mL with *carbon dioxide-free water R*. Add 0.1 mL of *bromocresol green solution R* and 0.40 mL of *0.01 M sodium hydroxide*; the solution is blue. Add 0.80 mL of *0.01 M hydrochloric acid*; the solution is yellow.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 30 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dissolve 3 mg of the substance to be examined and 3 mg of *prilocaine impurity E CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 10.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (c) Dissolve 30.0 mg of *prilocaine impurity B CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (d) Dissolve 15 mg of *prilocaine for peak identification CRS* (containing impurity G) in the mobile phase and dilute to 5.0 mL with the mobile phase.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped extra-dense bonded octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 26 volumes of *acetonitrile for chromatography R* and 74 volumes of a solution prepared as follows: dissolve 0.180 g of *sodium dihydrogen phosphate monohydrate R* and 2.89 g of *disodium hydrogen phosphate dihydrate R* in 1000 mL of *water for chromatography R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 240 nm.

Injection 20 µL.

Run time Twice the retention time of prilocaine.

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peak due to impurity B; use the chromatogram obtained with reference solution (a) to identify the peak due to impurity E; use the chromatogram obtained with reference solution (d) to identify the peak due to impurity G.

Relative retention With reference to prilocaine (retention time = about 25 min): impurity B = about 0.3; impurity G = about 0.8; impurity E = about 1.2.

System suitability Reference solution (a):

— *resolution*: minimum 3.0 between the peaks due to prilocaine and impurity E.

Limits:

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (100 ppm);

— *impurity G*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

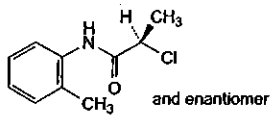
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.200 g in a mixture of 5.0 mL of *0.01 M hydrochloric acid* and 50 mL of *ethanol (96 per cent) R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

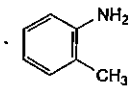
1 mL of *0.1 M sodium hydroxide* is equivalent to 25.68 mg of $C_{13}H_{21}ClN_2O$.

IMPURITIES
Specified impurities B, G.

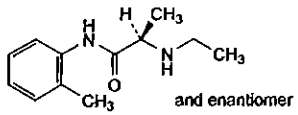
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) A, C, D, E, F.



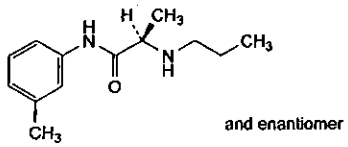
A. (2*RS*)-2-chloro-*N*-(2-methylphenyl)propanamide,



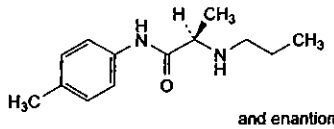
B. 2-methylbenzenamine (*o*-toluidine),



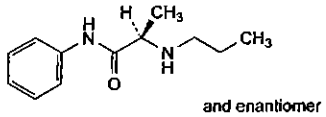
C. (2*RS*)-2-(ethylamino)-*N*-(2-methylphenyl)propanamide,



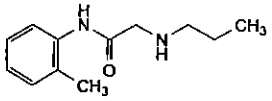
D. (2*RS*)-*N*-(3-methylphenyl)-2-(propylamino)propanamide,



E. (2*RS*)-*N*-(4-methylphenyl)-2-(propylamino)propanamide,



F. (2*RS*)-*N*-phenyl-2-(propylamino)propanamide,

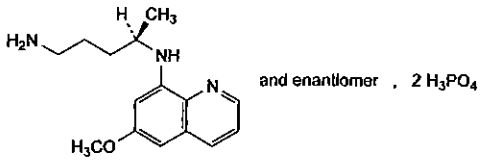


G. *N*-(2-methylphenyl)-2-(propylamino)acetamide.

Ph Eur

Primaquine Phosphate

(Primaquine Diphosphate, Ph. Eur. monograph 0635)



$C_{15}H_{27}N_3O_9P_2$ 455.3 *63-45-6*

Action and use
Antiprotozoal (malaria).

Ph Eur

DEFINITION
(4*RS*)-N^1-(6-Methoxyquinolin-8-yl)pentane-1,4-diamine bis (dihydrogen phosphate).

Content
98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS
Appearance
Orange, crystalline powder.

Solubility
Soluble in water, practically insoluble in ethanol (96 per cent).

mp
About 200 °C, with decomposition.

IDENTIFICATION
First identification: B, D.

Second identification: A, C, D.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution (a) Dissolve 15 mg in a 1.03 g/L solution of *hydrochloric acid R* and dilute to 100.0 mL with the same solution.

Test solution (b) Dilute 5.0 mL of test solution (a) to 50.0 mL with a 1.03 g/L solution of *hydrochloric acid R*.

Spectral range 310-450 nm for test solution (a); 215-310 nm for test solution (b).

Absorption maxima At 332 nm and 415 nm for test solution (a); at 225 nm, 265 nm and 282 nm for test solution (b).

Specific absorbance at the absorption maxima:
- — at 332 nm: 45 to 52 for test solution (a);
- — at 415 nm: 27 to 35 for test solution (a);
- — at 225 nm: 495 to 515 for test solution (b);
- — at 265 nm: 335 to 350 for test solution (b);
- — at 282 nm: 330 to 345 for test solution (b).

B. Infrared absorption spectrophotometry (*2.2.24*).

Examine the substances in discs prepared as follows: dissolve separately 0.1 g of the substance to be examined and 0.1 g of

the reference substance in 5 mL of *water R*, add 2 mL of *dilute ammonia R2* and 5 mL of *methylene chloride R*, then shake. Dry the methylene chloride layer over 0.5 g of *anhydrous sodium sulfate R*. Prepare a blank disc using about 0.3 g of *potassium bromide R*. Apply dropwise to the disc 0.1 mL of the methylene chloride layer, allowing the methylene chloride to evaporate between applications. Dry the disc at 50 °C for 2 min.

Comparison *primaquine diphosphate CRS.*

C. Thin-layer chromatography (*2.2.27*). *Carry out all operations as rapidly as possible, protected from light. Prepare the solutions immediately before use.*

Test solution Dissolve 0.20 g of the substance to be examined in 5 mL of *water R* and dilute to 10 mL with *methanol R*. Dilute 1 mL of this solution to 10 mL with a mixture of equal volumes of *methanol R* and *water R*.

Reference solution Dissolve 20 mg of *primaquine diphosphate CRS* in 5 mL of *water R* and dilute to 10 mL with *methanol R*.

Plate *TLC silica gel GF_{254} plate R.*

Pretreatment Wash the plate with the mobile phase and allow to dry in air.

Mobile phase *concentrated ammonia R, methanol R, methylene chloride R* (1:40:60 *V/V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve 50 mg in 5 mL of *water R*. Add 2 mL of *dilute sodium hydroxide solution R* and shake with 2 quantities, each of 5 mL, of *methylene chloride R*. The aqueous layer, acidified by addition of *nitric acid R*, gives reaction (b) of phosphates (*2.3.1*).

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution *Use a freshly prepared solution.* Dissolve 25.0 mg of the substance to be examined in mobile phase A, using sonication if necessary, and dilute to 50.0 mL with mobile phase A.

Reference solution (a) Dissolve 5 mg of *primaquine for system suitability CRS* (containing impurity A) in mobile phase A and dilute to 10 mL with mobile phase A.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octylsilyl silica gel for chromatography with embedded polar groups R* (5 µm);
- *temperature*: 25 °C.

Mobile phase:
- *mobile phase A*: *trifluoroacetic acid R, tetrahydrofuran R, acetonitrile R, water for chromatography R* (0.1/1/9/90 *V/V/V/V*);
- *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	100	0
15 - 40	100 → 50	0 → 50

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 265 nm.

Injection 25 µL.

Relative retention With reference to primaquine (retention time = about 13 min): impurity A = about 0.86.

System suitability Reference solution (a):
- *resolution*: minimum 2.0 between the peaks due to impurity A and primaquine.

Calculation of percentage contents:
- for each impurity, use the concentration of primaquine diphosphate in reference solution (b).

Limits:
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.5 per cent;
- *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.200 g in 40 mL of *anhydrous acetic acid R*, heating gently. Allow to cool and titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

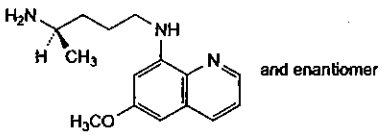
1 mL of *0.1 M perchloric acid* is equivalent to 22.77 mg of $C_{15}H_{27}N_3O_9P_2$.

STORAGE

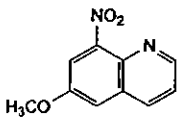
Protected from light.

IMPURITIES

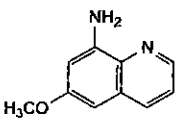
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D.*



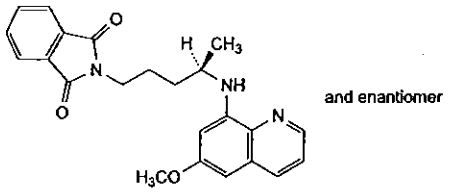
A. (4*RS*)-*N*^{1}-(6-methoxyquinolin-8-yl)pentane-1,4-diamine (quinocide),



B. 6-methoxy-8-nitroquinoline,



C. 6-methoxyquinolin-8-amine,

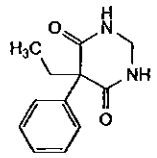


D. 2-[(4*RS*)-4-[(6-methoxyquinolin-8-yl)amino]pentyl]-1*H*-isoindole-1,3(2*H*)-dione.

Ph Eur

Primidone

(Ph. Eur. monograph 0584)



$C_{12}H_{14}N_2O_2$ 218.3 *125-33-7*

Action and use
Antiepileptic.

Preparations
Primidone Oral Suspension
Primidone Tablets

Ph Eur

DEFINITION
5-Ethyl-5-phenyl-1,3-diazinane-4,6-dione.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Very slightly soluble in water, slightly soluble in ethanol (96 per cent). It dissolves in alkaline solutions.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *primidone CRS.*

TESTS
Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in *methanol R* and dilute to 50.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *methanol R*. Dilute 1.0 mL of this solution to 10.0 mL with *methanol R*.

Reference solution (b) Dissolve 5 mg of *primidone for system suitability CRS* (containing impurities B and C) in *methanol R* and dilute to 5 mL with the same solvent.

Column:
— *size: l* = 0.10 m, Ø = 4.6 mm;
— *stationary phase: end-capped monolithic octadecylsilyl silica gel for chromatography R* (2 µm).

Mobile phase:
— *mobile phase A*: 1.36 g/L solution of *potassium dihydrogen phosphate R*;
— *mobile phase B*: *methanol R2*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 1	75	25
1 - 6	75 → 40	25 → 60
6 - 8	40	60

Flow rate 3.2 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 10 µL.

Identification of impurities Use the chromatogram supplied with *primidone for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B and C.

Relative retention With reference to primidone (retention time = about 2.2 min): impurity B = about 1.4; impurity C = about 1.6.

System suitability Reference solution (b):
— *resolution*: minimum 2.5 between the peaks due to impurities B and C.

Calculation of percentage contents:
— for each impurity, use the concentration of primidone in reference solution (a).

Limits:
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.3 per cent;
— *reporting threshold*: 0.05 per cent.

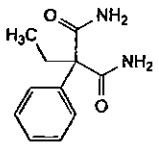
Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

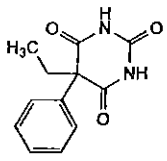
ASSAY
Dissolve 60.0 mg with heating in 70 mL of *ethanol (96 per cent) R*, cool and dilute to 100.0 mL with the same solvent. Prepare a reference solution in the same manner using 60.0 mg of *primidone CRS*. Measure the absorbance (*2.2.25*) of the 2 solutions at the absorption maximum at 257 nm.

Calculate the content of $C_{12}H_{14}N_2O_2$ from the absorbances measured and the concentrations of the solutions.

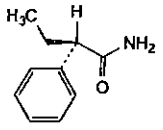
IMPURITIES
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, E, F.*



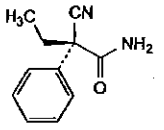
A. 2-ethyl-2-phenylpropanediamide (ethylphenylmalonamide),



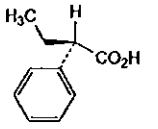
B. 5-ethyl-5-phenyl-1,3-diazinane-2,4,6-trione (phenobarbital),



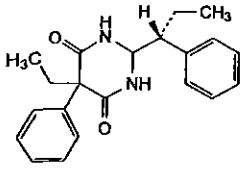
C. (2*RS*)-2-phenylbutanamide,



D. (2*RS*)-2-cyano-2-phenylbutanamide,



E. (2*RS*)-2-phenylbutanoic acid,

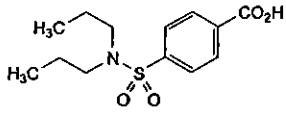


F. 5-ethyl-5-phenyl-2-[(1*RS*)-1-phenylpropyl]-1,3-diazinane-4,6-dione.

Ph Eur

Probenecid

(Ph. Eur. monograph 0243)



$C_{13}H_{19}NO_4S$ 285.4 *57-66-9*

Action and use
Uricosuric drug.

Preparation
Probenecid Tablets

Ph Eur

DEFINITION
4-(Dipropylsulfamoyl)benzoic acid.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder or small crystals.

Solubility
Practically insoluble in water, soluble in acetone, sparingly soluble in anhydrous ethanol.

IDENTIFICATION

First identification: A, C.

Second identification: A, B, D.

A. Melting point (*2.2.14*): 197 °C to 202 °C.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 20 mg in a mixture of 1 volume of *0.1 M hydrochloric acid* and 9 volumes of *ethanol (96 per cent) R* and dilute to 100.0 mL with the same mixture of solvents. Dilute 5.0 mL of the solution to 100.0 mL with a mixture of 1 volume of *0.1 M hydrochloric acid* and 9 volumes of *ethanol (96 per cent) R*.

Spectral range 220-350 nm.

Absorption maxima At 223 nm and 248 nm.

Specific absorbance at the absorption maximum at 248 nm 310 to 350.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *probenecid CRS*.

D. Dissolve 0.2 g in the smallest necessary quantity of *dilute ammonia R2* (about 0.6 mL). Add 3 mL of *silver nitrate solution R2*. A white precipitate is formed which dissolves in an excess of ammonia.

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Dissolve 1.0 g in *1 M sodium hydroxide* and dilute to 10 mL with the same solvent.

Acidity
To 2.0 g add 100 mL of *water R* and heat on a water-bath for 30 min. Make up to the original volume with *water R*, allow to cool to room temperature and filter. To 50 mL of the filtrate add 0.1 mL of *phenolphthalein solution R*. Not more than 0.5 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Related substances
Liquid chromatography (*2.2.29*).

Solution A Mix 1 volume of *glacial acetic acid R* and 100 volumes of *acetonitrile R*.

Solution B Mix 1 volume of *glacial acetic acid R* and 100 volumes of a 6.9 g/L solution of *sodium dihydrogen phosphate monohydrate R* in *water for chromatography R* and adjust to pH 3.0 with *dilute phosphoric acid R*.

Test solution Dissolve 25 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 5 mg of *potassium 4-sulfobenzoate R* (impurity A) in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 10.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the test solution.

Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: end-capped phenylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Solution A, solution B (50:50 *V/V*).

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 240 nm.

Injection 20 µL.

Run time 4 times the retention time of probenecid.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity A.

Relative retention With reference to probenecid (retention time = about 7 min): impurity A = about 0.3.

System suitability Reference solution (b):
— *resolution*: minimum 5.0 between the peaks due to impurity A and probenecid.

Calculation of percentage contents:
— *correction factor*: multiply the peak area of impurity A by 0.4;
— for each impurity, use the concentration of probenecid in reference solution (a).

Limits:
— *impurity A*: maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.2 per cent;
— *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

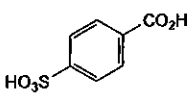
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.250 g in 50 mL of *ethanol (96 per cent) R*, shaking and heating slightly if necessary. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

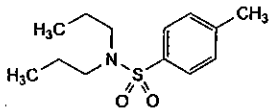
1 mL of *0.1 M sodium hydroxide* is equivalent to 28.54 mg of $C_{13}H_{19}NO_4S$.

IMPURITIES
Specified impurities *A.*

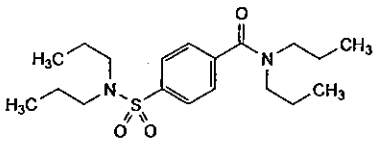
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D.*



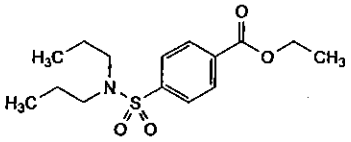
A. 4-sulfobenzoic acid,



B. 4-methyl-*N*,*N*-dipropylbenzenesulfonamide,



C. 4-(dipropylsulfamoyl)-*N*,*N*-dipropylbenzamide,

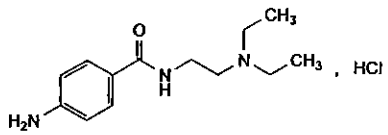


D. ethyl 4-(dipropylsulfamoyl)benzoate.

Ph Eur

Procainamide Hydrochloride

(Ph. Eur. monograph 0567)



$C_{13}H_{22}ClN_3O$ 271.8 *614-39-1*

Action and use
Class I antiarrhythmic.

Preparations
Procainamide Injection
Procainamide Tablets

Ph Eur

DEFINITION
Procainamide hydrochloride contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of 4-amino-*N*-[2-(diethylamino)ethyl] benzamide hydrochloride, calculated with reference to the dried substance.

CHARACTERS
A white or very slightly yellow, crystalline powder, hygroscopic, very soluble in water, freely soluble in alcohol, slightly soluble in acetone.

IDENTIFICATION
First identification: C, D.

Second identification: A, B, D, E.

A. Melting point (*2.2.14*): 166 °C to 170 °C.

B. Dissolve 10.0 mg in *0.1 M sodium hydroxide* and dilute to 100.0 mL with the same solvent. Dilute 10.0 mL of the solution to 100.0 mL with *0.1 M sodium hydroxide*. Examined between 220 nm and 350 nm (*2.2.25*), the solution shows an absorption maximum at 273 nm. The specific absorbance at the maximum is 580 to 610.

C. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *procainamide hydrochloride CRS*.

D. Dilute 1 mL of solution S to 5 mL with *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

E. Dilute 1 mL of solution S (see Tests) to 2 mL with *water R*. 1 mL of this solution gives the reaction of primary aromatic amines (*2.3.1*).

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_6 (*2.2.2, Method II*).

pH (*2.2.3*)

The pH of solution S is 5.6 to 6.3.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel GF_{254} R* as the coating substance.

Test solution Dissolve 0.10 g of the substance to be examined in *alcohol R* and dilute to 10 mL with the same solvent.

Reference solution Dilute 1 mL of the test solution to 200 mL with *alcohol R*.

Apply to the plate 5 µL of each solution. Develop over a path of 12 cm using a mixture of 15 volumes of *glacial acetic acid R*, 30 volumes of *water R* and 60 volumes of *butanol R*. Place the plate in a stream of cold air until the plate appears dry. Examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Loss on drying (*2.2.32*)

Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.2500 g in 50 mL of *dilute hydrochloric acid R*. Carry out the determination of primary aromatic amino-nitrogen (*2.5.8*).

1 mL of *0.1 M sodium nitrite* is equivalent to 27.18 mg of $C_{13}H_{22}ClN_3O$.

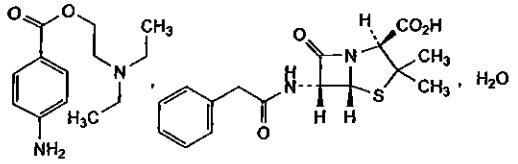
STORAGE

Store in an airtight container, protected from light.

Ph Eur

Benzylpenicillin (Procaine) Monohydrate

(Ph. Eur. monograph 0115)



$C_{29}H_{38}N_4O_6S,H_2O$ 588.7 *6130-64-9*

Action and use

Penicillin antibacterial.

Preparation

Procaine Benzylpenicillin Injection

Ph Eur

DEFINITION

2-(Diethylamino)ethyl 4-aminobenzoate (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylic acid monohydrate.

Salt obtained from *Benzylpenicillin sodium (0114)* or *Benzylpenicillin potassium (0113)* produced by the growth of certain strains of *Penicillium notatum* or related micro-organisms.

Content

— *procaine benzylpenicillin*: 96.0 per cent to 102.0 per cent (anhydrous substance) without correction for dispersing or suspending agents;

— *procaine* ($C_{13}H_{20}N_2O_2$; M_r 236.3): 39.0 per cent to 42.0 per cent (anhydrous substance).

Dispersing or suspending agents (for example, lecithin and polysorbate 80) may be added.

CHARACTERS

Appearance

White or almost white, slightly hygroscopic, crystalline powder.

Solubility

Slightly soluble in water, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: A.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *procaine benzylpenicillin CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 25 mg of the substance to be examined in 5 mL of *acetone R*.

Reference solution Dissolve 25 mg of *procaine benzylpenicillin CRS* in 5 mL of *acetone R*.

Plate *TLC silanised silica gel plate R.*

Mobile phase Mix 30 volumes of *acetone R* and 70 volumes of a 154 g/L solution of *ammonium acetate R* previously adjusted to pH 7.0 with *ammonia R*.

Application 1 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Expose to iodine vapour until the spots appear and examine in daylight.

System suitability Reference solution:

— the chromatogram shows 2 clearly separated spots.

Results The 2 principal spots in the chromatogram obtained with the test solution are similar in position, colour and size to the 2 principal spots in the chromatogram obtained with the reference solution.

C. Place about 2 mg in a test-tube about 150 mm long and 15 mm in diameter. Moisten with 0.05 mL of *water R* and add 2 mL of *sulfuric acid-formaldehyde reagent R*. Mix the contents of the tube by swirling; the solution is practically colourless. Place the test-tube on a water-bath for 1 min; a reddish-brown colour develops.

D. Dissolve 0.1 g in 2 mL of *dilute hydrochloric acid R*. The solution, which may be turbid, gives the reaction of primary aromatic amines (*2.3.1*).

TESTS

pH (*2.2.3*)
5.0 to 7.5.

Dissolve 50 mg in *carbon dioxide-free water R* and dilute to 15 mL with the same solvent. Shake until dissolution is complete.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the test solutions immediately before use.*

Solvent mixture *methanol R, water R* (50:50 *V/V*).

Test solution (a) Dissolve 50.0 mg of the substance to be examined in 25 mL of *methanol R* and dilute to 50.0 mL with *water R*.

Test solution (b) Dissolve 0.100 g of the substance to be examined in 10 mL of *methanol R* and dilute to 20.0 mL with *water R*.

Reference solution (a) Dissolve 50.0 mg of *procaine benzylpenicillin CRS* in 25 mL of *methanol R* and dilute to 50.0 mL with *water R*.

Reference solution (b) Dissolve 12.0 mg of *4-aminobenzoic acid R* (impurity A) in *water R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of the solution to 100.0 mL with *water R*.

Reference solution (c) Dissolve 10 mg of *procaine benzylpenicillin for peak identification A CRS* (containing impurities B, C, D, E, G, H, I and J) in 1 mL of *methanol R* and add 1 mL of *water R*.

Reference solution (d) Dissolve the contents of a vial of *procaine benzylpenicillin impurity F CRS* in 1 mL of reference solution (c).

Reference solution (e) Dilute 1.0 mL of reference solution (a) to 20.0 mL with the solvent mixture.

Reference solution (f) Dilute 1.0 mL of reference solution (e) to 20.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm);
— *temperature*: 50 °C.

Mobile phase:
— *mobile phase A*: mix 10 volumes of a 68 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 3.4 with a 500 g/L solution of *phosphoric acid R*, 30 volumes of *methanol R1* and 60 volumes of *water for chromatography R*;
— *mobile phase B*: mix 10 volumes of a 68 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 3.4 with a 500 g/L solution of *phosphoric acid R*, 35 volumes of *water for chromatography R* and 55 volumes of *methanol R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 7	70	30
7 - 17	70 → 0	30 → 100
17 - 22	0	100

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 225 nm.

Injection 20 µL of test solution (b) and reference solutions (b), (d), (e) and (f).

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity A; use the chromatogram supplied with *procaine benzylpenicillin for peak identification A CRS* and the chromatogram obtained with reference solution (d) to identify the peaks due to impurities B, C, D, E, F, G, H, I and J.

Relative retention With reference to benzylpenicillin (retention time = about 7 min): procaine = about 0.19; impurity A = about 0.22; impurity D = about 0.33; impurity F = about 0.35; impurity B = about 0.48 and 0.55; impurity E = about 0.62; impurity C = about 0.81 and 0.83; impurity I = about 0.93; impurity G = about 1.47; impurity H = about 1.90; impurity J = about 2.37.

System suitability:
— *resolution*: minimum 1.0 between the peaks due to the epimers of impurity C and minimum 1.2 between the peaks due to impurities D and F in the chromatogram obtained with reference solution (d);
— *signal-to-noise ratio*: minimum 10 for the peak due to benzylpenicillin in the chromatogram obtained with reference solution (f).

Calculation of percentage contents:
— *correction factor*: multiply the peak area of impurity D by 0.4;
— for impurity A, use the concentration of impurity A in reference solution (b);
— for impurities other than A, use the concentration of procaine benzylpenicillin monohydrate in reference solution (e) taking into account the area of the peak due to benzylpenicillin in the chromatogram obtained with reference solution (e).

Limits:
— *impurities F, G, H, I, J*: for each impurity, maximum 0.5 per cent;
— *impurities B (sum of isomers), C (sum of epimers)*: for each impurity, maximum 0.4 per cent;
— *impurities E, D*: for each impurity, maximum 0.2 per cent;
— *impurity A*: maximum 0.024 per cent;
— *any other impurity*: for each impurity, maximum 0.2 per cent;
— *total*: maximum 2.0 per cent;
— *reporting threshold*: 0.05 per cent; disregard the peak due to procaine.

Water (*2.5.12*)
2.8 per cent to 4.2 per cent, determined on 0.300 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Mobile phase Mobile phase B, mobile phase A (30:70 *V/V*).

Injection 10 µL of test solution (a) and reference solution (a).

Run time 3 times the retention time of benzylpenicillin.

Calculate the percentage content of procaine ($C_{13}H_{20}N_2O_2$) taking into account the area of the peak due to procaine and the assigned content of procaine ($C_{13}H_{20}N_2O_2$) in *procaine benzylpenicillin CRS*.

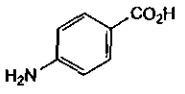
Calculate the percentage content of procaine benzylpenicillin ($C_{29}H_{38}N_4O_6S$) taking into account the area of the peak due to benzylpenicillin and the assigned content of procaine benzylpenicillin ($C_{29}H_{38}N_4O_6S$) in *procaine benzylpenicillin CRS*.

STORAGE

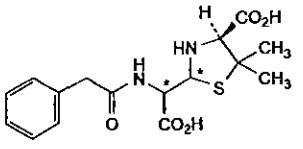
In an airtight container. If the substance is sterile, the container is also sterile and tamper-evident.

IMPURITIES

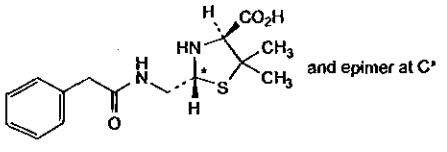
Specified impurities *A, B, C, D, E, F, G, H, I, J.*



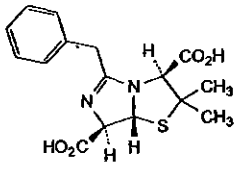
A. 4-aminobenzoic acid,



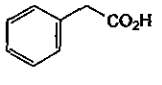
B. (2*S*,4*S*)-2-[(*S*)-carboxy(2-phenylacetamido)methyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid (penicilloic acids of benzylpenicillin),



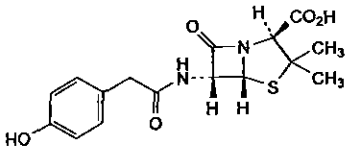
C. (2*RS*,4*S*)-5,5-dimethyl-2-[(2-phenylacetamido)methyl]-1,3-thiazolidine-4-carboxylic acid (penilloic acids of benzylpenicillin),



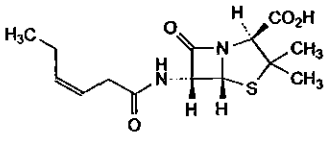
D. (3*S*,7*R*,7a*R*)-5-benzyl-2,2-dimethyl-2,3,7,7a-tetrahydroimidazo[5,1-*b*][1,3]thiazole-3,7-dicarboxylic acid (penillic acid of benzylpenicillin),



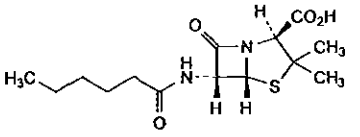
E. phenylacetic acid,



F. (2*S*,5*R*,6*R*)-6-[2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid,



G. (2*S*,5*R*,6*R*)-6-[(3*Z*)-hex-3-enamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (isopenicillin F),



H. (2*S*,5*R*,6*R*)-6-hexanamido-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (dihydropenicillin F),

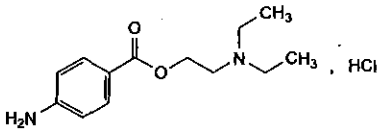
I. unknown structure,

J. unknown structure.

Ph Eur

Procaine Hydrochloride

(Ph. Eur. monograph 0050)



$C_{13}H_{21}ClN_2O_2$ 272.8 *51-05-8*

Action and use

Local anaesthetic.

Ph Eur

DEFINITION

Procaine hydrochloride contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 2-(diethylamino)ethyl 4-aminobenzoate hydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, very soluble in water, soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: A, B, E.

Second identification: A, C, D, E, F.

A. Melting point (*2.2.14*): 154 °C to 158 °C.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *procaine hydrochloride CRS*.

C. To about 5 mg add 0.5 mL of *fuming nitric acid R*. Evaporate to dryness on a water-bath, allow to cool and dissolve the residue in 5 mL of *acetone R*. Add 1 mL of *0.1 M alcoholic potassium hydroxide*. Only a brownish-red colour develops.

D. To 0.2 mL of solution S (see Tests) add 2 mL of *water R* and 0.5 mL of *dilute sulfuric acid R* and shake. Add 1 mL of a 1 g/L solution of *potassium permanganate R*. The colour is immediately discharged.

E. It gives reaction (a) of chlorides (*2.3.1*).

F. Dilute 1 mL of solution S to 100 mL with *water R*. 2 mL of this solution gives the reaction of primary aromatic amines (*2.3.1*).

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)
Dilute 4 mL of solution S to 10 mL with *carbon dioxide-free water R*. The pH of the solution is 5.0 to 6.5.

Related substances
Examine by thin-layer chromatography (*2.2.27*), using *silica gel GF_{254} R* as the coating substance.

Test solution Dissolve 1.0 g of the substance to be examined in *water R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 50 mg of *4-aminobenzoic acid R* in *water R* and dilute to 100 mL with the same solvent. Dilute 1 mL of the solution to 10 mL with *water R*.

Apply separately to the plate 5 µL of each solution. Develop over a path of 10 cm using a mixture of 4 volumes of *glacial acetic acid R*, 16 volumes of *hexane R* and 80 volumes of *dibutyl ether R*. Dry the plate at 100 °C to 105 °C for 10 min and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.05 per cent). The principal spot in the chromatogram obtained with the test solution remains on the point of application.

Loss on drying (*2.2.32*)
Not more than 0.5 per cent, determined on 1.00 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Not more than 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.400 g in 50 mL of *dilute hydrochloric acid R*. Carry out the determination of primary aromatic amino nitrogen (*2.5.8*).

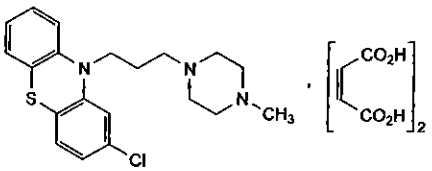
1 mL of *0.1 M sodium nitrite* is equivalent to 27.28 mg of $C_{13}H_{21}ClN_2O_2$.

STORAGE
Store protected from light.

Ph Eur

Prochlorperazine Maleate

(Ph. Eur. monograph 0244)



$C_{28}H_{32}ClN_3O_8S$ 606 *84-02-6*

Action and use
Dopamine receptor antagonist; neuroleptic.

Preparations
Prochlorperazine Tablets
Prochlorperazine Buccal Tablets

Ph Eur

DEFINITION
2-Chloro-10-[3-(4-methylpiperazin-1-yl)propyl]-10*H*-phenothiazine bis[hydrogen (2*Z*)-but-2-enedioate].

Content
98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or pale-yellow, crystalline powder.

Solubility
Very slightly soluble in water and in ethanol (96 per cent).

IDENTIFICATION
First identification: A, B.

Second identification: B.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison prochlorperazine maleate CRS.

B. Identification of phenothiazines by thin-layer chromatography (*2.3.3*) with the following modifications.

Test solution Dissolve 20 mg of the substance to be examined in a mixture of equal volumes of *methanol R* and *methylene chloride R*, and dilute to 20 mL with the same mixture of solvents.

Reference solution Dissolve 20 mg of *prochlorperazine maleate CRS* in a mixture of equal volumes of *methanol R* and *methylene chloride R*, and dilute to 20 mL with the same mixture of solvents.

Application 4 µL.

TESTS
pH (*2.2.3*)
3.0 to 4.0 for a freshly prepared saturated solution in *carbon dioxide-free water R*.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use and protect from light.*

Buffer solution Dissolve 0.96 g of *ammonium carbonate R* in about 800 mL of *water for chromatography R*. Adjust to pH 10.0 with *concentrated ammonia R* and dilute to 1000 mL with *water for chromatography R*.

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 5 mg of *prochlorperazine for system suitability CRS* (containing impurities B and D) in the mobile phase and dilute to 25 mL with the mobile phase.

Column:
— *size: l* = 0.15 m, Ø = 3.0 mm;
— *stationary phase: end-capped propyl-2-phenylsilyl amorphous organosilica polymer R* (3.5 µm);
— *temperature*: 40 °C.

Mobile phase *tetrahydrofuran R, methanol R*, buffer solution (15:33:52 *V/V/V*).

Flow rate 0.4 mL/min.

Detection Spectrophotometer at 252 nm.

Injection 10 µL.

Run time 1.5 times the retention time of prochlorperazine.

Identification of impurities Use the chromatogram supplied with *prochlorperazine for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B and D.

Relative retention With reference to prochlorperazine (retention time = about 24 min): maleic acid = about 0.06; impurity B = about 0.56; impurity D = about 1.11.

System suitability Reference solution (b):
— *peak-to-valley ratio*: minimum 3.0, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to prochlorperazine.

Calculation of percentage contents:
— for each impurity, use the concentration of prochlorperazine maleate in reference solution (a).

Limits:
— *impurity B*: maximum 0.3 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.5 per cent;
— *reporting threshold*: 0.05 per cent; disregard the peak due to maleic acid.

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

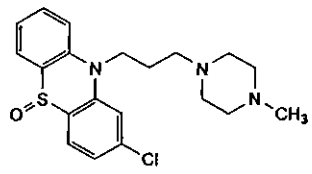
ASSAY
Dissolve 0.200 g of the powdered substance to be examined in 50 mL of *anhydrous acetic acid R*, warming on a water-bath. Allow to cool to room temperature. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid*, is equivalent to 30.31 mg of $C_{28}H_{32}ClN_3O_8S$.

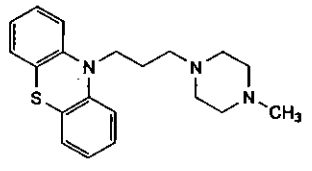
STORAGE
Protected from light.

IMPURITIES
Specified impurities *B.*

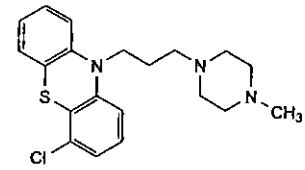
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, C, D.*



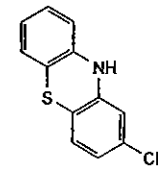
A. 2-chloro-10-[3-(4-methylpiperazin-1-yl)propyl]-5λ⁴-phenothiazin-5(10*H*)-one,



B. 10-[3-(4-methylpiperazin-1-yl)propyl]-10*H*-phenothiazine,



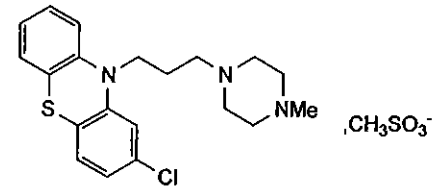
C. 4-chloro-10-[3-(4-methylpiperazin-1-yl)propyl]-10*H*-phenothiazine,



D. 2-chloro-10*H*-phenothiazine.

Ph Eur

Prochlorperazine Mesilate



$C_{20}H_{24}ClN_3S,2CH_4SO_3$ 566.2 *5132-55-8*

Action and use
Dopamine receptor antagonist; neuroleptic.

Preparations
Prochlorperazine Injection
Prochlorperazine Oral Solution

DEFINITION
Prochlorperazine Mesilate is 2-chloro-10-[3-(4-methylpiperazin-1-yl)propyl]phenothiazine di(methanesulfonate). It contains not less than 98.0% and not more than 101.0% of $C_{20}H_{24}ClN_3S,2CH_4SO_3$, calculated with reference to the dried substance.

PRODUCTION
Risk assessment should be used to evaluate the potential for genotoxic methanesulfonate esters to be formed in the presence of low molecular weight alcohols. If a risk of methanesulfonate ester formation is identified through risk assessment, these impurities should not exceed the threshold of toxicological concern.

CHARACTERISTICS
A white or almost white powder.

Very soluble in *water*; sparingly soluble in *ethanol (96%)*; practically insoluble in *ether*.

IDENTIFICATION
A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of prochlorperazine mesilate *(RS 290)*.

B. Dissolve 5 mg in 2 mL of *sulfuric acid* and allow to stand for 5 minutes. A red colour is produced.

TESTS

Acidity

pH of a 2% w/v solution, 2.0 to 3.0, Appendix V L.

Related substances

Complies with the test for *related substances in phenothiazines*, Appendix III A, using *mobile phase A* and dissolving the substance being examined in *methanol* containing 0.5% v/v of 13.5M *ammonia*.

Loss on drying

When dried to constant weight at 100° at a pressure not exceeding 0.7 kPa, loses not more than 1.0% of its weight. Use 1 g.

Sulfated ash

Not more than 0.1%, Appendix IX A.

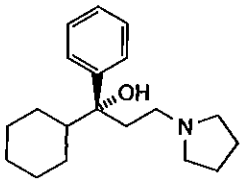
ASSAY

Dissolve 0.8 g in 10 mL of *water*, add 5 mL of 1M *sodium hydroxide* and extract by shaking with successive quantities of 50, 25, 25 and 25 mL of *ether*. Wash the combined ether extracts with 5 mL of *water*, shake the washings with 5 mL of *ether*, add the ether to the combined ether extracts and evaporate to dryness. Add 2 mL of *absolute ethanol* to the residue, evaporate to dryness and carry out Method I for *non-aqueous titration*, Appendix VIII A, using *1-naphtholbenzein solution* as indicator. Each mL of *0.1M perchloric acid VS* is equivalent to 28.31 mg of $C_{20}H_{24}ClN_3S,2CH_4SO_3$.

STORAGE

Prochlorperazine Mesilate should be protected from light.

Procyclidine Hydrochloride



$C_{19}H_{29}NO,HCl$ 323.9 *1508-76-5*

Action and use

Anticholinergic.

Preparations

Procyclidine Injection

Procyclidine Tablets

DEFINITION

Procyclidine Hydrochloride is (*RS*)-1-cyclohexyl-1-phenyl-3-pyrrolidin-1-ylpropan-1ol hydrochloride. It contains not less than 99.0% and not more than 101.0% of $C_{19}H_{29}NO,HCl$, calculated with reference to the dried substance.

CHARACTERISTICS

A white, crystalline powder.

Sparingly soluble in *water*; soluble in *ethanol (96%)*; practically insoluble in *acetone* and in *ether*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of procyclidine hydrochloride *(RS 292)*.

B. Dissolve 0.25 g in 10 mL of *water*, make alkaline with 5M *ammonia* and extract with three 10-mL quantities of *ether*. Dry the combined extracts over *anhydrous sodium sulfate*, filter, evaporate the ether and scratch the residue with a glass rod to induce solidification. The *melting point* of the residue is about 85°, Appendix V A.

C. Yields the reactions characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH of a 1% w/v solution, 4.5 to 6.5, Appendix V L.

Related substances

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *chloroform*.

(1) 2.0% w/v of the substance being examined.

(2) 0.0040% w/v of *1-phenyl-3-pyrrolidinopropan-1-one hydrochloride BPCRS*.

(3) 0.010% w/v of the substance being examined.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel F_{254}*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it at 105° for 15 minutes and examine under *ultraviolet light (254 nm)*. Spray the plate with *dilute potassium iodobismuthate solution* and examine.

MOBILE PHASE

1 volume of 13.5M *ammonia* and 100 volumes of *ether*.

LIMITS

When examined under UV light:

any spot corresponding to 1-phenyl-3-pyrrolidinopropan-1-one in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.2%).

When examined after spraying:

any other *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

B. Carry out the method for *gas chromatography*, Appendix III B. Use a solution containing 0.06% w/v of *triphenylethylene* in *ether* as the internal standard.

(1) Add 5 mL of 1.25M *sodium hydroxide* to 20 mL of a 0.50% w/v solution of the substance being examined and mix. Extract with two 20-mL quantities of *ether*, add to the combined extracts 5 mL of the internal standard, shake with *anhydrous sodium sulfate* and filter; evaporate the filtrate and dissolve the residue in 1 mL of *ether*.

(2) Prepare in the same manner as solution (1) but omitting the addition of the internal standard solution.

(3) Prepare in the same manner as solution (1) but using 20 mL of a 0.015% w/v solution of the substance being examined.

CHROMATOGRAPHIC CONDITIONS

(a) Use a fused silica column (15 m × 0.32 mm) bonded with a 0.25 µm film of *polyethylene glycol 20,000* (DB-Wax is suitable).

(b) Use *nitrogen* as the carrier gas at 0.8 mL per minute.

(c) Use isothermal conditions maintained at 210°.

(d) Use an inlet temperature of 250°.

(e) Use a flame ionisation detector at a temperature of 250°.

(f) Inject 1 µL of each solution.

(g) Use a split ratio of 1:5.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated peaks.

LIMITS

The ratio of the sum of the areas of any *secondary peaks* to the area of the peak due to the internal standard in the chromatogram obtained with solution (1) is not more than the ratio of the area of the principal peak to the area of the internal standard peak in the chromatogram obtained with solution (3) (3%).

Loss on drying

When dried to constant weight at 105°, loses not more than 0.5% of its weight. Use 1 g.

Sulfated ash

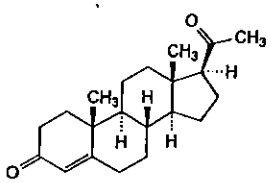
Not more than 0.1%, Appendix IX A.

ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.7 g and *crystal violet solution* as indicator. Each mL of *0.1M perchloric acid VS* is equivalent to 32.39 mg of $C_{19}H_{29}NO,HCl$.

Progesterone

(Ph. Eur. monograph 0429)



$C_{21}H_{30}O_2$ 314.5 *57-83-0*

Action and use

Progestogen.

Preparation

Progesterone Injection

Ph Eur

DEFINITION

Pregn-4-ene-3,20-dione.

Content

97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Practically insoluble in water, freely soluble in anhydrous ethanol, sparingly soluble in acetone and in fatty oils.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *progesterone CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *anhydrous ethanol R*, evaporate to dryness and record new spectra using the residues.

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 10 mL with the same mixture of solvents.

Reference solution Dissolve 10 mg of *progesterone CRS* in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 10 mL with the same mixture of solvents.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *ethyl acetate R, methylene chloride R* (33:66 *V/V*).

Application 5 µL.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Specific optical rotation (*2.2.7*)

+ 186 to + 194 (dried substance).

Dissolve 0.250 g in *anhydrous ethanol R* and dilute to 25.0 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in mobile phase B and dilute to 50.0 mL with mobile phase B.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase B. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase B.

Reference solution (b) Dissolve 2 mg of *progesterone for system suitability CRS* (containing impurities B, C, G, I and M) in mobile phase B and dilute to 5.0 mL with mobile phase B.

Reference solution (c) Dissolve 2 mg of *progesterone for peak identification CRS* (containing impurities D, E, J, K and L) in mobile phase B and dilute to 5.0 mL with mobile phase B.

Reference solution (d) Dissolve 10.0 mg of *progesterone for impurity H identification CRS* (with an assigned content of impurity H) in mobile phase B and dilute to 25.0 mL with mobile phase B.

Reference solution (e) Dissolve 20.0 mg of *progesterone CRS* in mobile phase B and dilute to 50.0 mL with mobile phase B.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped extra-dense bonded octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: *acetonitrile R, water R* (50:50 *V/V*);

— *mobile phase B*: *water R, acetonitrile R* (20:80 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	100	0
20 - 27	100 → 0	0 → 100
27 - 45	0	100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 241 nm and, for impurity H, at 286 nm.

Injection 10 µL of the test solution and reference solutions (a), (b), (c) and (d).

Identification of impurities Use the chromatogram supplied with *progesterone for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B, C, G, I and M; use the chromatogram supplied with *progesterone for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities D + E, J, K and L; use the chromatogram obtained with reference solution (d) to identify the peak due to impurity H.

Relative retention With reference to progesterone (retention time = about 14 min): impurity B = about 0.60; impurity J = about 0.65; impurity H = about 0.82; impurity K = about 0.85; impurity C = about 0.93; impurity M = about 1.1; impurity L = about 1.90; impurity I = about 1.95; impurities D and E = about 2.05; impurity G = about 2.65.

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 4.0, where H_p = height above the baseline of the peak due to impurity M and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to progesterone.

Limits:

— *impurity I (sum of the 2 epimers)*: not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.6 per cent);

— *impurity C*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *sum of impurities D and E*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);

— *impurities G, J, K, L, M*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);

— *impurity H at 286 nm*: maximum 0.15 per cent, calculated from the area of the corresponding peak in the chromatogram obtained with reference solution (d) and taking into account the assigned content of impurity H in *progesterone for impurity H identification CRS*;

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *sum of impurities other than H*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 0.500 g by drying in an oven at 105 °C for 2 h.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (e).

Calculate the percentage content of $C_{21}H_{30}O_2$ taking into account the assigned content of *progesterone CRS*.

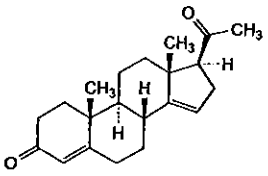
STORAGE

Protected from light.

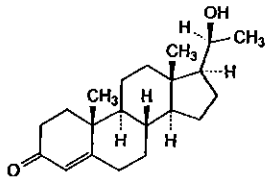
IMPURITIES

Specified impurities *B, C, D, E, G, H, I, J, K, L, M.*

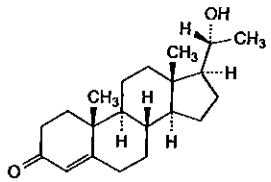
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A.*



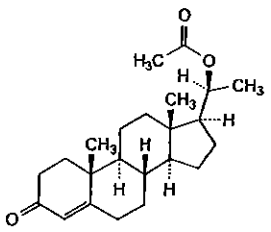
A. pregna-4,14-diene-3,20-dione,



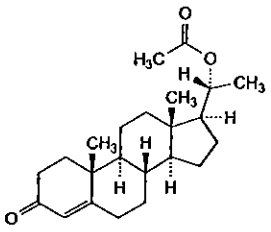
B. (20*S*)-20-hydroxypregn-4-en-3-one,



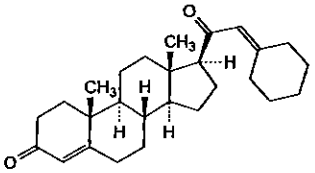
C. (20*R*)-20-hydroxypregn-4-en-3-one,



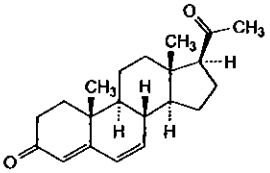
D. (20*S*)-3-oxopregn-4-en-20-yl acetate,



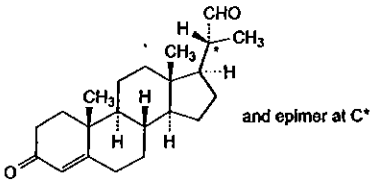
E. (20*R*)-3-oxopregn-4-en-20-yl acetate,



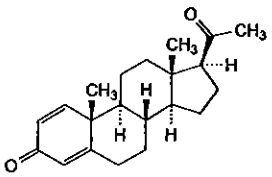
G. 21-(cyclohexylidene)pregn-4-ene-3,20-dione,



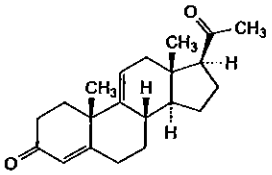
H. pregna-4,6-diene-3,20-dione (Δ6-progesterone),



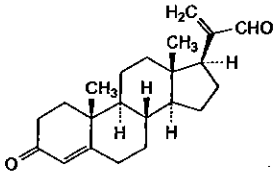
I. (20*RS*)-20-methyl-3-oxopregn-4-en-21-al,



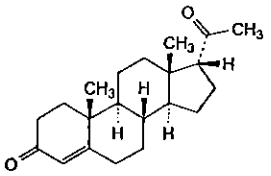
J. pregna-1,4-diene-3,20-dione,



K. pregna-4,9(11)-diene-3,20-dione,



L. 20-methylidene-3-oxopregn-4-en-21-al,

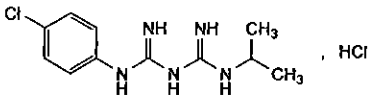


M. (17α)-pregn-4-ene-3,20-dione.

Ph Eur

Proguanil Hydrochloride

(Ph. Eur. monograph 2002)



$C_{11}H_{17}Cl_2N_5$ 290.2 *637-32-1*

Action and use
Antiprotozoal (malaria).

Preparation
Proguanil Tablets

Ph Eur

DEFINITION
N^1-(4-Chlorophenyl)-N^3-(propan-2-yl)imidodicarbonimidic diamide hydrochloride.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Slightly soluble in water, sparingly soluble in anhydrous ethanol, practically insoluble in methylene chloride.

IDENTIFICATION
First identification: A, C.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison proguanil hydrochloride CRS.

B. Dissolve 0.4 g in 50 mL of *water R* (solution A).
To 15 mL of solution A add 2 mL of *dilute sodium hydroxide solution R*. Extract with 20 mL of *ethyl acetate R*. Wash the organic layer with *water R*, evaporate to dryness and dry at 105 °C. The melting point (*2.2.14*) of the residue is 130 °C to 133 °C.

C. It gives reaction (a) of chlorides (*2.3.1*).

TESTS
Acidity or alkalinity
To 35 mL of *water R* maintained at 60-65 °C, add 0.2 mL of *methyl red mixed solution R*. Neutralise to a grey colour with either *0.01 M sodium hydroxide* or *0.01 M hydrochloric acid*.
Add 0.4 g of the substance to be examined and stir until completely dissolved. The solution is grey or green.
Not more than 0.2 mL of *0.01 M hydrochloric acid* is required to change the colour of the solution to reddish-violet.

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R, methanol R, water R* (20:20:60 *V/V/V*).

Test solution (a) Dissolve 20 mg of the substance to be examined in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Test solution (b) Dissolve 20 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dissolve 5 mg of *proguanil for system suitability CRS* (containing impurity G) in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 4.0 mg of *proguanil impurity B CRS* in the solvent mixture and dilute to 100.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 50.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Column:
- *size*: *l* = 0.075 m, Ø = 4.6 mm;
- *stationary phase*: *octylsilyl silica gel for chromatography R* (3.5 µm);
- *temperature*: 40 °C.

Mobile phase:
- *mobile phase A*: dissolve 0.65 g of *sodium pentanesulfonate R* and 7.0 g of *sodium perchlorate R* in *water for chromatography R* and dilute to 1000 mL with the same solvent; adjust to pH 3.0 with a 1 per cent *V/V* solution of *trifluoroacetic acid R*;
- *mobile phase B*: dissolve 0.65 g of *sodium pentanesulfonate R* and 7.0 g of *sodium perchlorate R* in a mixture of 20 volumes of *water for chromatography R*, 40 volumes of *acetonitrile for chromatography R* and 40 volumes of *methanol R1* and dilute to 1000 mL with the same solvent mixture; add 4 mL of a 1 per cent *V/V* solution of *trifluoroacetic acid R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	75	25
3 - 23	75 → 40	25 → 60

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 235 nm.

Injection 20 µL of test solution (a) and reference solutions (a) and (b).

Identification of impurities Use the chromatogram supplied with *proguanil for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peak due to impurity G.

Relative retention With reference to proguanil (retention time = about 16 min): impurity G = about 1.05.

System suitability Reference solution (a):
- *peak-to-valley ratio*: minimum 5.0, where H_p = height above the baseline of the peak due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to proguanil.

Calculation of percentage contents:
- for each impurity, use the concentration of proguanil hydrochloride in reference solution (b).

Limits:
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.2 per cent;
- *reporting threshold*: 0.05 per cent, except for impurity B.

Impurity B

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution (b) and reference solution (c).

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peak due to impurity B.

Relative retention With reference to proguanil (retention time = about 16 min): impurity B = about 0.2.

System suitability Reference solution (c):
- *signal-to-noise ratio*: minimum 10 for the peak due to impurity B.

Limit:
- *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (200 ppm).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Suspend 0.100 g in 20 mL of *anhydrous acetic acid R*, shake and heat at 50 °C for 5 min. Cool to room temperature and add 40 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 14.51 mg of $C_{11}H_{17}Cl_2N_5$.

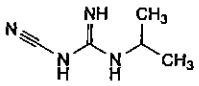
STORAGE

Protected from light.

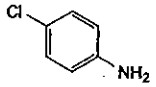
IMPURITIES

Specified impurities *B.*

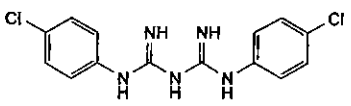
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, C, D, E, F, G.*



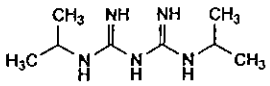
A. *N*-cyano-*N'*-(propan-2-yl)guanidine,



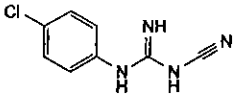
B. 4-chloroaniline,



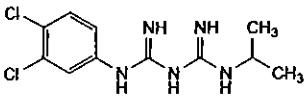
C. N^1,N^5-bis(4-chlorophenyl)imidodicarbonimidic diamide,



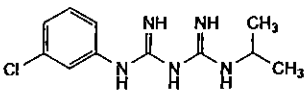
D. N^1,N^3-bis(propan-2-yl)imidodicarbonimidic diamide,



E. *N*-(4-chlorophenyl)-*N'*-cyanoguanidine,



F. N^1-(3,4-dichlorophenyl)-N^3-(propan-2-yl) imidodicarbonimidic diamide,

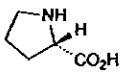


G. N^1-(3-chlorophenyl)-N^3-(propan-2-yl)imidodicarbonimidic diamide.

Ph Eur

Proline

(Ph. Eur. monograph 0785)



$C_5H_9NO_2$ 115.1 *147-85-3*

Action and use
Amino acid.

Ph Eur

DEFINITION

(2*S*)-Pyrrolidine-2-carboxylic acid.

Product of fermentation or of protein hydrolysis.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder or colourless crystals.

Solubility
Very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: A, B.

Second identification: A, C.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *proline CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in a 1 per cent *V/V* solution of *hydrochloric acid R* and dilute to 50 mL with the same solution.

Reference solution Dissolve 10 mg of *proline CRS* in a 1 per cent *V/V* solution of *hydrochloric acid R* and dilute to 50 mL with the same solution.

Plate *TLC silica gel plate R.*

Mobile phase *glacial acetic acid R, water R, butanol R* (20:20:60 *V/V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *ninhydrin solution R* and heat at 105 °C for 15 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Solution S
Dissolve 2.5 g in *distilled water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*)
−86.0 to −84.0 (dried substance).

Dissolve 1.00 g in *water R* and dilute to 25.0 mL with the same solvent.

Ninhydrin-positive substances
Amino acid analysis (*2.2.56*). For analysis, use Method 1.

The concentrations of the test solution and the reference solutions may be adapted according to the sensitivity of the equipment used. The concentrations of all solutions are adjusted so that the system suitability requirements described in general chapter *2.2.46* are fulfilled, keeping the ratios of concentrations between all solutions as described.

Solution A *dilute hydrochloric acid R1* or a sample preparation buffer suitable for the apparatus used.

Test solution Dissolve 30.0 mg of the substance to be examined in solution A and dilute to 50.0 mL with solution A.

Reference solution (a) Dissolve 30.0 mg of *alanine R* (impurity A) in solution A and dilute to 50.0 mL with solution A. Dilute 1.0 mL of the solution to 100.0 mL with solution A. Dilute 1.0 mL of this solution to 10.0 mL with solution A.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with solution A. Dilute 2.0 mL of this solution to 10.0 mL with solution A.

Reference solution (c) Dilute 6.0 mL of *ammonium standard solution (100 ppm NH_4) R* to 50.0 mL with solution A. Dilute 1.0 mL of this solution to 100.0 mL with solution A.

Reference solution (d) Dissolve 30 mg of *isoleucine R* and 30 mg of *leucine R* in solution A and dilute to 50.0 mL with solution A. Dilute 1.0 mL of the solution to 200.0 mL with solution A.

Blank solution Solution A.

Inject suitable, equal amounts of the test, blank and reference solutions into the amino acid analyser. Run a program suitable for the determination of physiological amino acids.

System suitability Reference solution (d):
— *resolution*: minimum 1.5 between the peaks due to isoleucine and leucine.

Calculation of percentage contents:
— for any ninhydrin-positive substance detected at 570 nm, use the concentration of impurity A in reference solution (a);
— for any ninhydrin-positive substance detected at 440 nm, use the concentration of proline in reference solution (b); if a peak is above the reporting threshold at both wavelengths, use the result obtained at 570 nm for quantification.

Limits:
— *any ninhydrin-positive substance:* for each impurity, maximum 0.2 per cent;
— *total:* maximum 0.5 per cent;
— *reporting threshold:* 0.05 per cent.

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Chlorides *(2.4.4)*
Maximum 200 ppm.

Dilute 5 mL of solution S to 15 mL with *water R.*

Sulfates *(2.4.13)*
Maximum 300 ppm.

Dilute 10 mL of solution S to 15 mL with *distilled water R.*

Ammonium
Amino acid analysis *(2.2.56)* as described in the test for ninhydrin-positive substances with the following modifications.

Injection Test solution, reference solution (c) and blank solution.

Limit:
— *ammonium at 570 nm:* not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.02 per cent), taking into account the peak due to ammonium in the chromatogram obtained with the blank solution.

Iron *(2.4.9)*
Maximum 10 ppm.

In a separating funnel, dissolve 1.0 g in 10 mL of *dilute hydrochloric acid R.* Shake with 3 quantities, each of 10 mL, of *methyl isobutyl ketone R1,* shaking for 3 min each time. To the combined organic layers add 10 mL of *water R* and shake for 3 min. Use the aqueous layer.

Loss on drying *(2.2.32)*
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

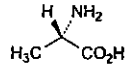
Sulfated ash *(2.4.14)*
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.100 g in 3 mL of *anhydrous formic acid R.* Add 30 mL of *anhydrous acetic acid R.* Titrate with *0.1 M perchloric acid,* determining the end-point potentiometrically *(2.2.20).*

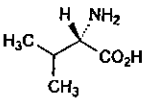
1 mL of *0.1 M perchloric acid* is equivalent to 11.51 mg of $C_5H_9NO_2$.

STORAGE
Protected from light.

IMPURITIES
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B.*



A. (2*S*)-2-aminopropanoic acid (alanine),

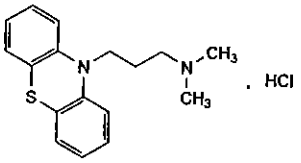


B. (2*S*)-2-amino-3-methylbutanoic acid (valine).

Ph Eur

Promazine Hydrochloride

(Ph. Eur. monograph 1365)



$C_{17}H_{21}ClN_2S$ 320.9 *53-60-1*

Action and use
Dopamine receptor antagonist; neuroleptic.

Preparations
Promazine Injection
Promazine Tablets

Ph Eur

DEFINITION
N,N-Dimethyl-3-(10*H*-phenothiazin-10-yl)propan-1-amine hydrochloride.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, slightly hygroscopic, crystalline powder.

Solubility
Very soluble in water, in ethanol (96 per cent) and in methylene chloride.

mp
About 179 °C.

IDENTIFICATION
First identification: A, D.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry *(2.2.24).*

Comparison: promazine hydrochloride CRS.

B. Identification test for phenothiazines by thin-layer chromatography *(2.3.3):* use *promazine hydrochloride CRS* to prepare the reference solution.

C. Dissolve about 5 mg in 2 mL of *sulfuric acid R* and allow to stand for 5 min. An orange colour is produced.

D. Dissolve 18 mg in 2 mL of *methanol R.* The solution gives reaction (a) of chlorides *(2.3.1).*

TESTS

pH
(*2.2.3*): 4.2 to 5.2, measured immediately after preparation.
Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Related substances
Thin-layer chromatography (*2.2.27*). *Prepare the solutions immediately before use and carry out the test protected from bright light.*
Solvent mixture *diethylamine R, methanol R* (5:95 *V/V*).
Test solution Dissolve 0.100 g of the substance to be examined in the solvent mixture and dilute to 10 mL with the solvent mixture.
Reference solution (a) Dilute 1 mL of the test solution to 200 mL with the solvent mixture.
Reference solution (b) Dissolve 10 mg of *chlorprothixene hydrochloride CRS* in the solvent mixture, add 1 mL of the test solution and dilute to 10 mL with the solvent mixture.
Plate *TLC silica gel F_{254} plate R.*
Mobile phase *acetone R, diethylamine R, cyclohexane R* (10:10:80 *V/V/V*).
Application 10 µL.
Development Over 2/3 of the plate.
Drying In air, until the solvents have evaporated.
Detection Examine in ultraviolet light at 254 nm.
Retardation factors Chlorprothixene = about 0.25; promazine = about 0.5.
System suitability Reference solution (b):
— the chromatogram shows 2 major spots (due to promazine and chlorprothixene) which are clearly separated.

Limit:
— *any impurity:* any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent); disregard any spot at the point of application.

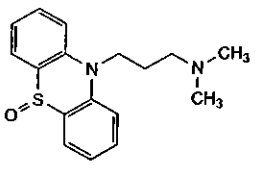
Loss on drying
(*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash
(*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.250 g in a mixture of 5.0 mL of *0.01 M hydrochloric acid* and 50 mL of *ethanol (96 per cent) R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.
1 mL of *0.1 M sodium hydroxide* is equivalent to 32.09 mg of $C_{17}H_{21}ClN_2S$.

STORAGE
In an airtight container protected from light.

IMPURITIES

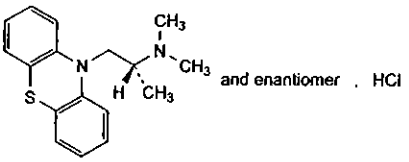


A. 10-[3-(dimethylamino)propyl]-5λ⁴-phenothiazin-5(10*H*)-one (promazine sulfoxide).

Ph Eur

Promethazine Hydrochloride

(Ph. Eur. monograph 0524)



$C_{17}H_{21}ClN_2S$ 320.9 *58-33-3*

Action and use
H_1 receptor antagonist; antihistamine.

Preparations
Promethazine Injection
Promethazine Oral Solution
Promethazine Hydrochloride Tablets

Ph Eur

DEFINITION
(2*RS*)-*N*,*N*-Dimethyl-1-(10*H*-phenothiazin-10-yl)propan-2-amine hydrochloride.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or faintly yellowish, crystalline powder.

Solubility
Very soluble in water, freely soluble in ethanol (96 per cent) and in methylene chloride.

mp
About 222 °C, with decomposition.

IDENTIFICATION
First identification: A, D.
Second identification: B, C, D.
A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *promethazine hydrochloride CRS.*
B. Identification test for phenothiazines by thin-layer chromatography (*2.3.3*): use *promethazine hydrochloride CRS* to prepare the reference solution.
C. Dissolve 0.1 g in 3 mL of *water R*. Add dropwise 1 mL of *nitric acid R*. A precipitate is formed which rapidly dissolves to give a red solution, becoming orange and then yellow. Heat to boiling. The solution becomes orange and an orange-red precipitate is formed.
D. Dissolve 18 mg in 2 mL of *methanol R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS
pH (*2.2.3*)
4.0 to 5.0, measured immediately after preparation.
Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use and carry out the test protected from light.*
Solvent mixture *triethylamine R, methanol R* (1:1000 *V/V*).
Test solution Dissolve 25.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dissolve 2.5 mg of *promethazine for peak identification CRS* (containing impurities A, B and C) in the solvent mixture and dilute to 5 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 5.0 mg of *promethazine impurity D CRS* in the solvent mixture and dilute to 100.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 100.0 mL with the solvent mixture.

Column:
- *size*: l = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octylsilyl silica gel for chromatography with embedded polar groups R* (5 µm).

Mobile phase Mix 20 volumes of *methanol R*, 30 volumes of *acetonitrile R* and 50 volumes of a 3.4 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 7.0 with *potassium hydroxide R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µL.

Run time 2.5 times the retention time of promethazine.

Identification of impurities Use the chromatogram supplied with *promethazine for peak identification CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B and C; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity D.

Relative retention With reference to promethazine (retention time = about 18 min): impurity D = about 0.2; impurity C = about 0.5; impurity B = about 1.4; impurity A = about 1.8.

System suitability Reference solution (a):
- *resolution*: minimum 2.0 between the peaks due to impurities B and A.

Limits:
- *correction factor*: for the calculation of content, multiply the peak area of impurity A by 0.5;
- *impurity B*: not more than 8 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.8 per cent);
- *impurity C*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *impurity D*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.1 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- *total*: not more than 12 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.2 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in a mixture of 5.0 mL of *0.01 M hydrochloric acid* and 50 mL of *ethanol (96 per cent) R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

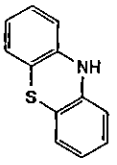
1 mL of *0.1 M sodium hydroxide* is equivalent to 32.09 mg of $C_{17}H_{21}ClN_2S$.

STORAGE

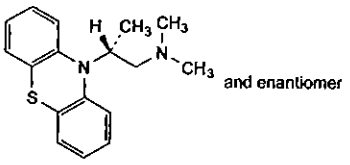
Protected from light.

IMPURITIES

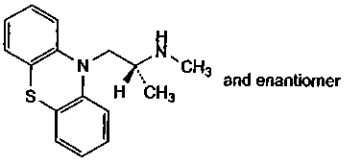
Specified impurities *A, B, C, D.*



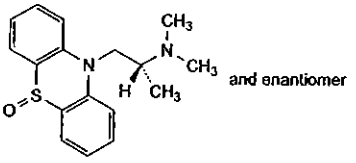
A. phenothiazine,



B. (2*RS*)-*N*,*N*-dimethyl-2-(10*H*-phenothiazin-10-yl)propan-1-amine (isopromethazine),

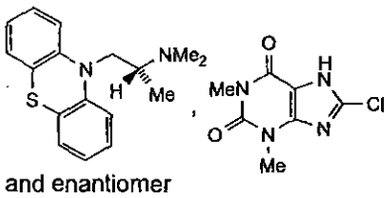


C. (2*RS*)-*N*-methyl-1-(10*H*-phenothiazin-10-yl)propan-2-amine,



D. 10-[(2*RS*)-2-(dimethylamino)propyl]-5λ⁴-phenothiazin-5(10*H*)-one.

Promethazine Teoclate



and enantiomer

$C_{17}H_{20}N_2S,C_7H_7ClN_4O_2$ 499.0 *17693-51-5*

Action and use
Histamine H_1 receptor antagonist; antihistamine.

Preparation
Promethazine Teoclate Tablets

DEFINITION
Promethazine Teoclate is the (*RS*)-dimethyl(2-phenothiazin-10-ylpropyl)amine salt of 8-chlorotheophylline. It contains not less than 98.0% and not more than 101.0% of $C_{17}H_{20}N_2S,C_7H_7ClN_4O_2$, calculated with reference to the dried substance.

CHARACTERISTICS
A white or almost white powder.

Very slightly soluble in *water*; sparingly soluble in *ethanol (96%)*; practically insoluble in *ether*.

IDENTIFICATION
A. Shake 0.15 g with 2.5 mL of *water*, add 1 mL of 5M *ammonia* and extract with 30 mL of *ether*. Wash the ether extract with 10 mL of *water*, dry with *anhydrous sodium sulfate* and evaporate the ether to dryness. Dissolve the residue in 1 mL of *chloroform IR*. The *infrared absorption spectrum* of the resulting solution, Appendix II A, is concordant with the *reference spectrum* of promethazine *(RS 297)*.

B. Dissolve 5 mg in 2 mL of *sulfuric acid* and allow to stand for 5 minutes. A red colour is produced.

C. Shake 0.4 g with 10 mL of *water*, add 4 mL of 5M *ammonia*, shake with two 30-mL quantities of *ether* and add 4 mL of *hydrochloric acid* to the aqueous solution. Filter the white precipitate, wash with *water* and dry at 105°. Dissolve 10 mg of the residue in 1 mL of *hydrochloric acid*, add 0.1 g of *potassium chlorate* and evaporate to dryness. A reddish residue remains which becomes purple on exposure to the vapour of ammonia.

TESTS
Chloride
Shake 0.3 g with 30 mL of *water* for 2 minutes and filter. 15 mL of the filtrate complies with the *limit test for chlorides*, Appendix VII, but using 2 mL of *nitric acid* in place of the 1 mL of *dilute nitric acid* (350 ppm).

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable) and a mixture of 5 volumes of *diethylamine*, 10 volumes of *acetone* and 85 volumes of *cyclohexane* as the mobile phase. Pour the mobile phase into an unlined tank, immediately place the prepared plate in the tank, close the tank and allow the solvent front to ascend 12 cm above the line of application. Apply separately to the plate 10 µL of each of the following solutions in a mixture of 5 volumes of *diethylamine* and 95 volumes of *methanol*. Solution (1) contains 2% w/v of the substance being examined. Solution (2) contains 0.02% w/v of *isopromethazine hydrochloride BPCRS*. For solution (3) dilute 1 volume of solution (1) to 200 volumes. For solution (4) dilute 1 volume of solution (1) to 500 volumes. Allow the plate to dry in air and examine under *ultraviolet light (254 nm)*. In the chromatogram obtained with solution (1) any spot corresponding to isopromethazine is not more intense than the spot in the chromatogram obtained with solution (2) (1%), any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%) and not more than three such spots are more intense than the spot in the chromatogram obtained with solution (4) (0.2%). Disregard any spot remaining on the line of application.

Loss on drying
When dried to constant weight at 105°, loses not more than 0.5% of its weight. Use 1 g.

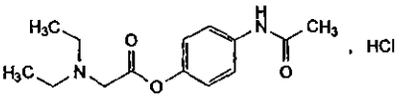
Sulfated ash
Not more than 0.1%, Appendix IX A.

ASSAY
Dissolve 1 g in 200 mL of *acetone* and carry out Method I for *non-aqueous titration*, Appendix VIII A, using 3 mL of a saturated solution of *methyl orange* in *acetone* as indicator. Each mL of *0.1M perchloric acid VS* is equivalent to 49.90 mg of $C_{17}H_{20}N_2S,C_7H_7ClN_4O_2$.

STORAGE
Promethazine Teoclate should be protected from light.

Propacetamol Hydrochloride

(Ph. Eur. monograph 1366)



$C_{14}H_{21}ClN_2O_3$ 300.8 *66532-86-3*

Action and use
Analgesic; antipyretic.

Ph Eur

DEFINITION
4-(Acetylamino)phenyl (diethylamino)acetate hydrochloride.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water, slightly soluble in anhydrous ethanol, practically insoluble in acetone.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of propacetamol hydrochloride.*

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS
Solution S
Prepare the solution immediately before use Dissolve 1.75 g in *water R* and dilute to 10.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 or BY_6 (*2.2.2, Method II*).

Absorbance (*2.2.25*)
Maximum 0.05, determined at 390 nm on solution S.

Impurity B
Thin-layer chromatography (*2.2.27*).

Test solution Suspend 4.00 g of the substance to be examined in 8 mL of *acetonitrile R*. Shake for 30 min and filter. Dilute to 10 mL with *acetonitrile R*.

Reference solution (a) Dissolve 25 mg of *4-aminophenol R* (impurity B) in *acetonitrile R* and dilute to 50 mL with the same solvent. Dilute 10 mL of this solution to 50 mL with *acetonitrile R*.

Reference solution (b) Dilute 5 mL of reference solution (a) to 50 mL with *acetonitrile R*.

Reference solution (c) Dilute 0.2 mL of reference solution (a) to 5 mL with the test solution.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *anhydrous formic acid R, water R, methanol R, methylene chloride R* (3:4:30:64 *V/V/V/V*).

Application 50 µL of the test solution and of reference solutions (b) and (c).

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm. Spray with a 10 g/L solution of *dimethylaminobenzaldehyde R* in *ethanol (96 per cent) R*.

Identification of spots Reference solution (c) shows 2 spots, one visible in ultraviolet light due to propacetamol and the other one yellow, visible after spraying due to impurity B. An additional spot may appear in ultraviolet light and corresponds to impurity A.

System suitability Reference solution (c):
— the chromatogram shows 2 clearly separated spots.

Limit:
— *impurity B*: any yellow spot due to impurity B not visible in ultraviolet light is not more intense than the corresponding spot in the chromatogram obtained with reference solution (b) (25 ppm).

Related substances
Liquid chromatography (*2.2.29*).

Solution A Dissolve 2.16 g of *sodium octanesulfonate R* in 900 mL of *water R* and dilute to 1000 mL with the same solvent. Adjust to pH 3.0 with *acetic acid R*.

Test solution Suspend 1.00 g of the substance to be examined in 10.0 mL of *acetonitrile R*. Shake for 10 min. Allow to stand. Take 3.0 mL of the supernatant solution and dilute to 10.0 mL with solution A. Inject immediately.

Reference solution (a) Dissolve 50 mg of *paracetamol R* (impurity A) in *acetonitrile R* and dilute to 50.0 mL with the same solvent. Dilute 1.0 mL of the solution to 50.0 mL with *acetonitrile R*. Dilute 3.0 mL of this solution to 10.0 mL with solution A.

Reference solution (b) Dissolve 10 mg of *paracetamol R* (impurity A) and 0.100 g of *4-aminophenol R* (impurity B) in *acetonitrile R* and dilute to 50.0 mL with the same solvent. Dilute 1.0 mL of the solution to 50.0 mL with *acetonitrile R*. Dilute 3.0 mL of this solution to 10.0 mL with solution A.

Column:
— *size*: l = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R*, solution A (30:70 *V/V*).

Flow rate 1 mL/min.

Detection Spectrophotometer at 246 nm.

Injection 20 µL.

Run time Twice the retention time of propacetamol.

Identification of impurities The chromatogram obtained with reference solution (b) shows a peak due to impurity A (1st peak) and a peak due to impurity B (2nd peak).

Relative retention With reference to impurity A: impurity B = about 1.6.

Limits:
— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (200 ppm);
— *unspecified impurities*: for each impurity, not more than 3.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent taking into account the response factor of paracetamol of 1.6);
— *total*: not more than 6.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent taking into account the relative response factor of paracetamol of 1.6);
— *disregard limit*: 0.01 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3 ppm taking into account the relative response factor of paracetamol of 1.6).

Methanol
Gas chromatography (*2.2.28*).

Internal standard solution Dilute 2.0 mL of *propanol R* to 20.0 mL with *water R*. Dilute 1.0 mL of the solution to 25.0 mL with *water R*. Dilute 1.0 mL of this solution to 25.0 mL with *water R*.

Test solution Dissolve 2.00 g of the substance to be examined in *water R*, add 2.0 mL of the internal standard solution and dilute to 10.0 mL with *water R*.

Reference solution Dilute 0.8 mL of *methanol R* to 50.0 mL with *water R*. Dilute 1.0 mL of the solution to 25.0 mL with *water R*. To 2.0 mL of this solution, add 2.0 mL of the internal standard solution and dilute to 10.0 mL with *water R*.

Column:
— *material*: glass;
— *size*: l = 2 m, Ø = 2 mm;
— *stationary phase*: carbon molecular sieve impregnated with 0.2 per cent of macrogol 1500.

Carrier gas *nitrogen for chromatography R*.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 1.5	60
	1.5 - 5.5	60 → 80
	5.5 - 15.5	80
Injection port		170
Detector		220

Detection Flame ionisation.

Injection 2 µL.

Limit:

— *methanol:* calculate the ratio (*R*) of the area of the peak due to methanol to the area of the peak due to the internal standard from the chromatogram obtained with the reference solution; from the chromatogram obtained with the test solution, calculate the ratio of the area of any peak due to methanol to the area of the peak due to the internal standard: this ratio is not greater than *R* (500 ppm).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

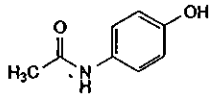
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.250 g in a mixture of 25 mL of *anhydrous acetic acid R* and 25 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

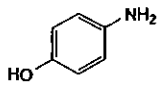
1 mL of *0.1 M perchloric acid* is equivalent to 30.08 mg of $C_{14}H_{21}ClN_2O_3$.

STORAGE
Protected from humidity.

IMPURITIES
Specified impurities *A, B.*



A. *N*-(4-hydroxyphenyl)acetamide (paracetamol),

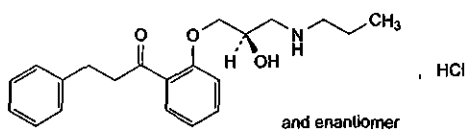


B. 4-aminophenol.

Ph Eur

Propafenone Hydrochloride

(Ph. Eur. monograph 2103)



$C_{21}H_{28}ClNO_3$ 377.9 *34183-22-7*

Action and use
Class I antiarrhythmic.

Ph Eur

DEFINITION
1-[2-[(2*RS*)-2-Hydroxy-3-(propylamino)propoxy]phenyl]-3-phenylpropan-1-one hydrochloride.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
Colourless crystals or white or almost white powder.

Solubility
Slightly soluble in cold water, soluble in methanol and in hot water, practically insoluble in ethanol (96 per cent).

mp
About 173 °C.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *propafenone hydrochloride CRS.*

B. To 5.0 mL of solution S (see Tests) add 2 drops of *dilute nitric acid R*. A precipitate is formed. After 10 min, filter. 2.0 mL of the clear filtrate gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S
To 0.500 g in a 100 mL volumetric flask add 50 mL of *water R*, and heat to boiling for 5 min. Allow to cool to room temperature and dilute to 100.0 mL with *carbon dioxide-free water R*.

Appearance of solution
Solution S is not more opalescent than reference suspension II (*2.2.1*) and is colourless (*2.2.2, Method II*).

pH (*2.2.3*)
5.0 to 6.2 for solution S.

Optical rotation (*2.2.7*)
−0.05° to + 0.05°.

Dissolve 1.00 g in *methanol R* and dilute to 100.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture Mobile phase B, mobile phase A (35:65 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 5.0 mg of the substance to be examined and 5.0 mg of *propafenone impurity B CRS* in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Column:
— *size:* l = 0.15 m, Ø = 4.6 mm,
— *stationary phase: end-capped octylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 320-350 m^2/g and a pore size of 12-13 nm,
— *temperature:* 30 °C.

Mobile phase:
— *mobile phase A:* 3.42 g/L solution of *dipotassium hydrogen phosphate trihydrate R* adjusted to pH 2.5 with *phosphoric acid R*,
— *mobile phase B: acetonitrile for chromatography R*,

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 8	65	35
8 - 20	65 → 30	35 → 70
20 - 30	30	70

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 220 nm.

Equilibration 60 min with the mobile phase at the initial composition, before each series of injections.

Injection 20 µL of the test solution, reference solutions (a), (b) and (c) and of the solvent mixture as a blank.

Relative retention With reference to propafenone (retention time = about 5 min): impurity B = about 0.8; impurity D = about 2.3; impurity G = about 3.6; impurity C = about 4.1; impurity F = about 5.3.

System suitability Reference solution (c):
— *resolution*: minimum 3.0 between the peaks due to impurity B and propafenone.

Limits:
— *impurities B, C, D, F, G*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent),
— *disregard limit*: 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.03 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

In order to avoid overheating during the titration, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.

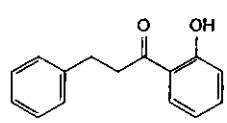
Dissolve 0.300 g in 2 mL of *anhydrous formic acid R*. Add 50 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 mL of *0.1 M perchloric acid* is equivalent to 37.79 mg of $C_{21}H_{28}ClNO_3$.

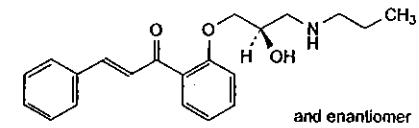
IMPURITIES

Specified impurities *B, C, D, F, G.*

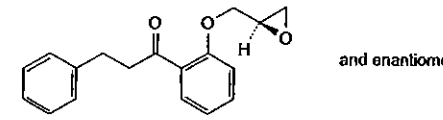
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, E, H.*



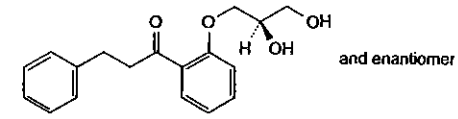
A. 1-(2-hydroxyphenyl)-3-phenylpropan-1-one,



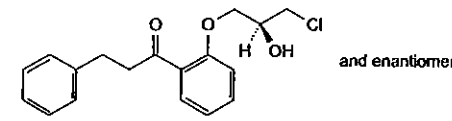
B. (2*E*)-1-[2-[(2*RS*)-2-hydroxy-3-(propylamino)propoxy]phenyl]-3-phenylprop-2-en-1-one,



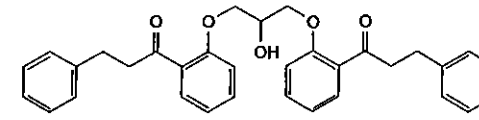
C. 1-[2-[[(2*RS*)-oxiranyl]methoxy]phenyl]-3-phenylpropan-1-one,



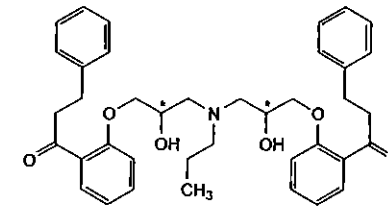
D. 1-[2-[(2*RS*)-2,3-dihydroxypropoxy]phenyl]-3-phenylpropan-1-one,



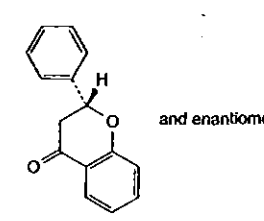
E. 1-[2-[(2*RS*)-3-chloro-2-hydroxypropoxy]phenyl]-3-phenylpropan-1-one,



F. 1,1′-[2-hydroxypropane-1,3-diylbis(oxy-2,1-phenylene)]bis(3-phenylpropan-1-one),



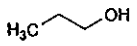
G. 1,1′-[propyliminobis[(2-hydroxypropane-3,1-diyl)oxy-2,1-phenylene]]bis(3-phenylpropan-1-one),



H. (2*RS*)-2-phenyl-2,3-dihydro-4*H*-1-benzopyran-4-one.

Propanol

(Ph. Eur. monograph 2036)



C_3H_8O 60.1 *71-23-8*

Ph Eur

DEFINITION

Propan-1-ol.

CHARACTERS

Appearance

Clear, colourless liquid.

Solubility

Miscible with water and with ethanol.

IDENTIFICATION

First identification: C, B.

Second identification: A, B, D.

A. Refractive index (*2.2.6*): 1.384 to 1.387.

B. Boiling point (*2.2.12*): 96 °C to 98 °C.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of propanol.*

D. To 1.0 mL add 0.10 g of *dinitrobenzoyl chloride R* and 0.05 mL of *sulfuric acid R*. Boil under reflux for 30 min. Evaporate until the excess of propanol is removed, add 5 mL of *heptane R* to the residue and heat to boiling. Filter the hot solution. Wash the crystals formed on cooling with *heptane R* and dry in vacuum (2 kPa, at room temperature for 24 h). The small, colourless, shiny plates melt (*2.2.14*) between 71 °C and 74 °C.

TESTS

Appearance

The substance to be examined is clear (*2.2.1*) and colourless (*2.2.2, Method II*). Dilute 2 mL to 10 mL with *water R*. After 5 min, the solution is clear (*2.2.1*).

Acidity or alkalinity

To 10.0 mL of *carbon dioxide-free water R* add 0.1 mL of *phenolphthalein solution R* and *0.01 M sodium hydroxide* until the solution becomes pale pink. After addition of 5.0 mL of the substance to be examined the colour of the solution does not become more intense. If the colour fades, add 0.2 mL of *0.01 M sodium hydroxide*. The solution is pink.

Absorbance (*2.2.25*)

Measure the absorbance between 230 nm and 310 nm using *water R* as the compensation liquid. The absorbance *A* is not greater than the following values.

Wavelength (nm)	Absorbance *A*
230	0.300
250	0.100
270	0.030
290	0.020
310	0.010

The absorption curve does not show any peaks.

Reducing substances

Place 10.0 mL in a test tube of about 20 mm in diameter in a water bath at 20 °C. Keep protected from actinic light and add 1.0 mL of a freshly prepared 0.16 g/L solution of *potassium permanganate R*. The mixture, maintained at 20 °C, slowly changes its colour from violet to red. After 30 min, the test solution is not less intensely coloured (*2.2.2, Method II*) than 10.0 mL of a reference solution prepared as follows: to 5.5 mL of primary solution yellow, add 13.0 mL of primary solution red and dilute to 100.0 mL with *water R*.

Related substances

Gas chromatography (*2.2.28*).

Test solution The substance to be examined.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *heptane R*. Dilute 1.0 mL of this solution to 10.0 mL with *heptane R*.

Reference solution (b) Mix 0.1 mL of *acetone R* and 0.1 mL of *2-propanol R* and dilute to 100 mL with the test solution.

Column:

— *material*: fused silica;
— *size*: *l* = 30 m, Ø = 0.25 mm;
— *stationary phase*: *cyanopropyl(3)phenyl(3)methyl(94) polysiloxane R* (film thickness 1.4 µm).

Carrier gas *helium for chromatography R.*

Linear velocity 25 cm/s.

Split ratio 1:200.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 12	40
	12 - 28	40 → 200
	28 - 38	200
Injection port		240
Detector		240

Detection Flame ionisation.

Injection 1 µL.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to impurities D and E.

Limits:

— *any impurity*: not more than the area of the peak due to propanol in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *total*: not more than 3 times the area of the peak due to propanol in the chromatogram obtained with reference solution (a) (0.3 per cent);
— *disregard limit*: 0.1 times the area of the peak due to propanol in the chromatogram obtained with reference solution (a) (0.01 per cent).

Non-volatile matter

Maximum 0.004 per cent.

Evaporate 50 mL of the substance to be examined to dryness at 100 °C and dry the residue in an oven at 100-105 °C to constant mass. The residue weighs a maximum of 2 mg.

Water (*2.5.12*)

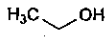
Maximum 0.2 per cent, determined on 10 g.

STORAGE

Protected from light.

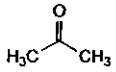
IMPURITIES

A. methanol,

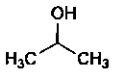


B. ethanol,

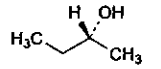
C. propanal,



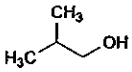
D. propanone (acetone),



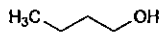
E. isopropyl alcohol (2-propanol),



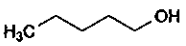
F. butan-2-ol (*sec*-butanol),



G. 2-methylpropan-1-ol (isobutanol),



H. butan-1-ol (*n*-butanol),



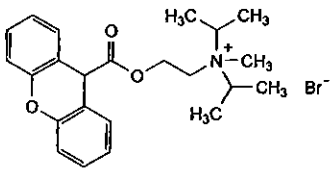
I. pentan-1-ol (*n*-pentanol),

J. hexan-1-ol (*n*-hexanol).

Ph Eur

Propantheline Bromide

(Ph. Eur. monograph 0857)



$C_{23}H_{30}BrNO_3$ 448.4 *50-34-0*

Action and use
Anticholinergic.

Preparation
Propantheline Tablets

Ph Eur

DEFINITION

N-Methyl-*N*,*N*-bis(1-methylethyl)-2-[(9*H*-xanthen-9-ylcarbonyl)oxy]ethanaminium bromide.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or yellowish-white, slightly hygroscopic powder.

Solubility
Very soluble in water, in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 60 mg in *methanol R* and dilute to 100.0 mL with the same solvent. Dilute 10.0 mL of this solution to 100.0 mL with *methanol R*.

Spectral range 230-350 nm.

Absorption maxima At 246 nm and 282 nm.

Specific absorbance at the absorption maxima:
— at 246 nm: 115 to 125;
— at 282 nm: 57 to 63.

B. Dissolve 0.2 g in 15 mL of *water R* and add 1 mL of *strong sodium hydroxide solution R*. Boil for 2 min and cool slightly. Add 7.5 mL of *dilute hydrochloric acid R* and filter. Wash the residue with *water R* and recrystallise from *ethanol (50 per cent V/V) R*. Dry at 100-105 °C for 1 h. Dissolve about 10 mg of the residue in 5 mL of *sulfuric acid R*. The solution has an intense yellow colour and shows an intense yellowish-green fluorescence when examined in ultraviolet light at 365 nm.

C. Dissolve 50 mg in 0.1 mL of *water R* in a 25 mL flask and add 1 mL of a saturated solution of *potassium permanganate R*. Attach a fractionating column and a condenser, with the end of the delivery tube immersed in 1 mL of *water R* in a test-tube placed in a bath of iced water. Distil fairly vigorously and continue heating for 1 min after a dry residue has been obtained in the flask. Prepare a blank by introducing into an identical test-tube a volume of *water R* equal to that of the distillate. Place the tubes in a bath of iced water. To each tube, add 0.5 mL of a 20 per cent *V/V* solution of *morpholine R* and 0.5 mL of a freshly prepared 50 g/L solution of *sodium nitroprusside R*. Mix and allow to stand at 0 °C for 5 min, and then at room temperature for 3 min. No blue colour develops in either tube. Add 1 g of *ammonium sulfate R*, mix and allow to stand for 15 min. A stable, intense pink colour develops in the test solution. A brownish-yellow colour develops in the blank.

D. It gives reaction (a) of bromides (*2.3.1*).

TESTS

Appearance of solution
The solution is clear (*2.2.1*).

Dissolve 0.6 g in *water R* and dilute to 20 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R*, *water R* (40:60 *V/V*).

Test solution (a) Dissolve 6 mg of the substance to be examined in the solvent mixture and dilute to 50 mL with the solvent mixture.

Test solution (b) Dissolve 6 mg of the substance to be examined in 30 mL of the solvent mixture. Add 5 mL of reference solution (b) and dilute to 50 mL with the solvent mixture.

Test solution (c) Dissolve 6 mg of *xanthydrol R1* and 6 mg of the substance to be examined in the solvent mixture, then dilute to 50 mL with the solvent mixture.

Reference solution (a) Dissolve 6 mg of *xanthydrol RI* in the solvent mixture and dilute to 50 mL with the solvent mixture.

Reference solution (b) Dilute 5 mL of reference solution (a) to 50 mL with the solvent mixture.

Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mixture of equal volumes of *acetonitrile R* and of a solution containing 28 g/L of *sodium perchlorate R* and 11 g/L of *phosphoric acid R*, adjusted to pH 3.8 with *strong sodium hydroxide solution R* and then with *0.1 M sodium hydroxide.*

Flow rate 1 mL/min.

Detection Spectrophotometer at 206 nm.

Injection 20 µL of test solutions (a), (b), (c) and reference solution (a).

Run time Twice the retention time of propantheline.

System suitability Test solution (c):
— in the chromatogram obtained with test solution (a), there is no peak corresponding to the principal peak in the chromatogram obtained with reference solution (a);
— *resolution*: minimum 8.0 between the peaks due to propantheline and xanthydrol.

Limits Test solution (b):
— *any impurity*: for each impurity, not more than the area of the peak due to xanthydrol (1.0 per cent), and not more than one such peak has an area greater than or equal to 0.5 times the area of the peak due to xanthydrol (0.5 per cent);
— *disregard limit*: disregard any peak with a retention time relative to propantheline of less than 0.2 (bromide); disregard the peak due to xanthydrol.

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.400 g in 50 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

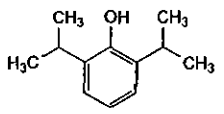
1 mL of *0.1 M perchloric acid* corresponds to 44.84 mg of $C_{23}H_{30}BrNO_3$.

STORAGE
In an airtight container.

Ph Eur

Propofol

(Ph. Eur. monograph 1558)



$C_{12}H_{18}O$ 178.3 *2078-54-8*

Action and use
Intravenous general anaesthetic.

Preparation
Propofol Injection

Ph Eur

DEFINITION
2,6-Bis(1-methylethyl)phenol.

Content
98.0 per cent to 102.0 per cent.

This monograph applies to propofol prepared using distillation for purification.

CHARACTERS

Appearance
Colourless or very light yellow, clear liquid.

Solubility
Very slightly soluble in water, miscible with hexane and with methanol.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *propofol CRS.*

TESTS

Refractive index (*2.2.6*)
1.5125 to 1.5145.

Related substances
Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 1.00 g of the substance to be examined in *hexane R* and dilute to 10.0 mL with the same solvent.

Test solution (b) Dissolve 0.240 g of the substance to be examined in *hexane R* and dilute to 100.0 mL with the same solvent.

Reference solution (a) Dissolve 5 µL of the substance to be examined and 15 µL of *propofol impurity J CRS* in *hexane R* and dilute to 50.0 mL with the same solvent.

Reference solution (b) Dilute 0.1 mL of *propofol for peak identification CRS* (containing impurities E and G) to 1.0 mL with *hexane R.*

Reference solution (c) Dilute 1.0 mL of test solution (a) to 100.0 mL with *hexane R.* Dilute 1.0 mL of this solution to 10.0 mL with *hexane R.*

Reference solution (d) Dissolve 0.240 g of *propofol CRS* in *hexane R* and dilute to 100.0 mL with the same solvent.

Column:
— *size: l* = 0.20 m, Ø = 4.6 mm;
— *stationary phase: silica gel for chromatography R* (5 µm).

Mobile phase *anhydrous ethanol R, acetonitrile R, hexane R* (1.0:7.5:990 *V/V/V*).

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 275 nm.

Injection 10 µL of test solution (a) and reference solutions (a), (b) and (c).

Run time 7 times the retention time of propofol.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities G and E.

Relative retention With reference to propofol (retention time = about 3 min): impurity G = about 0.5; impurity I = about 0.6; impurity B = about 0.7; impurity N = about 2.3; impurity D = about 2.5; impurity P = about 2.9; impurity A = about 3.0; impurity C = about 3.4; impurity E = about 4.0; impurity F = about 5.8; impurity H = about 6.4.

System suitability Reference solution (a):
— *resolution*: minimum 4.0 between the peaks due to impurity J and propofol.

Limits:
— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity E = 0.25; impurity G = 5.0;
— *impurity G*: not more than twice the area of the peak due to propofol in the chromatogram obtained with reference solution (c) (0.2 per cent);
— *impurity E*: not more than 0.1 times the area of the peak due to propofol in the chromatogram obtained with reference solution (c) (0.01 per cent);
— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the peak due to propofol in the chromatogram obtained with reference solution (c) (0.05 per cent);
— *total*: not more than 3 times the area of the peak due to propofol in the chromatogram obtained with reference solution (c) (0.3 per cent);
— *disregard limit*: 0.3 times the area of the peak due to propofol in the chromatogram obtained with reference solution (c) (0.03 per cent), except for impurity E.

Impurities J, K, L and O

Gas chromatography (*2.2.28*).

Test solution Dissolve 40.0 mg of the substance to be examined in *methylene chloride R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *methylene chloride R*. Dilute 1.0 mL of this solution to 10.0 mL with *methylene chloride R*.

Reference solution (b) Dissolve 5 µL of *propofol impurity J CRS* (corresponding to 5 mg) in *methylene chloride R* and dilute to 100 mL with the same solvent. Dilute 1.0 mL of this solution to 25 mL with *methylene chloride R*.

Reference solution (c) Dissolve 4 mg of *propofol CRS* in reference solution (b) and dilute to 1 mL with the same solution.

Column:
— *material*: fused silica;
— *size*: *l* = 30 m, Ø = 0.32 mm;
— *stationary phase*: *phenyl(50)methyl(50)polysiloxane R* (film thickness 0.5 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.7 mL/min.

Split ratio 1:5.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 3	80
	3 - 25	80 → 210
	25 - 40	210
Injection port		100
Detector		270

Detection Flame ionisation.

Injection 1 µL of the test solution and reference solutions (a) and (c).

Relative retention With reference to propofol (retention time = about 17 min): impurity K = about 0.76; impurity L = about 0.81; impurity J = about 1.01; impurity O = about 1.03.

System suitability Reference solution (c):
— *peak-to-valley ratio*: minimum 3.0, where H_p = height above the baseline of the peak due to impurity J, and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to propofol.

Limits:
— *impurities J, K, L, O*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (d).

Calculate the percentage content of $C_{12}H_{18}O$ using the declared content of *propofol CRS*.

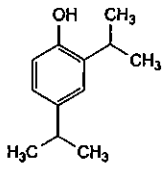
STORAGE

Protected from light under an inert gas.

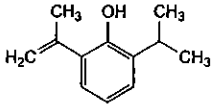
IMPURITIES

Specified impurities *E, G, J, K, L, O.*

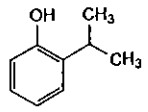
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, F, H, I, N, P.*



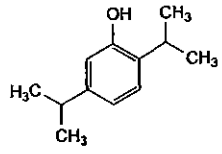
A. 2,4-bis(1-methylethyl)phenol,



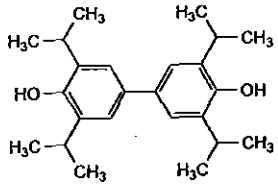
B. 2-(1-methylethenyl)-6-(1-methylethyl)phenol,



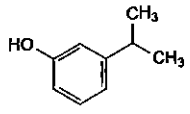
C. 2-(1-methylethyl)phenol,



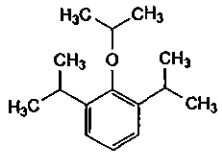
D. 2,5-bis(1-methylethyl)phenol,



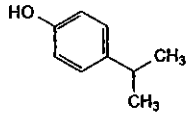
E. 3,3′,5,5′-tetrakis(1-methylethyl)biphenyl-4,4′-diol,



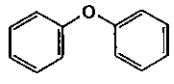
F. 3-(1-methylethyl)phenol,



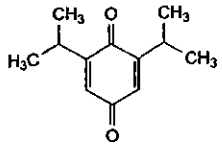
G. 2-(1-methylethoxy)-1,3-bis(1-methylethyl)benzene,



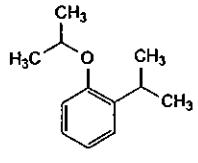
H. 4-(1-methylethyl)phenol,



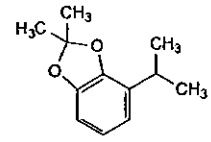
I. oxydibenzene,



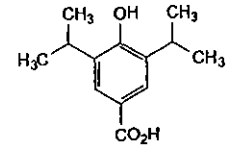
J. 2,6-bis(1-methylethyl)benzene-1,4-dione,



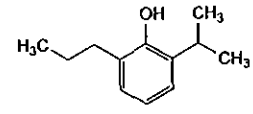
K. 1-(1-methylethoxy)-2-(1-methylethyl)benzene,



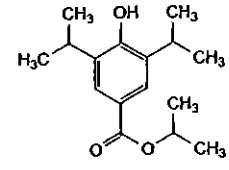
L. 2,2-dimethyl-4-(1-methylethyl)-1,3-benzodioxole,



N. 4-hydroxy-3,5-bis(1-methylethyl)benzoic acid,



O. 2-(1-methylethyl)-6-propylphenol,

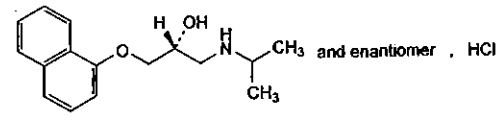


P. 1-methylethyl 4-hydroxy-3,5-bis(1-methylethyl)benzoate.

Ph Eur

Propranolol Hydrochloride

(Ph. Eur. monograph 0568)



$C_{16}H_{22}ClNO_2$ 295.8 *318-98-9*

Action and use
Beta-adrenoceptor antagonist.

Preparations
Propranolol Prolonged-release Capsules
Propranolol Injection
Propranolol Oral Solution
Propranolol Tablets

Ph Eur

DEFINITION
(2*RS*)-1-[(Propan-2-yl)amino]-3-[(naphthalen-1-yl)oxy] propan-2-ol hydrochloride.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white powder.

Solubility
Soluble in water and in ethanol (96 per cent), practically insoluble in heptane.

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 163 °C to 166 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *propranolol hydrochloride CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in 1 mL of *methanol R.*

Reference solution Dissolve 10 mg of *propranolol hydrochloride CRS* in 1 mL of *methanol R.*

Plate *TLC silica gel G plate R.*

Mobile phase *concentrated ammonia R1, methanol R* (1:99 *V/V*).

Application 10 µL.

Development Over 3/4 of the plate.

Drying At 100-105 °C.

Detection Spray with *anisaldehyde solution R* and heat at 100-105 °C until the colour of the spots reaches maximum intensity (10-15 min).

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than intensity 6 of the range of reference solutions of the most appropriate colour (*2.2.2, Method II*).

Dissolve 2.0 g in *methanol R* and dilute to 20 mL with the same solvent.

Acidity or alkalinity

Dissolve 0.20 g in *carbon dioxide-free water R* and dilute to 20 mL with the same solvent. Add 0.2 mL of *methyl red solution R* and 0.2 mL of *0.01 M hydrochloric acid*; the solution is red. Add 0.4 mL of *0.01 M sodium hydroxide*; the solution is yellow.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dissolve 10 mg of *propranolol for system suitability CRS* (containing impurity A) in the mobile phase and dilute to 10 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:

— *size: l* = 0.25 m; Ø = 4.6 mm;

— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 0.31 g of *tetrabutylammonium dihydrogen phosphate R* and 1.6 g of *sodium laurilsulfate R* in a mixture of 1 mL of *sulfuric acid R*, 450 mL of *water for chromatography R* and 550 mL of *acetonitrile R*; adjust to pH 3.3 with *dilute sodium hydroxide solution R.*

Flow rate 1.8 mL/min.

Detection Spectrophotometer at 292 nm.

Injection 20 µL.

Run time 7 times the retention time of propranolol.

Identification of impurities Use the chromatogram supplied with *propranolol for system suitability CRS* and the chromatogram obtained with reference solution (a)to identify the peak due to impurity A.

Relative retention With reference to propranolol (retention time = about 3 min): impurity A = about 0.6.

System suitability Reference solution (a):

— *resolution*: minimum 1.5 between the peaks due to impurity A and propranolol.

Calculation of percentage contents:

— for each impurity, use the concentration of propranolol hydrochloride in reference solution (b).

Limits:

— *unspecified impurities*: for each impurity, maximum 0.10 per cent;

— *total*: maximum 0.2 per cent;

— *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

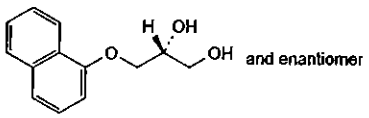
ASSAY

Dissolve 0.250 g in 25 mL of *ethanol (96 per cent) R.* Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

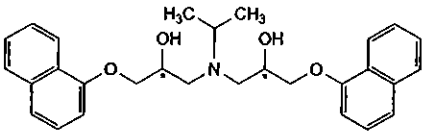
1 mL of *0.1 M sodium hydroxide* is equivalent to 29.58 mg of $C_{16}H_{22}ClNO_2$.

IMPURITIES

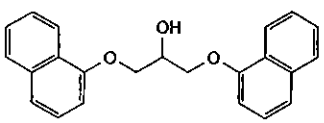
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C.*



A. (2*RS*)-3-[(naphthalen-1-yl)oxy]propane-1,2-diol,



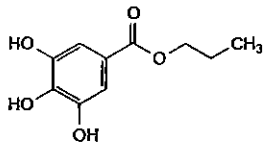
B. 1,1′-[(propan-2-yl)azanediyl]bis[(2Ξ)-3-[(naphthalen-1-yl)oxy]propan-2-ol,



C. 1,3-bis[(naphthalen-1-yl)oxy]propan-2-ol.

Propyl Gallate

(Ph. Eur. monograph 1039)



$C_{10}H_{12}O_5$ 212.2 *121-79-9*

Action and use
Antioxidant.

Ph Eur

DEFINITION
Propyl 3,4,5-trihydroxybenzoate.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Very slightly soluble in water, freely soluble in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION
First identification: B.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 148 °C to 151 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *propyl gallate for ID and assay CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *acetone R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 5 mg of *propyl gallate for ID and assay CRS* in *acetone R* and dilute to 5 mL with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *anhydrous formic acid R, ethyl formate R, toluene R* (10:40:50 *V/V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying In air for 10 min.

Detection Spray with a mixture of 1 volume of *ferric chloride solution R1* and 9 volumes of *ethanol (96 per cent) R.*

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve about 10 mg in 10 mL of *water R* by heating to about 70 °C. Cool and add 1 mL of *bismuth subnitrate solution R.* A bright yellow precipitate is formed.

TESTS
Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

Dissolve 1.0 g in *ethanol (96 per cent) R* and dilute to 20 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 25.0 mg of the substance to be examined in *methanol R* and dilute to 25.0 mL with the same solvent.

Test solution (b) Dilute 2.0 mL of test solution (a) to 20.0 mL with *methanol R.*

Reference solution (a) Dilute 1.0 mL of test solution (a) to 100.0 mL with *methanol R.* Dilute 1.0 mL of this solution to 10.0 mL with *methanol R.*

Reference solution (b) Dissolve 25.0 mg of *propyl gallate for ID and assay CRS* in *methanol R* and dilute to 25.0 mL with the same solvent. Dilute 2.0 mL of the solution to 20.0 mL with *methanol R.*

Reference solution (c) Dissolve 25 mg of *methyl parahydroxybenzoate R* in *methanol R* and dilute to 25 mL with the same solvent. Mix 1 mL of the solution and 1 mL of test solution (a), and dilute to 100 mL with *methanol R.*

Column:
— *size: l* = 0.10 m, Ø = 4.6 mm;
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography with embedded polar groups R* (3.5 µm);
— *temperature:* 28 °C.

Mobile phase:
— *mobile phase A:* 6.8 g/L solution of *potassium dihydrogen phosphate R;*
— *mobile phase B: acetonitrile R;*

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 6	95	5
6 - 25	95 → 45	5 → 55
25 - 40	45	55

Flow rate 0.6 mL/min.

Detection Spectrophotometer at 275 nm.

Injection 10 µL of test solution (a) and reference solutions (a) and (c).

Identification of peaks Use the chromatogram obtained with reference solution (c) to identify the peak due to methyl parahydroxybenzoate.

Relative retention With reference to propyl gallate (retention time = about 20 min): methyl parahydroxybenzoate = about 1.05.

System suitability Reference solution (c):
— *resolution:* minimum 4.0 between the peaks due to propyl gallate and methyl parahydroxybenzoate.

Calculation of percentage contents:
— for each impurity, use the concentration of propyl gallate in reference solution (a).

Limits:
— *unspecified impurities:* for each impurity, maximum 0.10 per cent;
— *total:* maximum 0.15 per cent;
— *reporting threshold:* 0.05 per cent.

Total chlorine
Maximum 200 ppm.

Mix 0.5 g with 2 g of *calcium carbonate R1.* Dry and ignite at 700 ± 50 °C. Take up the residue with 20 mL of *dilute nitric acid R* and dilute to 30 mL with *water R.* 15 mL of the solution, without further addition of *dilute nitric acid R,* complies with the test for chlorides (*2.4.4*).

Chlorides (*2.4.4*)
Maximum 100 ppm.

To 1.65 g add 50 mL of *water R*. Shake for 5 min. Filter.

Zinc
Maximum 25 ppm.

Atomic absorption spectrometry (*2.2.23, Method II*).

Test solution To 2.5 mL of the test solution prepared as described in general chapter *2.4.8* (method C) using 2.0 g of the substance to be examined, add 2.5 mL of *water R*.

Reference solutions Prepare the reference solutions using *zinc standard solution (10 ppm Zn) R*, diluting with *water R*.

Source Zinc hollow-cathode lamp.

Wavelength 213.9 nm.

Atomisation device Air-acetylene flame.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

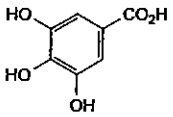
ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (b).

Calculate the percentage content of $C_{10}H_{12}O_5$ taking into account the assigned content of *propyl gallate for ID and assay CRS*.

STORAGE
Protected from light.

IMPURITIES
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) A.



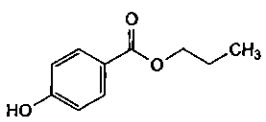
A. 3,4,5-trihydroxybenzoic acid (gallic acid).

Ph Eur

Propyl Hydroxybenzoate

Propylparaben

(Propyl Parahydroxybenzoate, Ph. Eur. monograph 0431)



$C_{10}H_{12}O_3$ 180.2 *94-13-3*

Action and use
Antimicrobial preservative.

Ph Eur

DEFINITION
Propyl 4-hydroxybenzoate.

Content
98.0 per cent to 102.0 per cent.

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Very slightly soluble in water, freely soluble in ethanol (96 per cent) and in methanol.

IDENTIFICATION
First identification: A, B.

Second identification: A, C.

A. Melting point (*2.2.14*): 96 °C to 99 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison propyl parahydroxybenzoate CRS.

C. Thin-layer chromatography (*2.2.27*).

Test solution (a) Dissolve 0.10 g of the substance to be examined in *acetone R* and dilute to 10 mL with the same solvent.

Test solution (b) Dilute 1 mL of test solution (a) to 10 mL with *acetone R*.

Reference solution (a) Dissolve 10 mg of *propyl parahydroxybenzoate CRS* in *acetone R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 10 mg of *ethyl parahydroxybenzoate CRS* in 1 mL of test solution (a) and dilute to 10 mL with *acetone R*.

Plate TLC octadecylsilyl silica gel F_{254} plate R.

Mobile phase glacial acetic acid R, water R, methanol R (1:30:70 *V/V/V*).

Application 2 µL of test solution (b) and reference solutions (a) and (b).

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):
— the chromatogram shows 2 clearly separated principal spots.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS
Solution S
Dissolve 1.0 g in *ethanol (96 per cent) R* and dilute to 10 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Acidity
To 2 mL of solution S add 3 mL of *ethanol (96 per cent) R*, 5 mL of *carbon dioxide-free water R* and 0.1 mL of *bromocresol green solution R*. Not more than 0.1 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator to blue.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in 2.5 mL of *methanol R* and dilute to 50.0 mL with the mobile phase. Dilute 10.0 mL of this solution to 100.0 mL with the mobile phase.

Reference solution (a) Dissolve 5 mg of *4-hydroxybenzoic acid R* (impurity A), 5 mg of *ethyl parahydroxybenzoate R* (impurity C) and 5 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 50.0 mg of *propyl parahydroxybenzoate CRS* in 2.5 mL of *methanol R* and dilute to 50.0 mL with the mobile phase. Dilute 10.0 mL of this solution to 100.0 mL with the mobile phase.

Reference solution (c) Dilute 1.0 mL of the test solution to 20.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase 6.8 g/L solution of *potassium dihydrogen phosphate R*, *methanol R* (35:65 *V/V*).

Flow rate 1.3 mL/min.

Detection Spectrophotometer at 272 nm.

Injection 10 µL of the test solution and reference solutions (a) and (c).

Run time 2.5 times the retention time of propyl parahydroxybenzoate.

Relative retention With reference to propyl parahydroxybenzoate (retention time = about 4.5 min): impurity A = about 0.3; impurity C = about 0.7.

System suitability Reference solution (a):
- *resolution*: minimum 3.0 between the peaks due to impurity C and propyl parahydroxybenzoate.

Limits:
- *correction factor*: for the calculation of content, multiply the peak area of impurity A by 1.4;
- *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent);
- *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

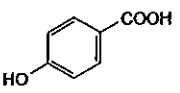
ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (b).

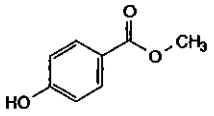
Calculate the percentage content of $C_{10}H_{12}O_3$ from the declared content of *propyl parahydroxybenzoate CRS*.

IMPURITIES
Specified impurities *A*.

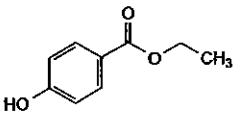
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B*, *C*, *D*.



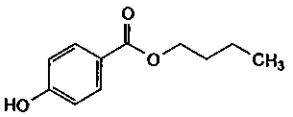
A. 4-hydroxybenzoic acid,



B. methyl 4-hydroxybenzoate (methyl parahydroxybenzoate),



C. ethyl 4-hydroxybenzoate (ethyl parahydroxybenzoate),

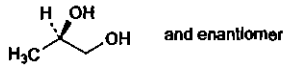


D. butyl 4-hydroxybenzoate (butyl parahydroxybenzoate).

Ph Eur

Propylene Glycol

(Ph. Eur. monograph 0430)



$C_3H_8O_2$ 76.1 *57-55-6*

Action and use
Excipient.

Preparation
Propylene Glycol Solution

Ph Eur

DEFINITION
Propylene glycol is (*RS*)-propane-1,2-diol.

CHARACTERS
A viscous, clear, colourless, hygroscopic liquid, miscible with water and with ethanol (96 per cent).

IDENTIFICATION
A. Relative density (see Tests).

B. Refractive index (see Tests).

C. Boiling point (*2.2.12*): 184 °C to 189 °C.

D. To 0.5 mL add 5 mL of *pyridine R* and 2 g of finely ground *nitrobenzoyl chloride R*. Boil for 1 min and pour into 15 mL of cold *water R* with shaking. Filter, wash the precipitate with 20 mL of a saturated solution of *sodium hydrogen carbonate R* and then with *water R* and dry. Dissolve

in boiling *ethanol (80 per cent V/V) R* and filter the hot solution. On cooling, crystals are formed which, after drying at 100-105 °C, melt (*2.2.14*) at 121 °C to 128 °C.

TESTS

Appearance
It is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Relative density (*2.2.5*)
1.035 to 1.040.

Refractive index (*2.2.6*)
1.431 to 1.433.

Acidity
To 10 mL add 40 mL of *water R* and 0.1 mL of *bromothymol blue solution R1*. The solution is greenish-yellow. Not more than 0.05 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator to blue.

Oxidising substances
To 10 mL add 5 mL of *water R*, 2 mL of *potassium iodide solution R* and 2 mL of *dilute sulfuric acid R* and allow to stand in a ground-glass-stoppered flask protected from light for 15 min. Titrate with *0.05 M sodium thiosulfate*, using 1 mL of *starch solution R* as indicator. Not more than 0.2 mL of *0.05 M sodium thiosulfate* is required.

Reducing substances
To 1 mL add 1 mL of *dilute ammonia R1* and heat in a water-bath at 60 °C for 5 min. The solution is not yellow. Immediately add 0.15 mL of *0.1 M silver nitrate* and allow to stand for 5 min. The solution does not change its appearance.

Water (*2.5.12*)
Not more than 0.2 per cent, determined on 5.00 g by the semi-micro determination of water.

Sulfated ash (*2.4.14*)
Heat 50 g until it burns and ignite. Allow to cool. Moisten the residue with *sulfuric acid R* and ignite; repeat the operations. The residue weighs not more than 5 mg (0.01 per cent).

STORAGE
Store in an airtight container.

Ph Eur

Propylene Glycol Dicaprylocaprate

(Ph. Eur. monograph 2122)

Action and use
Excipient.

Ph Eur

DEFINITION
Propylene glycol diesters of saturated fatty acids, mainly caprylic (octanoic) acid and capric (decanoic) acid, of vegetable origin.

CHARACTERS

Appearance
Almost colourless or light yellow, oily liquid.

Solubility
Practically insoluble in water, soluble in fatty oils and in light petroleum, slightly soluble in anhydrous ethanol.

IDENTIFICATION
First identification: C, D.
Second identification: A, B, C, E.
A. Refractive index (*2.2.6*): 1.439 to 1.442.
B. Relative density (*2.2.5*): 0.910 to 0.930.
C. Viscosity (*2.2.9*): 9 mPa·s to 13 mPa·s.
D. Composition of fatty acids (see Tests).
E. Saponification value (see Tests).

TESTS

Appearance
The substance to be examined is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Acid value (*2.5.1*)
Maximum 0.2.

Hydroxyl value (*2.5.3, Method A*)
Maximum 10.

Iodine value (*2.5.4*)
Maximum 1.0.

Peroxide value (*2.5.5, Method A*)
Maximum 1.0.

Saponification value (*2.5.6*)
315 to 340.

Alkaline impurities
Dissolve 2.00 g of the substance to be examined in a mixture of 1.5 mL of *ethanol (96 per cent) R* and 3.0 mL of *ether R*. Add 0.05 mL of *bromophenol blue solution R*. Not more than 0.15 mL of *0.01 M hydrochloric acid* is required to change the colour of the indicator to yellow.

Composition of fatty acids
Gas chromatography (*2.4.22, Method C*). Prepare reference solution (a) as indicated in Table 2.4.22.-2.

Column:
— *material*: fused silica;
— *size*: *l* = 30 m, Ø = 0.32 mm;
— *stationary phase*: *macrogol 20 000 R* (film thickness 0.5 µm).

Carrier gas *helium for chromatography R.*

Flow rate 1.3 mL/min.

Split ratio 1:100.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 1	70
	1 - 35	70 → 240
	35 - 50	240
Injection port		250
Detector		250

Detection Flame ionisation.

Composition of the fatty-acid fraction of the substance:
— *caproic acid*: maximum 2.0 per cent;
— *caprylic acid*: 50.0 per cent to 80.0 per cent;
— *capric acid*: 20.0 per cent to 50.0 per cent;
— *lauric acid*: maximum 3.0 per cent;
— *myristic acid*: maximum 1.0 per cent.

Water (*2.5.12*)
Maximum 0.1 per cent, determined on 5.00 g.

Total ash (*2.4.16*)
Maximum 0.1 per cent, determined on 2.0 g.

STORAGE
Protected from light.

Ph Eur

Propylene Glycol Dilaurate

(Ph. Eur. monograph 2087)

Action and use
Excipient.

Ph Eur

DEFINITION

Mixture of propylene glycol ((2*RS*)-propane-1,2-diol) mono- and diesters of lauric (dodecanoic) acid.

Content
Minimum 70.0 per cent of diesters and maximum 30.0 per cent of monoesters.

CHARACTERS

Appearance
Clear, oily liquid at 20 °C, colourless or slightly yellow.

Solubility
Practically insoluble in water, very soluble in ethanol (96 per cent), in methanol and in methylene chloride.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.1 g of the substance to be examined in *methylene chloride R* and dilute to 2 mL with the same solvent.

Reference solution Dissolve 0.1 g of *propylene glycol dilaurate CRS* in *methylene chloride R* and dilute to 2 mL with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *hexane R, ether R* (30:70 *V/V*).

Application 10 µL.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with a 0.1 g/L solution of *rhodamine 6 G R* in *ethanol (96 per cent) R* and examine in ultraviolet light at 365 nm.

Results The spots in the chromatogram obtained with the test solution are similar in position to those in the chromatogram obtained with the reference solution.

B. Composition of fatty acids (see Tests).

C. It complies with the assay (content of diesters).

TESTS

Acid value (*2.5.1*)
Maximum 4.0, determined on 5.00 g.

Iodine value (*2.5.4, Method A*)
Maximum 1.0.

Saponification value (*2.5.6*)
230 to 250.

Composition of fatty acids (*2.4.22, Method C*)
Use the mixture of calibrating substances in Table 2.4.22.-2.

Composition of the fatty acid fraction of the substance:
— *caprylic acid*: maximum 0.5 per cent;
— *capric acid*: maximum 2.0 per cent;
— *lauric acid*: minimum 95.0 per cent;
— *myristic acid*: maximum 3.0 per cent;
— *palmitic acid*: maximum 1.0 per cent.

Free propylene glycol
Maximum 2.0 per cent, determined as prescribed under Assay.

Water (*2.5.12*)
Maximum 1.0 per cent, determined on 1.00 g.

Total ash (*2.4.16*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Size-exclusion chromatography (*2.2.30*).

Stock solution Introduce 0.100 g of *propylene glycol R* into a flask and dilute to 25.0 mL with *tetrahydrofuran R.*

Test solution In a 15 mL flask, weigh 0.200 g (*m*). Add 5.0 mL of *tetrahydrofuran R* and shake to dissolve. Reweigh the flask and calculate the total mass of solvent and substance (*M*).

Reference solutions Into four 15 mL flasks, introduce respectively 0.25 mL, 0.5 mL, 1.0 mL and 2.5 mL of stock solution and add 5.0 mL of *tetrahydrofuran R*. Weigh each flask and calculate the concentration of propylene glycol in milligrams per gram for each reference solution.

Column:
— *size*: *l* = 0.6 m, Ø = 7 mm,
— *stationary phase*: *styrene-divinylbenzene copolymer R* (5 µm) with a pore size of 10 nm.

Mobile phase *tetrahydrofuran R.*

Flow rate 1 mL/min.

Detection Differential refractometer.

Injection 40 µL.

Relative retention With reference to propylene glycol: diesters = about 0.85; monoesters = about 0.90.

Calculations:
— *free propylene glycol*: from the calibration curve obtained with the reference solutions, determine the concentration of propylene glycol (*C*) in milligrams per gram in the test solution and calculate the percentage content of free propylene glycol (*A*) in the substance to be examined using the following expression:

$$\frac{C \times M}{m \times 10}$$

— *free fatty acids*: calculate the percentage content of free fatty acids (*D*) using the following expression:

$$\frac{I_A \times 200}{56.11 \times 10}$$

I_A = acid value (see Tests);
200 = rounded molar mass of lauric acid, in grams per mole;
56.11 = molar mass of potassium hydroxide, in grams per mole.

— *monoesters*: calculate the percentage content of monoesters using the following expression:

$$\left[\frac{X}{X+Y}\ (100 - A - B)\right] - D$$

A = percentage content of free propylene glycol;
B = percentage content of water (see Tests);
D = percentage content of free fatty acids;
X = area of the peak due to monoesters and free fatty acids;
Y = area of the peak due to diesters.

— *diesters*: calculate the percentage content of diesters using the following expression:

$$\frac{Y}{X+Y}\ (100 - A - B)$$

STORAGE

Protected from moisture.

Ph Eur

Propylene Glycol Monolaurate

(Ph. Eur. monograph 1915)

Action and use
Excipient.

Ph Eur

DEFINITION

Mixture of propylene glycol ((2*RS*)-propane-1,2-diol) mono- and diesters of lauric (dodecanoic) acid.

Content

— *propylene glycol monolaurate (type I)*: 45.0 per cent to 70.0 per cent of monoesters and 30.0 per cent to 55.0 per cent of diesters,
— *propylene glycol monolaurate (type II)*: minimum 90.0 per cent of monoesters and maximum 10.0 per cent of diesters.

CHARACTERS

Appearance
Clear, oily liquid at 20 °C, colourless or slightly yellow.

Solubility
Practically insoluble in water, very soluble in ethanol (96 per cent), in methanol and in methylene chloride.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.1 g of the substance to be examined in *methylene chloride R* and dilute to 2 mL with the same solvent.

Reference solution Dissolve 0.1 g of *propylene glycol monolaurate CRS* in *methylene chloride R* and dilute to 2 mL with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *hexane R, ether R* (30:70 *V/V*).

Application 10 µL.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with a 0.1 g/L solution of *rhodamine 6 G R* in *ethanol (96 per cent) R* and examine in ultraviolet light at 365 nm.

Results The spots in the chromatogram obtained with the test solution are similar in position to those in the chromatogram obtained with the reference solution.

B. Composition of fatty acids (see Tests).

C. It complies with the assay (content of monoesters).

TESTS

Acid value (*2.5.1*)
Maximum 4.0, determined on 5.00 g.

Iodine value (*2.5.4, Method A*)
Maximum 1.0.

Saponification value (*2.5.6*)
210 to 245 for propylene glycol monolaurate (type I) and 200 to 230 for propylene glycol monolaurate (type II).

Composition of fatty acids (*2.4.22, Method C*)
Use the mixture of calibrating substances in Table 2.4.22.-2.

Composition of the fatty acid fraction of the substance:
— *caprylic acid*: maximum 0.5 per cent;
— *capric acid*: maximum 2.0 per cent;
— *lauric acid*: minimum 95.0 per cent;
— *myristic acid*: maximum 3.0 per cent;
— *palmitic acid*: maximum 1.0 per cent.

Free propylene glycol
Maximum 5.0 per cent for propylene glycol monolaurate (type I) and maximum 1.0 per cent for propylene glycol monolaurate (type II), determined as prescribed under Assay.

Water (*2.5.12*)
Maximum 1.0 per cent, determined on 1.00 g.

Total ash (*2.4.16*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Size-exclusion chromatography (*2.2.30*).

Stock solution Introduce 0.100 g of *propylene glycol R* into a vial and dilute to 25.0 mL with *tetrahydrofuran R.*

Test solution In a 15 mL flask, weigh 0.200 g (*m*). Add 5.0 mL of *tetrahydrofuran R* and shake to dissolve. Reweigh the flask and calculate the total mass of solvent and substance (*M*).

Reference solutions Into four 15 mL flasks, introduce respectively 0.25 mL, 0.5 mL, 1.0 mL and 2.5 mL of stock solution and add 5.0 mL of *tetrahydrofuran R.* In a fifth 15 mL flask, introduce 5.0 mL of stock solution. Weigh each flask and calculate the concentration of propylene glycol in milligrams per gram for each reference solution.

Column:
— *size*: l = 0.6 m, Ø = 7 mm;
— *stationary phase*: *styrene-divinylbenzene copolymer R* (5 µm) with a pore size of 10 nm.

Mobile phase *tetrahydrofuran R.*

Flow rate 1 mL/min.

Detection Differential refractometer.

Injection 40 µL.

Relative retention With reference to propylene glycol: diesters = about 0.85; monoesters = about 0.90.

Calculations:
— *free propylene glycol*: from the calibration curve obtained with the reference solutions, determine the concentration of propylene glycol (*C*) in milligrams per gram in the test solution and calculate the percentage content of free propylene glycol (*A*) in the substance to be examined using the following expression:

$$\frac{C \times M}{m \times 10}$$

— *free fatty acids*: calculate the percentage content of free fatty acids (*D*) using the following expression:

$$\frac{I_A \times 200}{56.11 \times 10}$$

I_A = acid value (see Tests);
200 = rounded molar mass of lauric acid, in grams per mole;
56.11 = molar mass of potassium hydroxide, in grams per mole.

— *monoesters*: calculate the percentage content of monoesters using the following expression:

$$\left[\frac{X}{X+Y}\ (100 - A - B)\right] - D$$

A = percentage content of free propylene glycol;
B = percentage content of water (see Tests);
D = percentage content of free fatty acids;
X = area of the peak due to monoesters and free fatty acids;
Y = area of the peak due to diesters.

— *diesters*: calculate the percentage content of diesters using the following expression:

$$\frac{Y}{X+Y}\ (100-A-B)$$

STORAGE
Protected from moisture.

LABELLING
The label states the type of propylene glycol monolaurate (type I or type II).

Ph Eur

Propylene Glycol Monopalmitostearate

Propylene Glycol Monostearate

(Ph. Eur. monograph 1469)

Action and use
Excipient.

Ph Eur

DEFINITION
Mixture of propylene glycol ((2*RS*)-propane-1,2-diol) mono- and diesters of palmitic (hexadecanoic) and stearic (octadecanoic) acids, produced by the condensation of propylene glycol and stearic acid 50 of vegetable or animal origin (see *Stearic acid (1474)*).

Content
Minimum 50.0 per cent of monoesters.

CHARACTERS

Appearance
White or almost white, waxy solid.

Solubility
Practically insoluble in water, soluble in acetone and in hot ethanol (96 per cent).

IDENTIFICATION
A. Melting point (see Tests).
B. Composition of fatty acids (see Tests).
C. It complies with the assay (content of monoesters).

TESTS

Melting point (*2.2.15*)
33 °C to 40 °C.

Acid value (*2.5.1*)
Maximum 4.0.

Iodine value (*2.5.4*)
Maximum 3.0.

Saponification value (*2.5.6*)
170 to 185, determined on 2.0 g.

Composition of fatty acids (*2.4.22, Method A*)
Composition of the fatty-acid fraction of the substance:
— *stearic acid*: 40.0 per cent to 60.0 per cent;
— *sum of contents of palmitic acid and stearic acid*: minimum 90.0 per cent.

Free propylene glycol
Maximum 5.0 per cent, determined as prescribed under Assay.

Water (*2.5.12*)
Maximum 1.0 per cent, determined on 1.00 g. Use a mixture of equal volumes of *methanol R* and *methylene chloride R* as the solvent.

Total ash (*2.4.16*)
Maximum 0.1 per cent.

ASSAY
Size-exclusion chromatography (*2.2.30*).

Test solution In a 15 mL flask, weigh 0.200 g (*m*). Add 5.0 mL of *tetrahydrofuran R* and shake to dissolve. Heat gently, if necessary. Reweigh the flask and calculate the total mass of solvent and substance (*M*).

Reference solutions In four 15 mL flasks, weigh respectively 2.5 mg, 5.0 mg, 10.0 mg and 20.0 mg of *propylene glycol R*. Add 5.0 mL of *tetrahydrofuran R* and shake to dissolve. Weigh the flasks again and calculate the concentration of propylene glycol in milligrams per gram for each reference solution.

Column:
— *size*: l = 0.6 m, Ø = 7.5 mm;
— *stationary phase*: *styrene-divinylbenzene copolymer R* (5 µm) with a pore size of 10 nm.

Mobile phase *tetrahydrofuran R.*

Flow rate 1 mL/min.

Detection Differential refractometer.

Injection 40 µL.

Relative retention With reference to propylene glycol: diesters = about 0.78; monoesters = about 0.84.

Calculations:
— *free propylene glycol*: from the calibration curve obtained with the reference solutions, determine the concentration of propylene glycol (*C*) in milligrams per gram in the test solution and calculate the percentage content of free propylene glycol (*A*) in the substance to be examined using the following expression:

$$\frac{C \times M}{m \times 10}$$

— *free fatty acids*: calculate the percentage content of free fatty acids (*D*) using the following expression:

$$\frac{I_A \times 270}{56.11 \times 10}$$

I_A = acid value (see Tests);
270 = average rounded molar mass of palmitic acid and stearic acid, in grams per mole;
56.11 = molar mass of potassium hydroxide, in grams per mole.

— *monoesters*: calculate the percentage content of monoesters using the following expression:

$$\left[\frac{X}{X+Y}\ (100-A-B)\right]-D$$

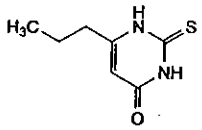
A = percentage content of free propylene glycol;
B = percentage content of water (see Tests);
D = percentage content of free fatty acids;
X = area of the peak due to monoesters and free fatty acids;
Y = area of the peak due to diesters.

STORAGE
Protected from light.

Ph Eur

Propylthiouracil

(Ph. Eur. monograph 0525)



$C_7H_{10}N_2OS$ 170.2 *51-52-5*

Action and use
Thiourea antithyroid drug.

Preparation
Propylthiouracil Tablets

Ph Eur

DEFINITION
Propylthiouracil contains not less than 98.0 per cent and not more than the equivalent of 100.5 per cent of 2,3-dihydro-6-propyl-2-thioxopyrimidin-4(1*H*)-one, calculated with reference to the dried substance.

CHARACTERS
White or almost white, crystalline powder or crystals, very slightly soluble in water, sparingly soluble in alcohol.
It dissolves in solutions of alkali hydroxides.

IDENTIFICATION
First identification: A, B.
Second identification: A, C, D.

A. Melting point (*2.2.14*): 217 °C to 221 °C.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *propylthiouracil CRS*. Examine as discs prepared using 1 mg of substance and 0.3 g of *potassium bromide R*.

C. Examine the chromatograms obtained in the test for impurity A and related substances in ultraviolet light at 254 nm before exposure of the plate to iodine vapour. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To about 20 mg add 8 mL of *bromine water R* and shake for a few minutes. Boil until the mixture is decolourised, allow to cool and filter. To the filtrate add 2 mL of *barium chloride solution R1*. A white precipitate is formed whose colour does not become violet on the addition of 5 mL of *dilute sodium hydroxide solution R*.

TESTS
Impurity A and related substances
Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel GF_{254} plate R*.

Test solution (a) Dissolve 0.1 g of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Test solution (b) Dilute 1 mL of test solution (a) to 10 mL with *methanol R*.

Reference solution (a) Dissolve 10 mg of *propylthiouracil CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 50 mg of *thiourea R* in *methanol R* and dilute to 100 mL with the same solvent. Dilute 1 mL of this solution to 100 mL with *methanol R*.

Reference solution (c) Dilute 1 mL of test solution (a) to 100 mL with *methanol R*.

Apply separately to the plate 10 µL of each solution. Develop over a path of 15 cm using a mixture of 0.1 volumes of *glacial acetic acid R*, 6 volumes of *2-propanol R* and 50 volumes of *chloroform R*. Allow the plate to dry in air. Examine in ultraviolet light at 254 nm. Expose the plate to iodine vapour for 10 min. In the chromatogram obtained with test solution (a), any spot corresponding to impurity A is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.05 per cent) and any spot apart from the principal spot and any spot corresponding to impurity A is not more intense than the spot in the chromatogram obtained with reference solution (c) (1.0 per cent).

Loss on drying (*2.2.32*)
Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

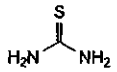
Sulfated ash (*2.4.14*)
Not more than 0.1 per cent, determined on 1.0 g.

ASSAY
To 0.300 g add 30 mL of *water R* and 30.0 mL of *0.1 M sodium hydroxide*. Boil and shake until dissolution is complete. Add 50 mL of *0.1 M silver nitrate* while stirring, boil gently for 5 min and cool. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). The volume of *0.1 M sodium hydroxide* used is equal to the sum of the volume added initially and the volume used in the final titration.

1 mL of *0.1 M sodium hydroxide* is equivalent to 8.511 mg of $C_7H_{10}N_2OS$.

STORAGE
Store protected from light.

IMPURITIES

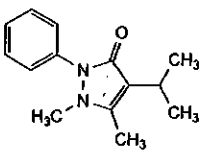


A. thiourea.

Ph Eur

Propyphenazone

(Ph. Eur. monograph 0636)



$C_{14}H_{18}N_2O$ 230.3 *479-92-5*

Action and use
Pyrazolone analgesic.

Ph Eur

DEFINITION
1,5-Dimethyl-2-phenyl-4-(propan-2-yl)-1,2-dihydro-3*H*-pyrazol-3-one.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or slightly yellowish, crystalline powder.

Solubility
Slightly soluble in water, freely soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

First identification: A, B.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 102 °C to 106 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *propyphenazone CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 80 mg of the substance to be examined in *methanol R* and dilute to 5 mL with the same solvent.

Reference solution Dissolve 80 mg of *propyphenazone CRS* in *methanol R* and dilute to 5 mL with the same solvent.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *butanol R, cyclohexane R, ethyl acetate R* (10:45:45 *V/V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying In a current of hot air for 15 min.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. To 1 mL of solution S (see Tests) add 0.1 mL of *ferric chloride solution R1*. A brownish-red colour appears which becomes yellow on addition of 1 mL of *dilute hydrochloric acid R*.

TESTS

Solution S
Dissolve 2 g in a mixture of equal volumes of *carbon dioxide-free water R* and *ethanol (96 per cent) R* and dilute to 50 mL with the same mixture of solvents.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity
To 10 mL of solution S add 0.1 mL of *phenolphthalein solution R*. The solution is colourless. Add 0.2 mL of a 0.40 g/L solution of *sodium hydroxide R*; the solution becomes pink. Add 0.4 mL of a 1.03 g/L solution of *hydrochloric acid R*; the solution becomes colourless. Add 0.2 mL of *methyl red solution R*; the solution becomes orange or red.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 20.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 1 mg of *phenazone R* (impurity A) in the mobile phase, add 1 mL of the test solution and dilute to 10 mL with the mobile phase.

Column:
— *size: l* = 0.25 m, Ø = 4.0 mm;
— *stationary phase: end-capped octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 13.7 g of *potassium dihydrogen phosphate R* in 900 mL of *water for chromatography R*, adjust to pH 5.2 with *dilute sodium hydroxide solution R* and dilute to 1000 mL with *water for chromatography R*. Mix 60 volumes of the solution and 40 volumes of *acetonitrile R1*.

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µL.

Run time 4 times the retention time of propyphenazone.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity A.

Relative retention With reference to propyphenazone (retention time = about 7 min): impurity A = about 0.4.

System suitability Reference solution (b):
— *resolution*: minimum 4.0 between the peaks due to impurity A and propyphenazone.

Limits:
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 4 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 0.5 g.

ASSAY

Dissolve 0.175 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

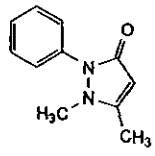
1 mL of *0.1 M perchloric acid* is equivalent to 23.03 mg of $C_{14}H_{18}N_2O$.

STORAGE

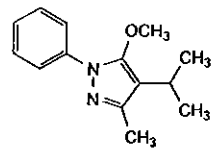
Protected from light.

IMPURITIES

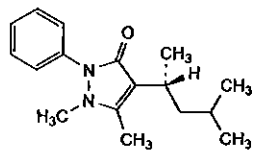
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C.*



A. 1,5-dimethyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one (phenazone),



B. 5-methoxy-3-methyl-1-phenyl-4-(propan-2-yl)-1*H*-pyrazole,



and enantiomer

C. 1,5-dimethyl-4-[(2*RS*)-4-methylpentan-2-yl]-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one.

Ph Eur

Protamine Sulfate

Protamine Sulphate

(Ph. Eur. monograph 0569)

9009-65-8

Action and use
Antidote to heparin.

Preparation
Protamine Sulfate Injection

Ph Eur

DEFINITION
Sulfated forms of basic peptides, comprising 4 major chemical species, extracted from the sperm or roe of *Salmonidae*. It binds with heparin in solution, inhibiting its anticoagulant activity; under the conditions of the potency assay this binding gives rise to a precipitate.

Content
90.0 per cent to 110.0 per cent (dried, sulfuric acid-free substance).

Potency
1 mg of protamine sulfate precipitates not less than 100 IU of heparin (dried substance).

PRODUCTION
The animals from which protamine sulfate is derived must fulfil the requirements for the health of animals suitable for human consumption.

CHARACTERS
Appearance
White or almost white, hygroscopic powder.

Solubility
Sparingly soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION
A. Examine the chromatograms obtained in the test for related substances and calculate individually the relative amounts of protamine peptides A, B, C and D.

Results:
— the principal peaks in the chromatogram obtained with the test solution (protamine peptides A to D) are similar in retention time to the principal peaks in the chromatogram obtained with reference solution (a);
— protamine peptide A content: 13-18 per cent;
— protamine peptide B content: 21-28 per cent;
— protamine peptide C content: 31-38 per cent;
— protamine peptide D content: 19-24 per cent.

B. It gives the appropriate response when examined as described in the potency assay.

C. It complies with the test for sulfate (see Tests).

TESTS
Solution S
Dissolve 0.20 g in *water R* and dilute to 10.0 mL with the same solvent.

Appearance of solution
The solution is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution BY_6 or Y_6 (*2.2.2, Method II*).

To 2.5 mL of solution S add 7.5 mL of *water R*.

Absorbance (*2.2.25*)
Maximum 0.1 between wavelengths of 260 nm and 280 nm.

Dilute 2.5 mL of solution S to 5.0 mL with *water R*.

Related substances
Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution Dissolve 30 mg of the substance to be examined in 400 µL of a 618 g/L solution of *hydrochloric acid R* and dilute to 100.0 mL with a 1.03 g/L solution of *hydrochloric acid R*.

Reference solution (a) Dissolve the contents of a vial of *protamine sulfate CRS* in 40 µL of a 618 g/L solution of *hydrochloric acid R* and dilute to 10.0 mL with a 1.03 g/L solution of *hydrochloric acid R*.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 50.0 mL with a 1.03 g/L solution of *hydrochloric acid R*.

Column:
— *size: l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase: end-capped solid core octadecylsilyl silica gel for chromatography R* (3.6 µm);
— *temperature*: 45 °C.

Mobile phase:
— *mobile phase A: trifluoroacetic acid R, acetonitrile R1, water for chromatography R* (0.1:5:95 *V/V/V*); degas;
— *mobile phase B: trifluoroacetic acid R, acetonitrile R1, water for chromatography R* (0.1:50:50 *V/V/V*); degas;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	95	5
2 - 17	95 → 80	5 → 20
17 - 17.2	80 → 20	20 → 80
17.2 - 20	20	80

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 210 nm.

Autosampler Set at 5 °C.

Injection 30 µL.

Relative retention With reference to protamine peptide A (retention time = about 9-13 min): protamine peptide B = about 1.05; protamine peptide C = about 1.10; protamine peptide D = about 1.15.

System suitability:
— the 4 principal peaks (protamine peptides A to D) are eluted between 8 min and 16.5 min in the chromatogram obtained with reference solution (a);
— *resolution*: minimum 2.0 between each of the adjacent principal peaks in the chromatogram obtained with reference solution (a);
— *signal-to-noise ratio*: minimum 10 for the peak due to protamine peptide A in the chromatogram obtained with reference solution (b);
— *symmetry factor*: maximum 1.8 for the peak due to protamine peptide A, maximum 1.5 for the peak due to protamine peptide B, maximum 2.5 for the peak due to protamine peptide C and maximum 2.2 for the peak due to protamine peptide D in the chromatogram obtained with reference solution (a).

Calculate the percentage content of related substances using the following expression (use the vertical drop approach for integration):

$$100 - \left(\frac{r_A + r_B + r_C + r_D}{r_T} \times 100\right)$$

$r_{A\text{-}D}$ = areas of the peaks due to protamine peptides A to D;
r_T = sum of the areas of all peaks in the chromatogram.

Limit:
— *total*: maximum 8.0 per cent;
— *reporting threshold*: 0.5 per cent.

Sulfate
16 per cent to 24 per cent (dried substance).

Dissolve 0.150 g in 15 mL of *distilled water R* in a beaker. Add 5 mL of *dilute hydrochloric acid R*. Heat to boiling and slowly add to the boiling solution 10 mL of a 100 g/L solution of *barium chloride R*. Cover the beaker and heat on a water-bath for 1 h. Filter. Wash the precipitate several times with small quantities of hot *water R*. Dry and ignite the residue at 600 ± 50 °C to constant mass.

1.0 g of residue is equivalent to 0.4117 g of SO_4.

Iron (*2.4.9*)
Maximum 10 ppm.

Dissolve 1.0 g with heating in *water R* and dilute to 10 mL with the same solvent.

Loss on drying (*2.2.32*)
Maximum 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

ASSAY

Protein
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (a).

System suitability Reference solution (a):
— *repeatability*: maximum relative standard deviation of 2.0 per cent for the total area of the peaks above the reporting threshold with a relative retention with reference to protamine peptide A of 0.80-1.30, determined on 6 injections.

Calculate the percentage content of protamine from the areas of all peaks above the reporting threshold with a relative retention with reference to protamine peptide A of 0.80-1.30, taking into account the assigned content of *protamine sulfate CRS*.

Potency
Test solution (a) Dissolve 15.0 mg of the substance to be examined in *water R* and dilute to 100.0 mL with the same solvent.

Test solution (b) Dilute 2.0 mL of test solution (a) to 3.0 mL with *water R*.

Test solution (c) Dilute 1.0 mL of test solution (a) to 3.0 mL with *water R*.

Use as titrant a 6-fold dilution of *heparin sodium BRP* in *water R* (for example, 1.7 mL diluted to 10.0 mL with *water R*). Titrate each test solution in duplicate as follows: introduce an accurately measured volume of the solution to be titrated, for example 1.5 mL, into the cell of a suitable colorimeter and set the apparatus for measurement at a suitable wavelength (none is critical) in the visible range. Add the titrant in small volumes until there is a sharp increase in the absorbance and note the volume of titrant added.

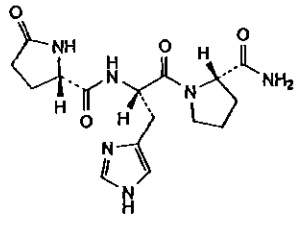
Carry out 3 independent assays. For each individual titration, calculate the number of International Units of heparin in the volume of titrant added at the end-point per milligram of the substance to be examined. Calculate the potency of the substance as the average of the 18 values. Test the linearity of the response by the usual statistical methods (for example, *5.3*). Calculate the 3 standard deviations for the results obtained with each of the 3 test solutions. Calculate the 3 standard deviations for the results obtained with each of the 3 independent assays. The assay is not valid unless each of the 6 standard deviations is less than 5 per cent of the average result.

STORAGE
In an airtight, tamper-evident container. If the substance is sterile, store in a sterile, airtight, tamper-evident container.

Ph Eur

Protirelin

(Ph. Eur. monograph 1144)



$C_{16}H_{22}N_6O_4$ 362.4 *24305-27-9*

Action and use
Thyrotrophin-releasing hormone.

Ph Eur

DEFINITION
5-Oxo-L-prolyl-L-histidyl-L-prolinamide.

Synthetic tripeptide with the same sequence of amino acids as the natural hypothalamic neurohormone, which stimulates the release and synthesis of thyrotropin.

Content
97.0 per cent to 102.0 per cent (anhydrous and acetic acid-free substance).

CHARACTERS

Appearance

White or yellowish-white powder, hygroscopic.

Solubility

Very soluble in water, freely soluble in methanol.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *protirelin CRS.*

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with the reference solution.

TESTS

Appearance of solution

A 10 g/L solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5 (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*)

−62 to −70 (anhydrous and acetic acid-free substance).

Dissolve 10 mg in 1.0 mL of *water R.*

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 5.0 mg of the substance to be examined in mobile phase A and dilute to 5.0 mL with mobile phase A.

Reference solution (a) Dissolve the contents of a vial of *D-His-protirelin CRS* in an appropriate volume of mobile phase A to obtain a concentration of 1 mg/mL.
Mix equal volumes of this solution and the test solution.

Reference solution (b) Dilute 0.2 mL of the test solution to 10.0 mL with mobile phase A.

Column:
- *size*: *l* = 0.25 m, Ø = 4.0 mm,
- *stationary phase*: spherical *octadecylsilyl silica gel for chromatography R* (5 µm) with a pore size of 12 nm.

Mobile phase:
- *mobile phase A*: a mixture of 100 mL of *acetonitrile for chromatography R*, 1900 mL of *water R* and 2.0 g of *sodium octanesulfonate R*, containing 2.5 mL/L of *tetraethylammonium hydroxide solution R*; adjust to pH 3.5 with *phosphoric acid R*,
- *mobile phase B*: a mixture of 300 mL of *acetonitrile for chromatography R*, 1700 mL of *water R* and 2.0 g of *sodium octanesulfonate R*, containing 2.5 mL/L of *tetraethylammonium hydroxide solution R*; adjust to pH 3.5 with *phosphoric acid R*,

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	74 → 41	26 → 59
30 - 35	41 → 74	59 → 26
35 - 50	74	26

Flow rate 1 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µL.

Relative retention With reference to protirelin (retention time = about 18 min): impurity C = about 0.2; impurity D = about 0.68; impurity A = about 0.91; impurity B = about 0.95; impurity E = about 1.08.

System suitability Reference solution (a):
- *resolution*: minimum 2.5 between the peaks due to impurity A and protirelin,
- *symmetry factor*: 0.9 to 1.2 for the peak due to protirelin.

Limits:
- *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent),
- *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3 per cent),
- *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Acetic acid (*2.5.34*)

Maximum 2.0 per cent.

Test solution Dissolve 40.0 mg of the substance to be examined in a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A and dilute to 10.0 mL with the same mixture of solvents.

Water (*2.5.12*)

Maximum 7.0 per cent, determined on 0.200 g.

Bacterial endotoxins (*2.6.14*)

Less than 0.7 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Reference solution Dissolve the contents of a vial of *protirelin CRS* in an appropriate volume of mobile phase A to obtain a concentration of 1.0 mg/mL.

Calculate the content of protirelin ($C_{16}H_{22}N_6O_4$) using the peak areas of the chromatograms obtained with the test solution and the reference solution and the declared content of $C_{16}H_{22}N_6O_4$ in *protirelin CRS.*

STORAGE

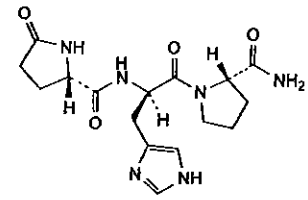
In an airtight container, protected from light at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-evident container.

LABELLING

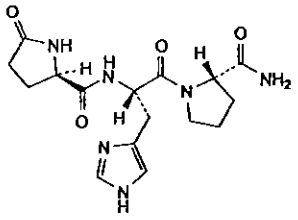
The label states the mass of peptide in the container.

IMPURITIES

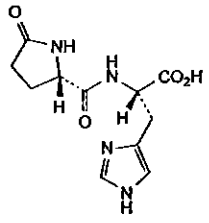
Specified impurities *A, B, C, D, E.*



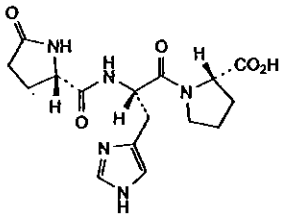
A. 5-oxo-L-prolyl-D-histidyl-L-prolinamide,



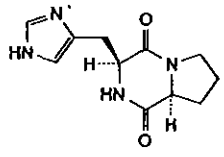
B. 5-oxo-D-prolyl-L-histidyl-L-prolinamide,



C. 5-oxo-L-prolyl-L-histidine,



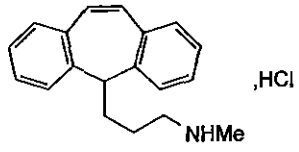
D. 5-oxo-L-prolyl-L-histidyl-L-proline,



E. (3*S*,8a*S*)-3-(1*H*-imidazol-4-ylmethyl) hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (cyclo(-L-histidyl-L-prolyl-)).

Ph Eur

Protriptyline Hydrochloride



$C_{19}H_{21}N,HCl$ 299.8 *1225-55-4*

Action and use
Monoamine reuptake inhibitor; tricyclic antidepressant.

DEFINITION
Protriptyline Hydrochloride is 3-(5*H*-dibenzo[*a*,*d*]cycloheptan-5-yl)propyl(methyl)amine hydrochloride. It contains not less than 99.0% and not more than 101.0% of $C_{19}H_{21}N,HCl$, calculated with reference to the dried substance.

CHARACTERISTICS
A white to yellowish white powder.

Freely soluble in *water* and in *ethanol (96%)*; practically insoluble in *ether*.

IDENTIFICATION
A. Dissolve 0.1 g in 10 mL of *water*, make alkaline with 1M *sodium hydroxide*, extract with 5 mL of *chloroform*, dry with *anhydrous sodium sulfate* and evaporate the solvent using a current of nitrogen. The *infrared absorption spectrum* of the oily residue, Appendix II A, is concordant with the *reference spectrum* of protriptyline *(RS 301)*.

B. Yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

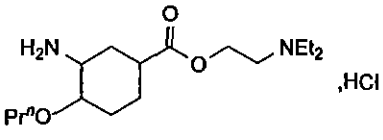
Acidity
pH of a 1% w/v solution, 5.0 to 6.5, Appendix V L.

Loss on drying
When dried to constant weight at 60° at a pressure not exceeding 0.7 kPa, loses not more than 0.5% of its weight. Use 1 g.

Sulfated ash
Not more than 0.1%, Appendix IX A.

ASSAY
Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.7 g and *crystal violet solution* as indicator. Each mL of *0.1M perchloric acid VS* is equivalent to 29.98 mg of $C_{19}H_{21}N,HCl$.

Proxymetacaine Hydrochloride



$C_{16}H_{26}N_2O_3HCl$ 330.9 *5875-06-9*

Action and use
Local anaesthetic.

Preparation
Proxymetacaine Eye Drops

DEFINITION
Proxymetacaine Hydrochloride is 2-diethylaminoethyl 3-amino-4-propoxybenzoate hydrochloride. It contains not less than 98.0% and not more than 102.0% of $C_{16}H_{26}N_2O_3$, HCl, calculated with reference to the dried substance.

CHARACTERISTICS
A white or almost white, crystalline powder.

Soluble in *water*; very soluble in *absolute ethanol*; practically insoluble in *ether*.

IDENTIFICATION
A. The *light absorption*, Appendix II B, in the range 220 to 350 nm of a 0.002% w/v solution exhibits three maxima, at 231, 268 and 310 nm. The *absorbances* at the maxima at 268 nm and at 310 nm are about 0.58 and about 0.32, respectively.

B. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of proxymetacaine hydrochloride *(RS 303)*.

C. A 5% w/v solution yields the reaction characteristic of *primary aromatic amines* and the reactions characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH of a 1% w/v solution, 5.7 to 6.4, Appendix V L.

Related substances

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions of the substance being examined in *methanol*.

(1) 2.0% w/v of the substance being examined.

(2) 0.020% w/v of the substance being examined.

(3) 0.010% w/v of the substance being examined.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* GF_{254}.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, heat at 105° for 10 minutes, allow to cool and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

5 volumes of *diethylamine*, 30 volumes of *ethyl acetate* and 75 volumes of *toluene*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1);

is not more intense than the spot in the chromatogram obtained with solution (2) (1%);

not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (0.5%). Disregard any spot remaining on the line of application.

B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *methanol*.

(1) 2.0% w/v of the substance being examined.

(2) 0.0050% w/v of *3-amino-4-propoxybenzoic acid BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* GF_{254}.

(b) Use the mobile phase as described below.

(c) Apply 10 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

4 volumes of *glacial acetic acid*, 20 volumes of *cyclohexane* and 80 volumes of *1,4-dioxan*.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.25%). The principal spot remains on or near the line of application.

Loss on drying

When dried at 105° for 3 hours, loses not more than 0.5% of its weight. Use 1 g.

Sulfated ash

Not more than 0.15%, Appendix IX A.

ASSAY

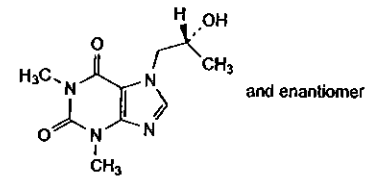
Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.25 g, 20 mL of *mercury(II) acetate solution* and *1-naphtholbenzein solution* as indicator. Each mL of *0.1M perchloric acid VS* is equivalent to 16.54 mg of $C_{16}H_{26}N_2O_3,HCl$.

STORAGE

Proxymetacaine Hydrochloride should be protected from light.

Proxyphylline

(Ph. Eur. monograph 0526)



and enantiomer

$C_{10}H_{14}N_4O_3$ 238.2 *603-00-9*

Action and use

Non selective phospho-diesterase inhibitor; treatment of reversible airways obstruction.

Ph Eur

DEFINITION

Proxyphylline contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 7-[(2*RS*)-2-hydroxypropyl]-1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, very soluble in water, soluble in alcohol.

IDENTIFICATION

First identification: B, C.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 134 °C to 136 °C.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *proxyphylline CRS*. Examine the substances as discs prepared using 0.5 mg to 1 mg of the substance to be examined in 0.3 g of *potassium bromide R*.

C. Dissolve 1 g in 5 mL of *acetic anhydride R* and boil under a reflux condenser for 15 min. Allow to cool and add 100 mL of a mixture of 20 volumes of *ether R* and 80 volumes of *light petroleum R*. Cool in iced water for at least 20 min, shaking from time to time. Filter, wash the precipitate with a mixture of 20 volumes of *ether R* and 80 volumes of *light petroleum R*, recrystallise from *alcohol R* and dry *in vacuo*. The crystals melt (*2.2.14*) at 87 °C to 92 °C.

D. It gives the reaction of xanthines (*2.3.1*).

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 10 mL of solution S add 0.25 mL of *bromothymol blue solution R1*. The solution is yellow or green. Not more than 0.4 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to blue.

Related substances
Examine by thin-layer chromatography (*2.2.27*), using *silica gel HF_{254} R* as the coating substance.

Test solution Dissolve 0.3 g of the substance to be examined in a mixture of 20 volumes of *water R* and 30 volumes of *methanol R* and dilute to 10 mL with the same mixture of solvents. Prepare immediately before use.

Reference solution (a) Dilute 1 mL of the test solution to 100 mL with *methanol R*.

Reference solution (b) Dilute 0.2 mL of the test solution to 100 mL with *methanol R*.

Reference solution (c) Dissolve 10 mg of *theophylline R* in *methanol R*, add 0.3 mL of the test solution and dilute to 10 mL with *methanol R*.

Apply separately to the plate 10 µL of each solution. Develop over a path of 15 cm using a mixture of 1 volume of *concentrated ammonia R*, 10 volumes of *ethanol R* and 90 volumes of *chloroform R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (1 per cent) and at most one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Chlorides (*2.4.4*)
Dilute 2.5 mL of solution S to 15 mL with *water R*. The solution complies with the limit test for chlorides (400 ppm).

Loss on drying (*2.2.32*)
Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Not more than 0.1 per cent, determined on 1.0 g.

ASSAY
In order to avoid overheating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.

Dissolve 0.200 g in 3.0 mL of *anhydrous formic acid R* and add 50.0 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (*2.2.20*).

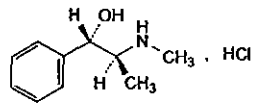
1 mL of *0.1 M perchloric acid* is equivalent to 23.82 mg of $C_{10}H_{14}N_4O_3$.

STORAGE
Store protected from light.

Ph Eur

Pseudoephedrine Hydrochloride

(Ph. Eur. monograph 1367)



$C_{10}H_{16}ClNO$ 201.7 *345-78-8*

Action and use
Adrenoceptor agonist.

Preparations
Pseudoephedrine Oral Solution
Pseudoephedrine Tablets

Ph Eur

DEFINITION
(*1S,2S*)-2-(Methylamino)-1-phenylpropan-1-ol hydrochloride.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder or colourless crystals.

Solubility
Freely soluble in water and in ethanol (96 per cent), sparingly soluble in methylene chloride.

mp
About 184 °C.

IDENTIFICATION
First identification: A, B, D.
Second identification: A, C, D.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison pseudoephedrine hydrochloride CRS.

C. Thin-layer chromatography (*2.2.27*).
Test solution Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.
Reference solution (a) Dissolve 20 mg of *pseudoephedrine hydrochloride CRS* in *methanol R* and dilute to 10 mL with the same solvent.
Reference solution (b) Dissolve 10 mg of *ephedrine hydrochloride CRS* in reference solution (a) and dilute to 5 mL with reference solution (a).
Plate TLC silica gel plate R.
Mobile phase methylene chloride R, concentrated ammonia R, 2-propanol R (5:15:80 *V/V/V*).
Application 10 µL.
Development Over 2/3 of the plate.
Drying In air.
Detection Spray with *ninhydrin solution R* and heat at 110 °C for 5 min.
System suitability Reference solution (b):
— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Solution S (see Tests) gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 1.25 g in *carbon dioxide-free water R* and dilute to 25.0 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

Dilute 2 mL of solution S to 10 mL with *carbon dioxide-free water R*. Add 0.1 mL of *methyl red solution R* and 0.1 mL of *0.01 M sodium hydroxide*; the solution is yellow. Add 0.2 mL of *0.01 M hydrochloric acid*; the solution is red.

Specific optical rotation (*2.2.7*)

+ 61.0 to + 62.5 (dried substance), determined on solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 mL with the mobile phase.

Reference solution (a) Dissolve 20.0 mg of *ephedrine hydrochloride CRS* (impurity A) in the mobile phase and dilute to 20.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 200.0 mL with the mobile phase.

Reference solution (c) Dissolve 10 mg of *ephedrine hydrochloride CRS* (impurity A) in 5 mL of the test solution and dilute to 100 mL with the mobile phase.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *phenylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 6 volumes of *methanol R* and 94 volumes of an 11.6 g/L solution of *ammonium acetate R* previously adjusted to pH 4.0 with *glacial acetic acid R*.

Flow rate 1 mL/min.

Detection Spectrophotometer at 257 nm.

Injection 20 µL.

Run time 1.5 times the retention time of pseudoephedrine.

Relative retention With reference to pseudoephedrine (retention time = about 18 min): impurity A = about 0.9.

System suitability Reference solution (c):
- *resolution*: minimum 2.0 between the peaks due to impurity A and pseudoephedrine; if necessary, reduce the content of methanol in the mobile phase.

Limits:
- *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
- *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *sum of impurities other than A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.170 g in 30 mL of *ethanol (96 per cent) R*. Add 5.0 mL of *0.01 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

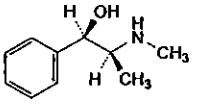
1 mL of *0.1 M sodium hydroxide* is equivalent to 20.17 mg of $C_{10}H_{16}ClNO$.

STORAGE

Protected from light.

IMPURITIES

Specified impurities *A*.

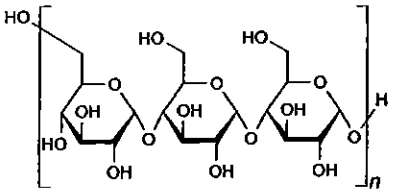


A. (1*R*,2*S*)-2-(methylamino)-1-phenylpropan-1-ol (ephedrine).

Ph Eur

Pullulan

(Ph. Eur. monograph 2603)



$C_{18n}H_{30n+2}O_{15n+1}$ *9057-02-7*

Action and use

Excipient.

Ph Eur

DEFINITION

Neutral polysaccharide produced by the growth of *Aureobasidium pullulans*. It has a chain structure consisting of α-(1→6)-linked maltotriose units, each of which is composed of three α-(1→4)-linked D-glucopyranose units. It may contain some maltotetraose units.

CHARACTERS

Appearance
White or almost white, very hygroscopic powder.

Solubility
Freely soluble in water, practically insoluble in anhydrous ethanol and in methylene chloride.

IDENTIFICATION

A. Dissolve 10 g in 100 mL of *water R*, adding the substance to be examined in small quantities while stirring. A viscous solution is produced.

B. Mix 10 mL of the solution obtained in identification test A with 0.1 mL of a 10 units/mL solution of *pullulanase R*. Incubate at 25 °C for about 20 min. A substantial decrease in viscosity is observed.

C. Dissolve 2 g in 100 mL of *water R*. To 10 mL of the solution, add 2 mL of *macrogol 600 R*. A white precipitate is formed immediately.

TESTS

pH (*2.2.3*)
4.5 to 6.5.

Dissolve 1.0 g in 10 mL of *carbon dioxide-free water R*.

Viscosity (*2.2.9*)
100 $mm^2 \cdot s^{-1}$ to 180 $mm^2 \cdot s^{-1}$.

Dissolve 10.0 g (dried substance) in *water R* to make 100 g of solution, adding the substance to be examined in small quantities while stirring. Carry out the test at 30 ± 0.1 °C.

Mono-, di- and oligosaccharides
Maximum 10.0 per cent for the sum of mono-, di- and oligosaccharides.

Stock solution Dissolve 0.80 g (dried substance) in *water R*, adding the substance to be examined in small quantities while stirring, and dilute to 100.0 mL with the same solvent.

Test solution To 1.0 mL of the stock solution, add 0.1 mL of a saturated solution of *potassium chloride R* (2 g in 5 mL). Mix vigorously with 3 mL of *methanol R*. Centrifuge at 12 000-16 000 *g* for 10 min maintaining at 4 °C. Use the supernatant.

Reference solution Dilute 1.0 mL of the stock solution to 50.0 mL with *water R*.

Blank solution *water R*.

Transfer 0.2 mL of the test solution, the reference solution and the blank solution to separate test tubes, placed in a bath of iced water with each containing 5 mL of a 2 g/L solution of *anthrone R* in *sulfuric acid R1*. Stir immediately, then heat at 90 °C for 10 min. Cool the tubes under running water.

Measure the absorbance (*2.2.25*) of the test solution, the reference solution and the blank solution at 620 nm, using *water R* as the compensation liquid.

Calculate the percentage content of mono-, di- and oligosaccharides using the following expression:

$$100 \times \frac{4.1}{50} \times \frac{A_1 - A_2}{A_3 - A_2}$$

4.1	=	dilution factor for the test solution;
50	=	dilution factor for the reference solution;
A_1	=	absorbance of the test solution;
A_2	=	absorbance of the blank solution;
A_3	=	absorbance of the reference solution.

Loss on drying (*2.2.32*)
Maximum 6.0 per cent, determined on 1.000 g by drying *in vacuo* at 90 °C for 6 h.

Sulfated ash (*2.4.14*)
Maximum 0.3 per cent, determined on 2.0 g.

Nitrogen (*2.5.9*)
Maximum 0.05 per cent (dried substance), determined on 4.0 g.

Carry out the determination of nitrogen using 40 mL of *sulfuric acid R* and heating until a green colour is obtained (about 1.5 h) to complete the decomposition and add *strong sodium hydroxide solution R* until a dark brown colour is obtained (about 90 mL).

Microbial contamination
TAMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

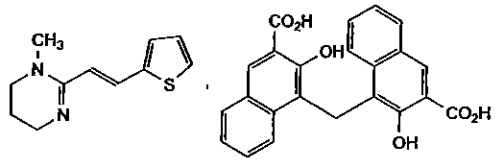
STORAGE

In an airtight container.

Ph Eur

Pyrantel Embonate

(Ph. Eur. monograph 1680)



$C_{34}H_{30}N_2O_6S$ 594.7 *22204-24-6*

Action and use
Anthelminthic.

Ph Eur

DEFINITION

1-Methyl-2-[(*E*)-2-(thiophen-2-yl)eth-1-en-1-yl]-1,4,5,6-tetrahydropyrimidine hydrogen 4,4′-methylenebis(3-hydroxynaphthalene-2-carboxylate).

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
Pale yellow or yellow powder.

Solubility
Practically insoluble in water, soluble in dimethyl sulfoxide, practically insoluble in methanol.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *pyrantel embonate CRS*.

TESTS

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use and protect from light.*

Solvent mixture Mix 5 volumes of *glacial acetic acid R* and 5 volumes of *water for chromatography R*, then add 2 volumes of *diethylamine R* with cooling.

Test solution (a) Dissolve 0.800 g of the substance to be examined in 7 mL of the solvent mixture and dilute to 100.0 mL with *acetonitrile R*.

Test solution (b) Dilute 1.0 mL of test solution (a) to 10.0 mL with the mobile phase.

Reference solution (a) Dissolve 10 mg of *pyrantel impurity A CRS* in the solvent mixture, add 2.5 mL of test solution (b) and dilute to 50 mL with the solvent mixture. Dilute 2 mL of this solution to 100 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of test solution (b) to 200.0 mL with the mobile phase.

Reference solution (c) Dissolve 8.0 mg of *pyrantel impurity D CRS* in *acetonitrile R* and dilute to 10.0 mL with the same solvent. Dilute 1.0 mL of the solution to 100.0 mL with *acetonitrile R*.

Reference solution (d) Dissolve 8.0 mg of *pyrantel impurity C CRS* in *acetonitrile R* and dilute to 10.0 mL with the same solvent. Dilute 1.0 mL of the solution to 100.0 mL with *acetonitrile R*.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *silica gel for chromatography R* (5 µm);
— *temperature*: 30 °C.

Mobile phase Solvent mixture, *acetonitrile for chromatography R* (72:928 *V/V*).

Flow rate 1 mL/min.

Detection Spectrophotometer at 288 nm and, for impurity D, at 238 nm.

Injection 20 µL of test solution (b) and reference solutions (a), (b) and (d); for impurity D, 50 µL of test solution (a) and reference solution (c).

Run time 4 times the retention time of pyrantel.

Identification of impurities Use the chromatogram obtained with reference solution (d) to identify the peak due to impurity C; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity D.

Relative retention With reference to pyrantel (retention time = about 11 min): impurity C = about 0.3; embonic acid = about 0.5; impurity A = about 1.3; impurity D = about 2.2.

System suitability Reference solution (a):
— *resolution*: minimum 4.0 between the peaks due to pyrantel and impurity A.

Calculation of percentage contents:
— for impurity D, use the concentration of impurity D in reference solution (c);
— for impurity C, use the concentration of impurity C in reference solution (d);
— for impurities other than C and D, use the concentration of pyrantel embonate in reference solution (b).

Limits:
— *impurity D*: maximum 0.2 per cent;
— *impurity C*: maximum 0.10 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *sum of impurities other than C and D (excluding embonic acid)*: maximum 0.3 per cent;
— *reporting threshold*: 0.05 per cent.

Chlorides (*2.4.4*)
Maximum 360 ppm.

To 0.46 g add 10 mL of *dilute nitric acid R* and 30 mL of *water R*. Heat on a water-bath for 5 min. Cool, dilute to 50 mL with *water R*, mix well and filter.

Sulfates (*2.4.13*)
Maximum 0.1 per cent.

To 0.50 g add 2.5 mL of *dilute nitric acid R* and dilute to 50 mL with *distilled water R*. Heat on a water-bath for 5 min, shake for 2 min, cool and filter.

Iron (*2.4.9*)
Maximum 75 ppm.

Ignite 0.66 g at 800 ± 50 °C for 2 h. Dissolve the residue in 2.5 mL of *dilute hydrochloric acid R* with gentle heating for 10 min. Cool and dilute to 50 mL with *water R*.

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 3 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

To 0.450 g add 10 mL of *acetic anhydride R* and 50 mL of *glacial acetic acid R*, heat at 50 °C and stir for 10 min. Allow to cool (a clear solution is not obtained). Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 mL of *0.1 M perchloric acid* is equivalent to 59.47 mg of $C_{34}H_{30}N_2O_6S$.

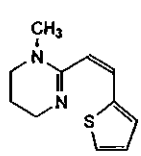
STORAGE

Protected from light.

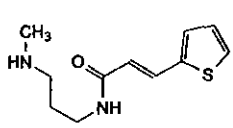
IMPURITIES

Specified impurities *C, D.*

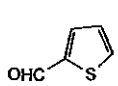
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B.*



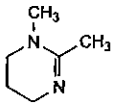
A. 1-methyl-2-[(*Z*)-2-(thiophen-2-yl)eth-1-en-1-yl]-1,4,5,6-tetrahydropyrimidine,



B. (*E*)-*N*-[3-(methylamino)propyl]-3-(thiophen-2-yl)prop-2-enamide,



C. thiophene-2-carbaldehyde,

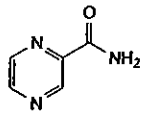


D. 1,2-dimethyl-1,4,5,6-tetrahydropyrimidine.

Ph Eur

Pyrazinamide

(Ph. Eur. monograph 0859)



$C_5H_5N_3O$ 123.1 *98-96-4*

Action and use
Antituberculosis drug.

Preparations
Pyrazinamide Oral Suspension
Pyrazinamide Tablets

Ph Eur

DEFINITION
Pyrazine-2-carboxamide.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Sparingly soluble in water, slightly soluble in ethanol (96 per cent) and in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION
First identification: C.

Second identification: A, B, D.

A. Melting point (*2.2.14*): 188 °C to 191 °C.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution (a) Dissolve 50.0 mg in *water R* and dilute to 100.0 mL with the same solvent.

Test solution (b) Dilute 1.0 mL of test solution (a) to 10.0 mL with *water R*.

Test solution (c) Dilute 2.0 mL of test solution (a) to 100.0 mL with *water R*.

Spectral range 290-350 nm for test solution (b); 230-290 nm for test solution (c).

Absorption maxima At 310 nm for test solution (b); at 268 nm for test solution (c).

Specific absorbance at the absorption maximum at 268 nm
640 to 680 for test solution (c).

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *pyrazinamide CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *ethanol (96 per cent) R*, evaporate to dryness and record new spectra using the residues.

D. Dissolve 0.1 g in 5 mL of *water R*. Add 1 mL of ferrous sulfate solution R2. The solution becomes orange. Add 1 mL of *dilute sodium hydroxide solution R*. The solution becomes dark blue.

TESTS
Solution S
Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity
To 25 mL of solution S add 0.05 mL of *phenolphthalein solution R1* and 0.2 mL of *0.01 M sodium hydroxide*. The solution is red. Add 1.0 mL of *0.01 M hydrochloric acid*. The solution is colourless. Add 0.15 mL of *methyl red solution R*. The solution is red.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 50 mg of the substance to be examined in *water R* and dilute to 25.0 mL with the same solvent. Dilute 5.0 mL of the solution to 25.0 mL with *water R*.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *water R*. Dilute 1.0 mL of this solution to 10.0 mL with *water R*.

Reference solution (b) Dissolve 10 mg of *pyrazine-2-carbonitrile R* (impurity B) in *water R* and dilute to 50.0 mL with the same solvent. Dilute 5.0 mL of the solution to 50.0 mL with *water R*. To 5.0 mL of this solution add 5.0 mL of the test solution and dilute to 25.0 mL with *water R*.

Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 30 °C.

Mobile phase Dissolve 6.80 g of *potassium dihydrogen phosphate R* in 800 mL of *water R*, add 1.84 g of *sodium hydroxide R*, adjust to pH 3.0 with *dilute phosphoric acid R* and dilute to 1000 mL with *water R*; add 10.0 mL of *acetonitrile R* and 1.0 mL of *tetrahydrofuran R*.

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 270 nm.

Injection 40 µL.

Run time 4 times the retention time of pyrazinamide.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity B.

Relative retention With reference to pyrazinamide (retention time = about 5 min): impurity B = about 1.6.

System suitability Reference solution (b):
— *resolution*: minimum 4.0 between the peaks due to pyrazinamide and impurity B.

Limits:
- — *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- — *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent);
- — *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- — *disregard limit*: 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.03 per cent).

Water (*2.5.12*)
Maximum 0.5 per cent, determined on 2.00 g.

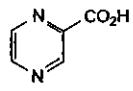
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.100 g in 50 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

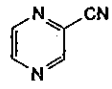
1 mL of *0.1 M perchloric acid* is equivalent to 12.31 mg of $C_5H_5N_3O$.

IMPURITIES
Specified impurities *B*.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A*.



A. pyrazine-2-carboxylic acid,

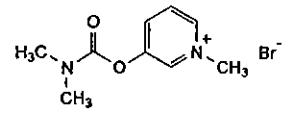


B. pyrazine-2-carbonitrile.

Ph Eur

Pyridostigmine Bromide

(Ph. Eur. monograph 1255)



$C_9H_{13}BrN_2O_2$ 261.1 *101-26-8*

Action and use
Cholinesterase inhibitor.

Preparation
Pyridostigmine Tablets

Ph Eur

DEFINITION
3-[(Dimethylcarbamoyl)oxy]-1-methylpyridinium bromide.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline, deliquescent powder.

Solubility
Very soluble in water and in ethanol (96 per cent).

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *pyridostigmine bromide CRS*.

B. It gives reaction (a) of bromides (*2.3.1*).

TESTS
Solution S
Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity
To 40 mL of solution S add a few drops of *methyl red solution R*. To 20 mL of this solution add 0.2 mL of *0.02 M sodium hydroxide*. The solution is yellow. To the other 20 mL add 0.2 mL of *0.02 M hydrochloric acid*. The solution is red.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 50 mg of the substance to be examined in the mobile phase at about 40 °C. Allow to cool and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 4 mg of *pyridostigmine bromide CRS*, 4 mg of *pyridostigmine impurity A CRS* and 4 mg of *pyridostigmine impurity B CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 5.0 mL of the solution to 100.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 10.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution (c) Dilute 5.0 mL of reference solution (b) to 20.0 mL with the mobile phase.

Column:
- — *size*: *l* = 0.25 m, Ø = 4.0 mm;
- — *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (5-10 µm).

Mobile phase Mix 30 volumes of *acetonitrile R* and 70 volumes of a 4.33 g/L solution of *sodium dodecyl sulfate R* previously adjusted to pH 2.0 with *phosphoric acid R*.

Flow rate 1.1 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µL.

Run time Twice the retention time of pyridostigmine.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A and B.

Relative retention With reference to pyridostigmine (retention time = about 32 min): impurity B = about 0.7; impurity A = about 0.9.

System suitability Reference solution (a):
— *resolution*: minimum 1.5 between the peaks due to impurity A and pyridostigmine.

Limits:
— *impurities A, B*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent); at most one such peak has an area greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent); disregard the peak due to the bromide ion.

Loss on drying *(2.2.32)*
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash *(2.4.14)*
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.230 g in 10 mL of *anhydrous acetic acid R*. Add 40 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically *(2.2.20)*.

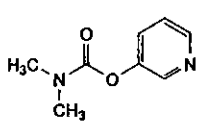
1 mL of *0.1 M perchloric acid* is equivalent to 26.11 mg of $C_9H_{13}BrN_2O_2$.

STORAGE

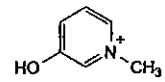
In an airtight container, protected from light. If the substance is sterile, store in a sterile, airtight, tamper-evident container, protected from light.

IMPURITIES

Specified impurities *A, B.*



A. pyridin-3-yl dimethylcarbamate,

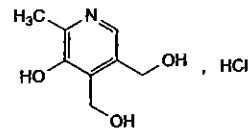


B. 3-hydroxy-1-methylpyridinium.

Ph Eur

Pyridoxine Hydrochloride

(Ph. Eur. monograph 0245)



$C_8H_{12}ClNO_3$ 205.6 *58-56-0*

Action and use
Vitamin B6.

Preparations
Pyridoxine Tablets
Vitamins B and C Injection

When vitamin B_6 is prescribed or demanded, Pyridoxine Hydrochloride shall be dispensed or supplied.

Ph Eur

DEFINITION

(5-Hydroxy-6-methylpyridine-3,4-diyl)dimethanol hydrochloride.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water, slightly soluble in ethanol (96 per cent).

mp
About 205 °C, with decomposition.

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Ultraviolet and visible absorption spectrophotometry *(2.2.25)*.

Solution A Dilute 1.0 mL of solution S (see Tests) to 50.0 mL with a 10.3 g/L solution of *hydrochloric acid R*.

Solution B Dilute 1.0 mL of solution A to 100.0 mL with a 10.3 g/L solution of *hydrochloric acid R*.

Solution C Dilute 1.0 mL of solution A to 100.0 mL with the potassium dihydrogen phosphate 0.025 M + disodium hydrogen phosphate 0.025 M solution described in general chapter *2.2.3*.

Spectral ranges 250-350 nm for solution B; 220-350 nm for solution C.

Absorption maxima 288-296 nm for solution B; 248-256 nm and 320-327 nm for solution C.

Specific absorbances at the absorption maxima:
— 425 to 445 for solution B at 288-296 nm;
— 175 to 195 for solution C at 248-256 nm;
— 345 to 365 for solution C at 320-327 nm.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *pyridoxine hydrochloride CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 1.0 g of the substance to be examined in *water R* and dilute to 10 mL with the same solvent. Dilute 1 mL of this solution to 10 mL with *water R.*

Reference solution Dissolve 0.10 g of *pyridoxine hydrochloride CRS* in *water R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *concentrated ammonia R, methylene chloride R, tetrahydrofuran R, acetone R* (9:13:13:65 *V/V/V/V*).

Application 2 µL.

Development In an unsaturated tank, over a path of 15 cm.

Drying In air.

Detection Spray with a 50 g/L solution of *sodium carbonate R* in a mixture of 30 volumes of *ethanol (96 per cent) R* and 70 volumes of *water R*; dry in a current of air, spray with a 1 g/L solution of *dichloroquinonechlorimide R* in *ethanol (96 per cent) R* and examine immediately.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Solution S gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 50.0 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

pH (*2.2.3*)

2.4 to 3.0 for solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 25 mg of the substance to be examined in *water R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *water R*. Dilute 1.0 mL of this solution to 10.0 mL with *water R.*

Reference solution (b) Dissolve 2.5 mg of *pyridoxine impurity A CRS* and 2.5 mg of *4-deoxypyridoxine hydrochloride R* (impurity B) in *water R* and dilute to 10 mL with the same solvent. Dilute 2 mL of the solution to 10 mL with *water R.*

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 2.72 g of *potassium dihydrogen phosphate R* in 900 mL of *water for chromatography R*, adjust to pH 3.0 with *dilute phosphoric acid R* and dilute to 1000 mL with *water for chromatography R.*

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 5 µL.

Run time 2.5 times the retention time of pyridoxine.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and B.

Relative retention With reference to pyridoxine (retention time = about 12 min): impurity A = about 1.7; impurity B = about 1.9.

System suitability Reference solution (b):
— *resolution*: minimum 1.5 between the peaks due to impurities A and B.

Calculation of percentage contents:
— for each impurity, use the concentration of pyridoxine hydrochloride in reference solution (a).

Limits:
— *impurity B*: maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.2 per cent;
— *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

In order to avoid overheating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.

Dissolve 0.150 g in 5 mL of *anhydrous formic acid R.* Add 50 mL of *acetic anhydride R.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 mL of *0.1 M perchloric acid* is equivalent to 20.56 mg of $C_8H_{12}ClNO_3$.

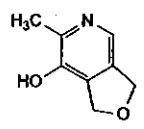
STORAGE

Protected from light.

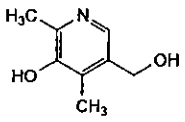
IMPURITIES

Specified impurities *B.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A.*



A. 6-methyl-1,3-dihydrofuro[3,4-*c*]pyridin-7-ol,

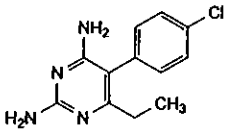


B. 5-(hydroxymethyl)-2,4-dimethylpyridin-3-ol.

Ph Eur

Pyrimethamine

(Ph. Eur. monograph 0288)



$C_{12}H_{13}ClN_4$ 248.7 *58-14-0*

Action and use
Dihydrofolate reductase inhibitor; antiprotozoal (malaria).

Preparation
Pyrimethamine Tablets

Ph Eur

DEFINITION
5-(4-Chlorophenyl)-6-ethylpyrimidine-2,4-diamine.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder or colourless crystals.

Solubility
Practically insoluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION
First identification: B.
Second identification: A, C.
A. Melting point (*2.2.14*): 239 °C to 243 °C.
B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *pyrimethamine CRS.*
C. Thin-layer chromatography (*2.2.27*).
Solvent mixture *methanol R, methylene chloride R* (10:90 *V/V*).
Test solution Dissolve 0.1 g of the substance to be examined in the solvent mixture and dilute to 100 mL with the solvent mixture.
Reference solution Dissolve 0.1 g of *pyrimethamine CRS* in the solvent mixture and dilute to 100 mL with the solvent mixture.
Plate *TLC silica gel F_{254} plate R.*
Mobile phase *methylene chloride R, propanol R, glacial acetic acid R, toluene R* (4:8:12:76 *V/V/V/V*).
Application 20 µL.
Development Over 2/3 of the plate.
Drying In air.
Detection Examine in ultraviolet light at 254 nm.
Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Solution S
Shake 1.0 g with 50 mL of *carbon dioxide-free water R* for 2 min and filter.

Appearance of solution
Prepare the solution immediately before use Dissolve 0.25 g in a mixture of 1 volume of *methanol R* and 3 volumes of *methylene chloride R* and dilute to 10 mL with the same mixture of solvents. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Acidity or alkalinity
To 10 mL of solution S add 0.05 mL of *phenolphthalein solution R1*. The solution is colourless. Not more than 0.2 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink. Add 0.4 mL of *0.01 M hydrochloric acid* and 0.05 mL of *methyl red solution R*. The solution is red or orange.

Related substances
Liquid chromatography (*2.2.29*).
Solvent mixture Mobile phase A, mobile phase B (50:50 *V/V*).
Test solution Dissolve 10.0 mg of the substance to be examined in 4 mL of the solvent mixture using sonication and dilute to 10.0 mL with the solvent mixture.
Reference solution (a) Dissolve 10 mg of the substance to be examined and 10 mg of *pyrimethamine impurity B CRS* in 50 mL of the solvent mixture using sonication and dilute to 100 mL with the solvent mixture.
Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.
Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R1* (5 µm);
— *temperature*: 30 °C.
Mobile phase:
— *mobile phase A*: dissolve 2.72 g of *potassium dihydrogen phosphate R* in 900 mL of *water for chromatography R*, adjust to pH 8.0 with *ammonia R* and dilute to 1000 mL with *water for chromatography R*;
— *mobile phase B*: *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 6	65	35
6 - 20	65 → 40	35 → 60
20 - 35	40 → 55	60 → 45

Flow rate 1.0 mL/min.
Detection Spectrophotometer at 210 nm.
Injection 10 µL.
Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity B.

Relative retention With reference to pyrimethamine (retention time = about 15 min): impurity B = about 0.8.

System suitability Reference solution (a):
— *resolution*: minimum 5.0 between the peaks due to impurity B and pyrimethamine.

Calculation of percentage contents:
— for each impurity, use the concentration of pyrimethamine in reference solution (b).

Limits:
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.3 per cent;
— *reporting threshold*: 0.05 per cent.

Sulfates (*2.4.13*)
Maximum 80 ppm, determined on solution S. Prepare the standard using a mixture of 2.5 mL of *sulfate standard solution (10 ppm SO_4) R* and 12.5 mL of *distilled water R*.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 0.500 g by drying in an oven at 105 °C for 4 h.

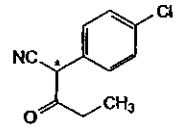
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.200 g in 25 mL of *anhydrous acetic acid R* with gentle heating and allow to cool. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (*2.2.20*).

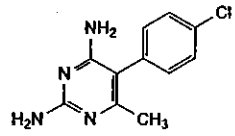
1 mL of *0.1 M perchloric acid* is equivalent to 24.87 mg of $C_{12}H_{13}ClN_4$.

STORAGE
Protected from light.

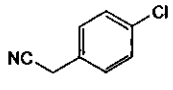
IMPURITIES
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D.*



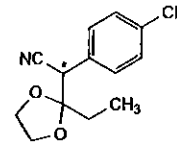
A. (2Ξ)-2-(4-chlorophenyl)-3-oxopentanenitrile,



B. 5-(4-chlorophenyl)-6-methylpyrimidine-2,4-diamine,



C. (4-chlorophenyl)acetonitrile,



D. (Ξ)-(4-chlorophenyl)(2-ethyl-1,3-dioxolan-2-yl) acetonitrile.

Ph Eur

Pyroxylin
Cellulose Nitrate

Preparation
Flexible Collodion

DEFINITION
Pyroxylin is a nitrated cellulose obtained by the action of a mixture of nitric and sulfuric acids on wood pulp or cotton linters that have been freed from fatty matter. It must be damped with not less than 25% by weight of Isopropyl Alcohol or of Industrial Methylated Spirit.

CAUTION *Compliance is required with the provisions of relevant legislation relating to the storage of, use of and containers used for Pyroxylin.*

In the following tests, particular care should be exercised when drying pyroxylin. The material so obtained is explosive and sensitive to ignition by impact or friction and it should be handled as carefully as possible.

CHARACTERISTICS
White or almost white, cuboid granules or fibrous material, the latter resembling absorbent cotton but harsher to the touch and more powdery. Both the granules and the fibrous material appear moist. Highly flammable.

Soluble in *glacial acetic acid* and in *acetone*.

IDENTIFICATION
Add *water* to a solution in *acetone*. A white, viscid mass is readily precipitated.

TESTS
Clarity and colour of solution
A 10% w/v solution dissolves at 20° in a mixture of 1 volume of *ethanol (90%)* and 3 volumes of *ether* to produce an almost clear and colourless to pale straw-coloured solution.

Kinematic viscosity
1160 to 2900 mm^2s^{-1} when determined in the following manner. To 20 g, previously dried to constant weight by heating on a water-bath at 80° and allowing to cool in a desiccator over *silica gel*, add 200 mL of a mixture of 19 volumes of *acetone* and 1 volume of *water*. Shake vigorously to prevent the formation of aggregates of partly solvated pyroxylin and rotate suitably to obtain a homogeneous solution. Measure the viscosity of the solution within 48 hours using a falling sphere viscometer complying with British Standard 188: 1977 (Methods for the determination of viscosity of liquids). Fill the fall tube with the solution being examined to about 10 mm above the 220-mm mark, place vertically in the bath and allow to stand for air bubbles to clear and for temperature equilibrium to be attained. Clean the sphere, immerse it in a portion of the

liquid being examined maintained at a temperature of 19.9° to 20.1° and when it is at this temperature introduce it, without wiping, into the delivery tube. Observe the time for the lowest part of the sphere to pass through the planes of the tops of the 175-mm mark and the 25-mm mark, using a telescope or other suitable device to avoid errors due to parallax. The average of three readings concordant to within 0.5% is taken as the time of fall. Calculate the kinematic viscosity (v) in square millimetres per second (mm^2s^{-1}) from the expression:

$$\nu = \frac{d^2 g(\delta - \rho)}{0.18 v \rho} \times 0.867$$

where d = the diameter of the sphere in cm,
δ = density of the sphere in g cm^{-3},
ρ = density of the liquid being tested in g cm^{-3},
v = velocity of fall in cm s^{-1},
g = local acceleration due to gravity in cm s^{-2}.

Nitrogen

11.7 to 12.2%, calculated with reference to the material dried to constant weight by heating on a water-bath at 80° and allowing to cool in a desiccator over silica gel, when determined by the following method. Transfer 0.4 g of the dried substance to a 750 mL round-bottomed flask using 60 mL of *water*, add 20 mL of *hydrogen peroxide solution (20 vol)* and then add slowly, with shaking, 50 mL of a 30% w/w solution of *potassium hydroxide* and 5 mL of *ethanol (96%)*. Agitate slowly for 3 hours or until the substance being examined has dissolved. Add 3 g of *Devarda's alloy* and immediately connect the flask to the spray trap of an ammonia-distillation apparatus, the receiver of which contains 50 mL of *0.1M hydrochloric acid VS* and 0.15 mL of *methyl red solution*. Allow the reaction to proceed until no further evolution of gas occurs and then heat the contents of the flask to boiling and distil carefully until 30 mL remains in the round-bottomed flask. Rinse the delivery tube into the receiver with *water* and titrate the combined distillate and rinsings with *0.1M sodium hydroxide VS*. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of acid required to neutralise the ammonia formed. Each mL of *0.1M hydrochloric acid VS* is equivalent to 1.401 mg of nitrogen.

STORAGE

Pyroxylin should be loosely packed, protected from light and stored at a temperature not exceeding 15°, remote from fire. The container should be suitably designed to disrupt should the internal pressure reach or exceed 1400 kPa. The amount of damping fluid must not be allowed to fall below 25% w/w; should this happen, the material should be either rewetted or used immediately for the preparation of Collodion.

Pyrrolidone

(Ph. Eur. monograph 2180)

C_4H_7NO 85.1 *616-45-5*

Ph Eur

DEFINITION

Pyrrolidin-2-one.

CHARACTERS

Appearance

Clear, colourless or slightly greyish liquid, or white or almost white crystals, or colourless crystal needles.

Solubility

Miscible with water, with ethanol (96 per cent) and with most common organic solvents.

mp

About 25 °C; the molten substance remains liquid at temperatures below the melting point.

bp

About 245 °C.

IDENTIFICATION

First identification: A.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *pyrrolidone CRS.*

B. Relative density (*2.2.5*): 1.112 to 1.115.

C. Refractive index (*2.2.6*): 1.487 to 1.490.

TESTS

Use the molten substance for all tests.

Appearance

The substance to be examined is clear (*2.2.1*) and not more intensely coloured than intensity 7 of the range of reference solutions of the most appropriate colour (*2.2.2, Method II*).

Alkalinity

To 100 mL of *water R* add 1.0 mL of *bromothymol blue solution R1* and adjust to a green colour with *0.02 M potassium hydroxide* or *0.02 M hydrochloric acid*. To 50 mL of this solution add 20 mL of the substance to be examined and titrate with *0.02 M hydrochloric acid* to the initial colour. Not more than 8.0 mL of *0.02 M hydrochloric acid* is required.

Related substances

Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution The substance to be examined.

Reference solution (a) Dissolve 1 mL of the substance to be examined and 1 mL of *N-methylpyrrolidone R* (impurity C) in *methylene chloride R* and dilute to 20 mL with the same solvent.

Reference solution (b) Dissolve 1.1 g of the substance to be examined in *methylene chloride R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of this solution to 20.0 mL with *methylene chloride R*.

Reference solution (c) Dissolve 1 mL of *butyrolactone R* (impurity B) and 1 mL of *butane-1,4-diol R* (impurity A) in *methylene chloride R* and dilute to 20 mL with the same solvent.

Column:
— *material*: fused silica;
— *size*: *l* = 30 m, Ø = 0.32 mm;
— *stationary phase*: *methylpolysiloxane R* (film thickness 5 µm).

Carrier gas *nitrogen for chromatography R.*

Flow rate 1.3 mL/min.

Split ratio 1:80.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 18.75	100 → 250
	18.75 - 30	250
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 0.1 µL.

Relative retention With reference to pyrrolidone (retention time = about 13 min): impurity B = about 0.73; impurity A = about 0.76; impurity C = about 0.97.

Use the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A and B; use the chromatogram obtained with reference solution (a) to identify the peak due to impurity C.

System suitability Reference solution (a):
— *resolution*: minimum 2.0 between the peaks due to impurity C and pyrrolidone.

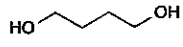
Limits:
— *impurity B*: maximum 0.5 per cent;
— *impurities A, C*: for each impurity, maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.7 per cent;
— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.32*)
Maximum 0.1 per cent, determined on 1.00 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

STORAGE
Protected from light.

IMPURITIES
Specified impurities *A, B, C.*



A. butane-1,4-diol,

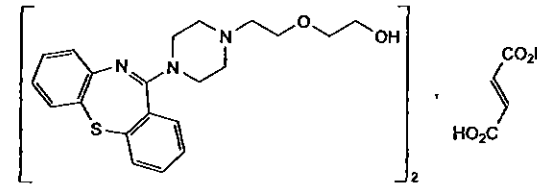
B. dihydrofuran-2(3*H*)-one (γ-butyrolactone),

C. 1-methylpyrrolidin-2-one (*N*-methylpyrrolidone).

Ph Eur

Quetiapine Fumarate

(Ph. Eur. monograph 2541)



$C_{46}H_{54}N_6O_8S_2$ 883 *111974-72-2*

Action and use
Dopamine receptor antagonist; neuroleptic.

Preparations
Quetiapine Prolonged-release Tablets
Quetiapine Tablets

Ph Eur

DEFINITION
Bis[2-[2-[4-(dibenzo[*b*,*f*][1,4]thiazepin-11-yl)piperazin-1-yl]ethoxy]ethanol] (2*E*)-but-2-enedioate.

Content
— *quetiapine fumarate* ($C_{46}H_{54}N_6O_8S_2$; M_r 883): 99.0 per cent to 101.0 per cent (dried substance);
— *fumaric acid* ($C_4H_4O_4$; M_r 116.1): 12.5 per cent to 13.8 per cent (dried substance).

CHARACTERS
Appearance
White or almost white powder.

Solubility
Slightly soluble in water, in anhydrous ethanol and in methanol.

It shows polymorphism (*5.9*).

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *quetiapine fumarate CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

TESTS
Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R, water R* (50:50 *V/V*).

Test solution Dissolve 50 mg of the substance to be examined in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

Reference solution (a) Dissolve the contents of a vial of *quetiapine for system suitability CRS* (containing impurities G and N) in 1.0 mL of the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.10 m, Ø = 2.1 mm;
— *stationary phase*: *end-capped, charged surface, ethylene-bridged phenylhexylsilyl silica gel for chromatography (hybrid material) R* (1.7 µm);
— *temperature*: 50 °C.

Mobile phase:
— *mobile phase A*: mix 10 volumes of *methanol R1* and 90 volumes of a 3.85 g/L solution of *ammonium acetate R*, previously adjusted to pH 9.0 with *ammonia R*;
— *mobile phase B*: *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 8	80	20
8 - 14.50	80 → 60	20 → 40
14.50 - 22.60	60 → 50	40 → 50
22.60 - 26	50 → 30	50 → 70
26 - 29	30 → 10	70 → 90
29 - 30	10	90

Flow rate 0.5 mL/min.

Detection Spectrophotometer at 240 nm.

Injection 3.0 µL.

Identification of impurities Use the chromatogram supplied with *quetiapine for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities G and N.

Relative retention With reference to quetiapine (retention time = about 13 min): fumaric acid = about 0.05; impurity G = about 0.5; impurity N = about 1.04.

System suitability:
— *signal-to-noise ratio*: minimum 40 for the principal peak in the chromatogram obtained with reference solution (b);
— *peak-to-valley ratio*: minimum 5.0, where H_p = height above the baseline of the peak due to impurity N and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to quetiapine in the chromatogram obtained with reference solution (a).

Calculation of percentage contents:
— *correction factors*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity G = 0.5; impurity N = 2.0;
— for each impurity, use the concentration of quetiapine fumarate in reference solution (b).

Limits:
— *impurities G, N*: for each impurity, maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.5 per cent;
— *reporting threshold*: 0.05 per cent; disregard any peak due to fumaric acid.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Quetiapine fumarate
Dissolve 0.170 g in 40 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 22.08 mg of $C_{46}H_{54}N_6O_8S_2$.

Fumaric acid
Dissolve 0.350 g in 70 mL of a mixture of equal volumes of *methanol R* and *water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M sodium hydroxide* is equivalent to 5.804 mg of $C_4H_4O_4$.

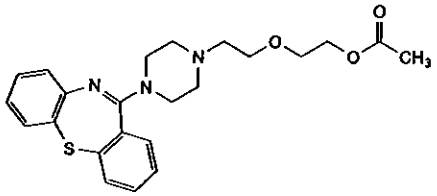
STORAGE

Protected from light.

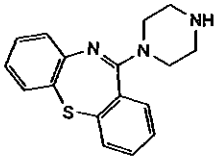
IMPURITIES

Specified impurities *G, N.*

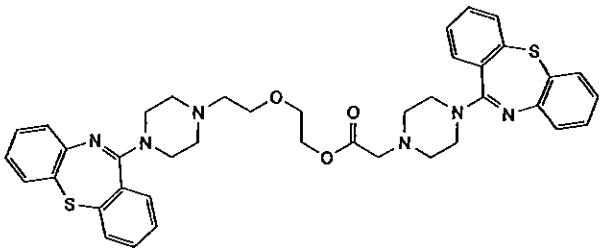
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, E, F, H, I, J, K, L, O, P, Q, S, T, U, V, W.*



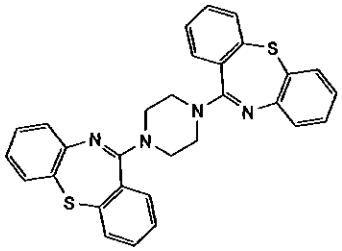
A. 2-[2-[4-(dibenzo[*b*,*f*][1,4]thiazepin-11-yl)piperazin-1-yl] ethoxy]ethyl acetate,



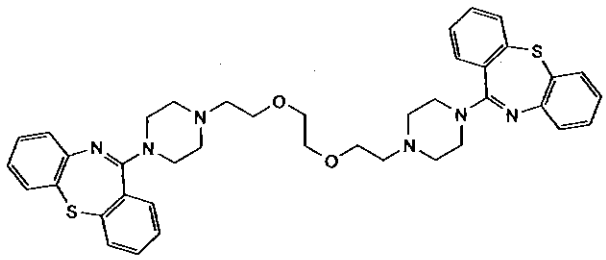
B. 11-(piperazin-1-yl)dibenzo[*b*,*f*][1,4]thiazepine,



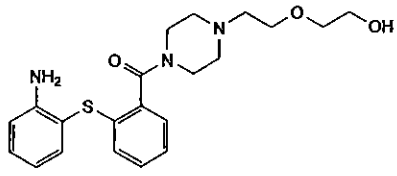
C. 2-[2-[4-(dibenzo[*b*,*f*][1,4]thiazepin-11-yl)piperazin-1-yl] ethoxy]ethyl 2-[4-(dibenzo[*b*,*f*][1,4]thiazepin-11-yl) piperazin-1-yl]acetate,



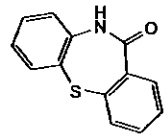
D. 11,11′-(piperazine-1,4-diyl)bis(dibenzo[*b*,*f*][1,4] thiazepine),



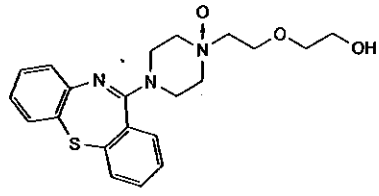
E. 11,11′-[ethylenebis(oxyethylenepiperazine-4,1-diyl)]bis (dibenzo[*b*,*f*][1,4]thiazepine),



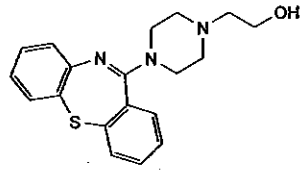
F. [2-[(2-aminophenyl)sulfanyl]phenyl][4-[2-(2-hydroxyethoxy)ethyl]piperazin-1-yl]methanone,



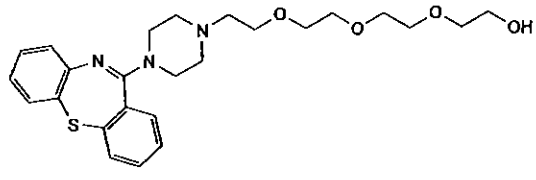
G. dibenzo[*b*,*f*][1,4]thiazepin-11(10*H*)-one,



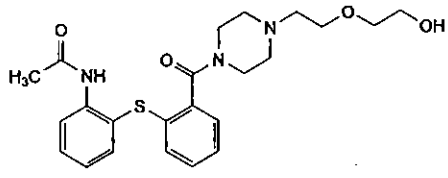
H. 2-[2-[4-(dibenzo[*b*,*f*][1,4]thiazepin-11-yl)-1-oxidopiperazin-1-yl]ethoxy]ethanol,



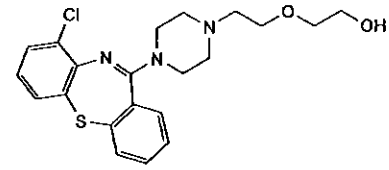
I. 2-[4-(dibenzo[*b*,*f*][1,4]thiazepin-11-yl)piperazin-1-yl] ethanol,



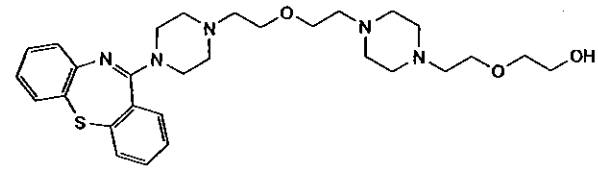
J. 2-[2-[2-[2-[4-(dibenzo[*b*,*f*][1,4]thiazepin-11-yl)piperazin-1-yl]ethoxy]ethoxy]ethoxy]ethanol,



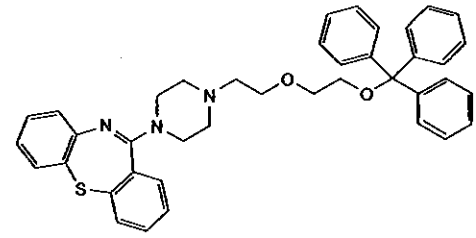
K. *N*-[2-[[2-[[4-[2-(2-hydroxyethoxy)ethyl]piperazin-1-yl] carbonyl]phenyl]sulfanyl]phenyl]acetamide,



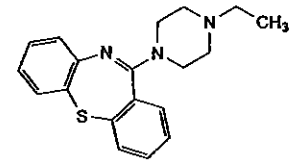
L. 2-[2-[4-(9-chlorodibenzo[*b*,*f*][1,4]thiazepin-11-yl) piperazin-1-yl]ethoxy]ethanol,



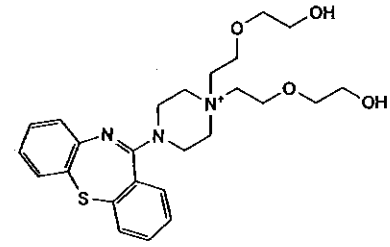
N. 2-[2-[4-[2-[2-[4-(dibenzo[*b*,*f*][1,4]thiazepin-11-yl) piperazin-1-yl]ethoxy]ethyl]piperazin-1-yl]ethoxy]ethanol,



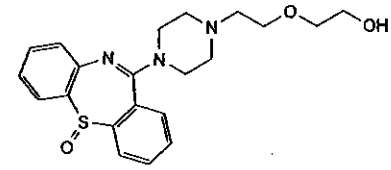
O. 11-[4-[2-[2-(triphenylmethoxy)ethoxy]ethyl]piperazin-1-yl] dibenzo[*b*,*f*][1,4]thiazepine,



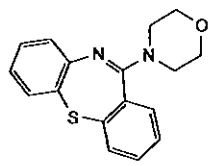
P. 11-(4-ethylpiperazin-1-yl)dibenzo[*b*,*f*][1,4]thiazepine,



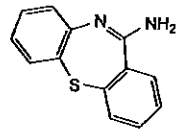
Q. 4-(dibenzo[*b*,*f*][1,4]thiazepin-11-yl)-1,1-bis[2-(2-hydroxyethoxy)ethyl]piperazin-1-ium,



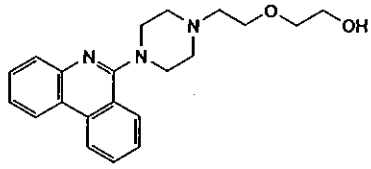
S. 2-[2-[4-(5-oxidodibenzo[*b*,*f*][1,4]thiazepin-11-yl)piperazin-1-yl]ethoxy]ethanol,



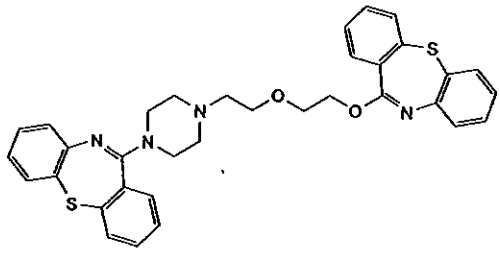
T. 11-(morpholin-4-yl)dibenzo[*b*,*f*][1,4]thiazepine,



U. dibenzo[*b*,*f*][1,4]thiazepin-11-amine,



V. 2-[2-[4-(phenanthridin-6-yl)piperazin-1-yl]ethoxy]ethanol,

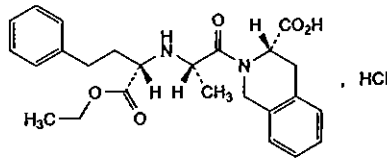


W. 11-(4-[2-[2-(dibenzo[*b*,*f*][1,4]thiazepin-11-yloxy)ethoxy]ethyl]piperazin-1-yl)dibenzo[*b*,*f*][1,4]thiazepine.

Ph Eur

Quinapril Hydrochloride

(Ph. Eur. monograph 1763)



$C_{25}H_{31}ClN_2O_5$ 475.0 *82586-55-8*

Action and use
Angiotensin converting enzyme inhibitor.

Preparation
Quinapril Tablets

Ph Eur

DEFINITION

(3*S*)-2-[(2*S*)-2-[[(1*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]propanoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid hydrochloride.

Content
98.5 per cent to 101.5 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white or slightly pink, hygroscopic powder.

Solubility
Freely soluble in water and in ethanol (96 per cent), very slightly soluble in acetone.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *quinapril hydrochloride CRS.*

B. Specific optical rotation (see Tests).

C. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Specific optical rotation (*2.2.7*)
+ 14.4 to + 16.6 (anhydrous substance).

Dissolve 0.500 g in *methanol R* and dilute to 25.0 mL with the same solvent.

Diastereoisomers
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Solvent mixture Adjust 500 mL of the mobile phase to pH 6.5 with *concentrated ammonia R.*

Test solution Dissolve 100 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 20.0 mL with the solvent mixture.

Reference solution (b) Dissolve the contents of a vial of *quinapril for peak identification CRS* (containing impurities G, H and I) in the solvent mixture and dilute to 5.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.25 m, Ø = 4.0 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 25 °C.

Mobile phase Mix 260 mL of *tetrahydrofuran R* (non-stabilised) with 740 mL of a freshly prepared solution containing 1.08 g/L of *sodium octanesulfonate R* and 2.88 g/L of *ammonium dihydrogen phosphate R*, previously adjusted to pH 4.5 with *phosphoric acid R.*

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µL.

Run time 3.5 times the retention time of quinapril.

Identification of impurities Use the chromatogram supplied with *quinapril for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities G, H and I.

Relative retention With reference to quinapril (retention time = about 18 min): impurity G = about 0.9; impurity H = about 1.2; impurity I = about 1.3.

System suitability Reference solution (b):
— *resolution*: minimum 1.5 between the peaks due to impurity G and quinapril;
— *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity H and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to quinapril.

Limits:
— *impurities G, H, I:* for each impurity, not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent).

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture Mix 40 volumes of *acetonitrile R1* and 60 volumes of a 2.88 g/L solution of *ammonium dihydrogen phosphate R* previously adjusted to pH 6.5 with *dilute ammonia R1*.

Test solution Dissolve 50 mg of the substance to be examined in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve the contents of a vial of *quinapril for system suitability CRS* (containing impurities A, C, D, E and G) in the solvent mixture and dilute to 5.0 mL with the solvent mixture.

Reference solution (c) In order to prepare impurity M *in situ*, dissolve 250 mg of the substance to be examined in *methylene chloride R* and dilute to 5.0 mL with the same solvent. Expose this solution to a source of ultraviolet light for 2.5 h and evaporate the solvent. Dissolve 40 mg of the remaining substance in the solvent mixture and dilute to 20.0 mL with the solvent mixture.

Column:
— *size:* l = 0.15 m, Ø = 3.9 mm;
— *stationary phase: end-capped octylsilyl silica gel for chromatography R* (5 µm).

Temperature:
— *column:* 30 °C;
— *autosampler:* 5 °C.

Mobile phase *acetonitrile R1*, 5.77 g/L solution of *sodium dodecyl sulfate R* adjusted to pH 2.2 with *phosphoric acid R* (48:52 *V/V*).

Flow rate 1.4 mL/min.

Detection Spectrophotometer at 214 nm.

Injection 10 µL.

Run time 3 times the retention time of quinapril.

Identification of impurities Use the chromatogram supplied with *quinapril for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, C, D, E and G; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity M.

Relative retention With reference to quinapril (retention time = about 12 min): impurity A = about 0.1; impurity C = about 0.3; impurity D = about 0.4; impurity M = about 0.7; impurities G + H = about 0.9; impurity E = about 2.3.

System suitability Reference solution (b):
— *resolution:* minimum 1.5 between the peaks due to impurities C and D; minimum 1.5 between the peaks due to impurity G and quinapril.

Limits:
— *correction factor:* for the calculation of content, multiply the peak area of impurity E by 1.5;
— *impurities C, D:* for each impurity, not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *impurity A:* not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
— *impurities E, M:* for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);
— *unspecified impurities:* for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total:* not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
— *disregard limit:* 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent); disregard any peak due to impurities G + H.

Water (*2.5.12*)
Maximum 1.0 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

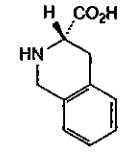
ASSAY
Dissolve 0.200 g in 50 mL of *water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M sodium hydroxide* is equivalent to 23.75 mg of $C_{25}H_{31}ClN_2O_5$.

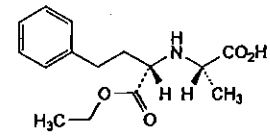
STORAGE
In an airtight container at a temperature of 2 °C to 8 °C.

IMPURITIES
Specified impurities *A, C, D, E, G, H, I, M.*

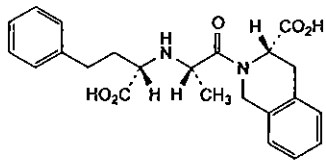
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, J.*



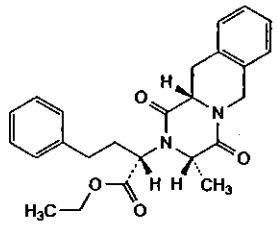
A. (3*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid,



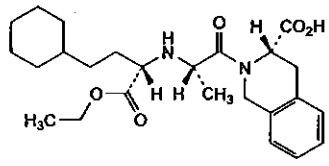
B. (2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)-3-phenylpropyl] amino]propanoic acid,



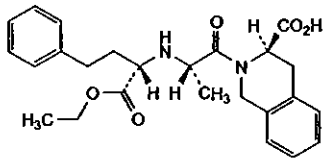
C. (3*S*)-2-[(2*S*)-2-[[(1*S*)-1-carboxy-3-phenylpropyl]amino]propanoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid,



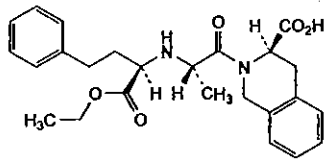
D. ethyl (2*S*)-2-[(3*S*,11a*S*)-3-methyl-1,4-dioxo-1,3,4,6,11,11a-hexahydro-2*H*-pyrazino[1,2-*b*]isoquinolin-2-yl]-4-phenylbutanoate,



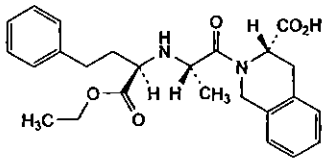
E. (3*S*)-2-[(2*S*)-2-[[(1*S*)-3-cyclohexyl-1-(ethoxycarbonyl)propyl]amino]propanoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid,



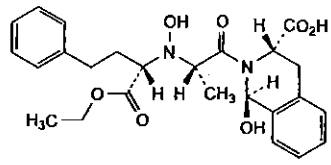
G. (3*R*)-2-[(2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]propanoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid,



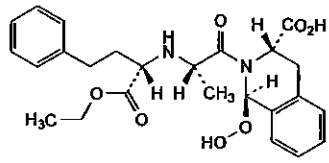
H. (3*R*)-2-[(2*S*)-2-[[(1*R*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]propanoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid,



I. (3*S*)-2-[(2*S*)-2-[[(1*R*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]propanoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid,



J. (1*R*,3*S*)-2-[(2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)-3-phenylpropyl](hydroxy)amino]propanoyl]-1-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid,



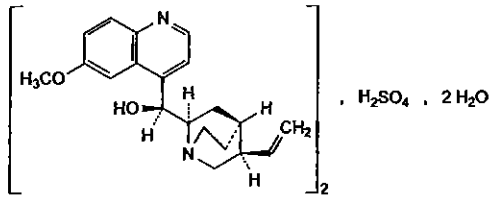
M. (1*R*,3*S*)-2-[(2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]propanoyl]-1-hydroperoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.

Ph Eur

Quinidine Sulfate

Quinidine Sulphate

(Ph. Eur. monograph 0017)



$C_{40}H_{50}N_4O_8S,2H_2O$ 783 *6591-63-5*

Action and use
Class I antiarrhythmic.

Preparation
Quinidine Sulfate Tablets

Ph Eur

DEFINITION
Alkaloid monosulfates, expressed as bis[(*S*)-[(2*R*,4*S*,5*R*)-5-ethenyl-1-azabicyclo[2.2.2]oct-2-yl](6-methoxyquinolin-4-yl)methanol] sulfate dihydrate.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder or silky, colourless needles.

Solubility
Slightly soluble in water, soluble in boiling water and in ethanol (96 per cent), practically insoluble in acetone.

IDENTIFICATION
A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.10 g of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 0.10 g of *quinidine sulfate CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *diethylamine R, ether R, toluene R* (10:24:40 *V/V/V*).

Application 5 µL.

Development Twice over a path of 15 cm; dry in a current of air for 15 min between the 2 developments.

Drying At 105 °C for 30 min and allow to cool.

Detection Spray with *iodoplatinate reagent R.*

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

B. Dissolve about 5 mg in 5 mL of *water R.* Add 0.2 mL of *bromine water R* and 1 mL of *dilute ammonia R2.* A green colour develops.

C. Dissolve 0.1 g in 3 mL of *dilute sulfuric acid R* and dilute to 100 mL with *water R.* When examined in ultraviolet light at 366 nm, an intense blue fluorescence appears which disappears almost completely on addition of 1 mL of *hydrochloric acid R.*

D. Dissolve about 50 mg in 5 mL of hot *water R,* cool, add 1 mL of *silver nitrate solution R1* and stir with a glass rod. After a few minutes, a white precipitate is formed that dissolves on the addition of *dilute nitric acid R.*

E. It gives reaction (a) of sulfates (*2.3.1*).

F. pH (see Tests).

TESTS

Solution S

Dissolve 0.500 g in *0.1 M hydrochloric acid* and dilute to 25.0 mL with the same acid.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution GY_6 (*2.2.2, Method II*).

pH (*2.2.3*)

6.0 to 6.8.

Dissolve 0.10 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Specific optical rotation (*2.2.7*)

+ 275 to + 290 (dried substance), determined on solution S.

Other cinchona alkaloids

Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution Dissolve 20 mg of the substance to be examined in 5 mL of the mobile phase, with gentle heating if necessary, and dilute to 10 mL with the mobile phase.

Reference solution (a) Dissolve 20 mg of *quinine sulfate CRS* (impurity A) in 5 mL of the mobile phase, with gentle heating if necessary, and dilute to 10 mL with the mobile phase.

Reference solution (b) Dissolve 20 mg of *quinidine sulfate CRS* in 5 mL of the mobile phase, with gentle heating if necessary, and dilute to 10 mL with the mobile phase.

Reference solution (c) To 1 mL of reference solution (a) add 1 mL of reference solution (b).

Reference solution (d) Dilute 1.0 mL of reference solution (a) to 10.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution (e) Dissolve 10 mg of *thiourea R* in the mobile phase and dilute to 10 mL with the mobile phase.

Column:
- *size:* l = 0.15-0.25 m, Ø = 4.6 mm;
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5-10 µm).

Mobile phase Dissolve 6.8 g of *potassium dihydrogen phosphate R* and 3.0 g of *hexylamine R* in 700 mL of *water R,* adjust to pH 2.8 with *dilute phosphoric acid R,* add 60 mL of *acetonitrile R* and dilute to 1000 mL with *water R.*

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 250 nm for reference solution (e) and at 316 nm for the other solutions.

Injection 10 µL.

Run time 2.5 times the retention time of quinidine.

Identification of peaks Use the chromatogram obtained with reference solution (a) to identify the peaks due to impurity A and dihydroquinine; use the chromatogram obtained with reference solution (b) to identify the peaks due to quinidine and impurity C; the chromatogram obtained with reference solution (c) shows 4 peaks due to quinidine, impurity A, impurity C and dihydroquinine which are identified by comparison of their retention times with those of the corresponding peaks in the chromatograms obtained with reference solutions (a) and (b).

Relative retention With reference to impurity A: dihydroquinine = about 1.4.

Relative retention With reference to quinidine: impurity C = about 1.5.

System suitability:
- *resolution:* minimum 3.0 between the peaks due to impurity A and quinidine and minimum 2.0 between the peaks due to impurities C and A in the chromatogram obtained with reference solution (c);
- *signal-to-noise ratio:* minimum 4 for the principal peak in the chromatogram obtained with reference solution (d);
- *mass distribution ratio:* 3.5 to 4.5 for the peak due to quinidine in the chromatogram obtained with reference solution (b), t_R, being calculated from the peak due to thiourea in the chromatogram obtained with reference solution (e); if necessary, adjust the concentration of acetonitrile in the mobile phase.

Limits:
- *impurity C:* maximum 15 per cent;
- *any impurity eluted before quinidine:* for each impurity, maximum 5 per cent;
- *any other impurity:* for each impurity, maximum 2.5 per cent;
- *disregard limit:* the area of the principal peak in the chromatogram obtained with reference solution (d) (0.2 per cent).

Boron

Maximum 5 ppm. *Avoid where possible the use of glassware.*

Test solution Dissolve 1.00 g in a mixture of 0.5 mL of *hydrochloric acid R* and 4.0 mL of *water R.*

Reference solution Dissolve 0.572 g of *boric acid R* in *water R* and dilute to 1000.0 mL with the same solvent. Dilute 5.0 mL of the solution to 100.0 mL with *water R.* To 1.0 mL of this solution add 3.0 mL of *water R* and 0.5 mL of *hydrochloric acid R.*

Blank solution Add 0.5 mL of *hydrochloric acid R* to 4.0 mL of *water R.*

Add 3.0 mL of a 100 g/L solution of *2-ethylhexane-1,3-diol R* in *methylene chloride R* to the test solution, to the reference solution and to the blank solution, then shake for 1 min.

Allow to stand for 6 min. To 1.0 mL of the lower layer, add 2.0 mL of a 3.75 g/L solution of *curcumin R* in *anhydrous acetic acid R* and 0.3 mL of *sulfuric acid R*. Mix and after 20 min add 25.0 mL of *ethanol (96 per cent) R*. Mix. The blank solution is yellow. Any red colour in the test solution is not more intense than that in the reference solution.

Loss on drying (*2.2.32*)
3.0 per cent to 5.0 per cent, determined on 1.000 g by drying in an oven at 130 °C.

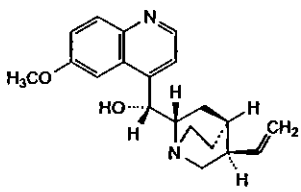
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.200 g in 20 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, using 0.15 mL of *naphtholbenzein solution R* as indicator.

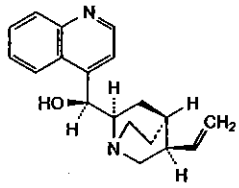
1 mL of *0.1 M perchloric acid* is equivalent to 24.90 mg of $C_{40}H_{50}N_4O_8S$.

STORAGE
Protected from light.

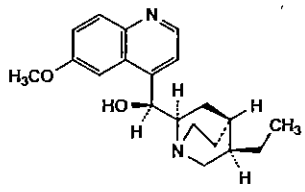
IMPURITIES



A. (*R*)-[(2*S*,4*S*,5*R*)-5-ethenyl-1-azabicyclo[2.2.2]oct-2-yl](6-methoxyquinolin-4-yl)methanol (quinine),



B. (*S*)-[(2*R*,4*S*,5*R*)-5-ethenyl-1-azabicyclo[2.2.2]oct-2-yl](quinolin-4-yl)methanol (cinchonine),

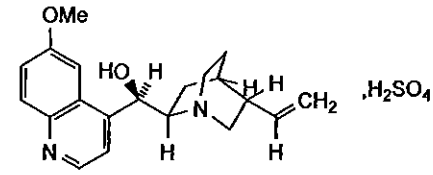


C. (*S*)-[(2*R*,4*S*,5*R*)-5-ethyl-1-azabicyclo[2.2.2]oct-2-yl](6-methoxyquinolin-4-yl)methanol (dihydroquinidine).

Ph Eur

Quinine Bisulfate

Quinine Bisulphate



$C_{20}H_{24}N_2O_2,H_2SO_4,7H_2O$ 548.6 *549-56-4*

Action and use
Antiprotozoal (malaria).

Preparation
Quinine Bisulfate Tablets

DEFINITION
Quinine Bisulfate is (8*S*,9*R*)-6′-methoxycinchonan-9-ol hydrogen sulfate heptahydrate. It contains not less than 98.5% and not more than 101.5% of alkaloid hydrogen sulfates, calculated as $C_{20}H_{24}N_2O_2,H_2SO_4$ with reference to the anhydrous substance.

CHARACTERISTICS
Colourless crystals or a white, crystalline powder; efflorescent in dry air.

Freely soluble in *water*; sparingly soluble in *ethanol (96%)*.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 15 volumes of *diethylamine*, 36 volumes of *ether* and 60 volumes of *toluene* as the mobile phase. Apply separately to the plate 4 µL of each of three solutions in *methanol* containing (1) 1.0% w/v of the substance being examined, (2) 1.0% w/v of *quinine sulfate BPCRS* and (3) 1.0% w/v each of *quinidine sulfate BPCRS* and *quinine sulfate BPCRS*. After removal of the plate, dry it in a current of air for 15 minutes and repeat the development. Dry the plate at 105° for 30 minutes, allow it to cool and spray with *iodoplatinate reagent*. The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

B. Complies with the test for Acidity.

C. Yields the reactions characteristic of sulfates, Appendix VI.

TESTS

Acidity
pH of a 1% w/v solution, 2.8 to 3.4, Appendix V L.

Specific optical rotation
In a 3% w/v solution in 0.1M *hydrochloric acid*, -208 to -216, calculated with reference to the anhydrous substance, Appendix V F.

Other cinchona alkaloids
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dissolve 20 mg of the substance being examined, with gentle heating if necessary, in 5 mL of the mobile phase and dilute to 10 mL with the mobile phase. Prepare solutions (2) and (3) in the same manner using *quinine sulfate BPCRS* and *quinidine sulfate BPCRS* respectively in place of the substance being examined. Solution (4) is a mixture of equal volumes

of solutions (2) and (3). For solution (5) dilute 1 volume of solution (2) to 10 volumes with the mobile phase and dilute 1 volume of the resulting solution to 50 volumes with the mobile phase. Solution (6) contains 0.10% w/v of *thiourea* in the mobile phase.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS 5 µm is suitable), (b) as the mobile phase with a flow rate of 1.5 mL per minute a solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 3.0 g of *hexylamine* in 700 mL of *water*, adjusting the pH to 2.8 with 1M *orthophosphoric acid*, adding 60 mL of *acetonitrile* and diluting to 1000 mL with *water* and (c) a detection wavelength of 250 nm for recording the chromatogram obtained with solution (6) and 316 nm for the other solutions.

Inject separately 10 µL of each of solutions (3) and (6). If necessary adjust the concentration of *acetonitrile* in the mobile phase so that in the chromatogram obtained with solution (3) the *capacity factor* of the peak due to quinidine is 3.5 to 4.5, V_O being calculated from the peak due to thiourea in the chromatogram obtained with solution (6). Inject 10 µL of each of solutions (2), (3), (4) and (5). The chromatogram obtained with solution (2) shows a principal peak due to quinidine and a peak due to dihydroquinine with a retention time relative to quinine of about 1.4. The chromatogram obtained with solution (3) shows a principal peak due to quinidine and a peak due to dihydroquinidine, with a retention time relative to quinidine of about 1.2. The chromatogram obtained with solution (4) shows four peaks due to quinine, dihydroquinine, quinidine and dihydroquinidine which are identified by comparison of their retention times with those of the corresponding peaks in the chromatograms obtained with solutions (2) and (3).

The test is not valid unless (a) in the chromatogram obtained with solution (4) the *resolution factor* between the peaks due to quinine and quinidine is at least 1.5 and the *resolution factor* between the peaks due to dihydroquinidine and quinine is at least 1.0 and (b) the *signal-to-noise ratio* of the principal peak in the chromatogram obtained with solution (5) is at least 5.

Inject 10 µL of solution (1) and allow the chromatography to proceed for 2.5 times the retention time of the principal peak. Calculate the percentage content of related substances by *normalisation*, disregarding any peaks the areas of which are less than that of the peak in the chromatogram obtained with solution (5) (0.2%). The content of dihydroquinine is not greater than 10%, the content of any related substances eluting before quinine is not greater than 5% and the content of any other related substances is not greater than 2.5%.

Sulfated ash
Not more than 0.1%, Appendix IX A.

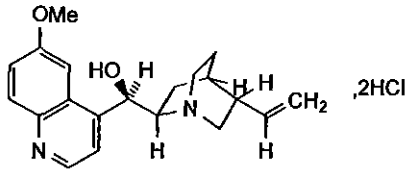
Water
19.0 to 25.0% w/w, Appendix IX C. Use 0.2 g.

Titratable cation
75.3 to 79.6%, calculated with reference to the anhydrous substance, when determined by the following method.
Add to the combined aqueous solutions reserved in the Assay 0.1 mL of *phenolphthalein solution R1* and titrate with *0.1M hydrochloric acid VS*. Each mL of *0.1M sodium hydroxide VS* is equivalent to 16.32 mg of $[C_{20}H_{26}N_2O_2]^{2+}$.

ASSAY
Dissolve 0.45 g in 15 mL of *water*. Add 25 mL of *0.1M sodium hydroxide VS* and extract with three 25 mL quantities of *chloroform*. Wash the combined chloroform extracts with 20 mL of *water*, combine the aqueous solutions and reserve for the test for Titratable cation. Dry the chloroform extracts with *anhydrous sodium sulfate*, evaporate to dryness at a pressure of 2 kPa and dissolve the residue in 50 mL of *anhydrous acetic acid*. Carry out method I for *non-aqueous titration*, Appendix VIII A, using *crystal violet solution* as indicator. Each mL of *0.1M perchloric acid VS* is equivalent to 21.13 mg of $C_{20}H_{24}N_2O_2,H_2SO_4$.

STORAGE
Quinine Bisulfate should be protected from light.

Quinine Dihydrochloride



$C_{20}H_{24}N_2O_2,2HCl$ 397.3 *60-93-5*

Action and use
Antiprotozoal (malaria).

Preparation
Quinine Dihydrochloride Infusion

DEFINITION
Quinine Dihydrochloride is (8S,9R)-6′-methoxycinchonan-9-ol dihydrochloride. It contains not less than 99.0% and not more than 101.0% of alkaloid dihydrochlorides, calculated as $C_{20}H_{24}N_2O_2,2HCl$, with reference to the dried substance.

CHARACTERISTICS
A white or almost white powder.

Very soluble in water; soluble in *ethanol (96%)*.

IDENTIFICATION
A. Carry out the method for *thin-layer chromatography* (*2.2.27*), Appendix III A, using the following solutions in *methanol*.

(1) 1.0% w/v of the substance being examined.

(2) 1.0% w/v of *quinine sulfate BPCRS*.

(3) 1.0% w/v each of *quinidine sulfate BPCRS* and *quinine sulfate BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.

(b) Use the mobile phase as described below.

(c) Apply 4 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of air for 15 minutes and repeat the development. Dry the plate at 105° for 30 minutes, allow to cool and spray with *iodoplatinate reagent*.

MOBILE PHASE

15 volumes of *diethylamine*, 36 volumes of *ether* and 60 volumes of *toluene*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position, colour and size to that in the chromatogram obtained with solution (2).

B. Complies with the test for Acidity.

C. Yields reaction A characteristic of *chlorides*, (*2.3.1*) Appendix VI.

TESTS

Acidity

pH of a 3% w/v solution, 2.0 to 3.0, Appendix V L.

Specific optical rotation (*2.2.7*)

In a 3% w/v solution in 0.1M *hydrochloric acid*, -223 to -229 calculated with reference to the dried substance, Appendix V F.

Barium

To 15 mL of a 2.0% w/v solution add 1 mL of 1M *sulfuric acid*. The solution remains clear for at least 15 minutes.

Sulfate (*2.4.13*)

0.125 g complies with *the limit test for sulfates*, Appendix VII (0.12%).

Other cinchona alkaloids

Carry out the method for *liquid chromatography* (*2.2.29*) Appendix III D, using the *normalisation procedure* and the following solutions in the mobile phase.

(1) Dissolve 20 mg of the substance being examined, with gentle heating if necessary, in 5 mL and dilute to 10 mL.

(2) Prepare as for solution (1) but using *quinine sulfate BPCRS* in place of the substance being examined.

(3) Prepare as for solution (1) but using *quinidine sulfate BPCRS* in place of the substance being examined.

(4) Mix equal volumes of solutions (2) and (3).

(5) Dilute 1 volume of solution (2) to 10 volumes and dilute 1 volume of the resulting solution to 50 volumes.

(6) 0.10% w/v of *thiourea*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil ODS 5 µm is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 250 nm for recording the chromatogram obtained with solution (6) and a detection wavelength of 316 nm for the other solutions.

(f) Inject 10 µL of each solution. Inject separately 10 µL of each of solutions (3) and (6).

(g) For solution (1) allow the chromatography to proceed for 2.5 times the retention time of the principal peak.

MOBILE PHASE

Dissolve 6.8 g of *potassium dihydrogen orthophosphate* and 3.0 g of *hexylamine* in 700 mL of *water*, adjust to pH 2.8 with 1M *orthophosphoric acid*, add 60 mL of *acetonitrile* and dilute to 1000 mL with *water*. If necessary adjust the concentration of acetonitrile in the mobile phase so that in the chromatogram obtained with solution (3) the *capacity factor* of the peak due to quinidine is 3.5 to 4.5, V_0 being calculated from the peak due to thiourea in the chromatogram obtained with solution (6).

SYSTEM SUITABILITY

The test is not valid unless (a) in the chromatogram obtained with solution (4) the *resolution* between the peaks due to quinine and quinidine is at least 1.5 and the *resolution* between the peaks due to dihydroquinidine and quinine is at least 1.0 and (b) the *signal-to-noise ratio* of the principal peak in the chromatogram obtained with solution (5) is at least 5.

The chromatogram obtained with solution (2) shows a principal peak due to quinidine and a peak due to dihydroquinine with a retention time relative to quinine of about 1.4. The chromatogram obtained with solution (3) shows a principal peak due to quinidine and a peak due to dihydroquinidine, with a retention time relative to quinidine of about 1.2. The chromatogram obtained with solution (4) shows four peaks due to quinine, dihydroquinine, quinidine and dihydroquinidine which are identified by comparison of their retention times with those of the corresponding peaks in the chromatograms obtained with solutions (2) and (3).

LIMITS

In the chromatogram obtained with solution (1):

the content of dihydroquinine is not greater than 10%;

the content of any related substance eluting before quinine is not more than 5%;

the content of any other related substance is not more than 2.5%.

Disregard any peak with an area less than that of the peak in the chromatogram obtained with solution (5).

Loss on drying (*2.2.32*)

When dried to constant weight at 105°, loses not more than 3.0% of its weight. Use 1 g.

Sulfated ash (*2.4.14*)

Not more than 0.1%, Appendix IX A.

ASSAY

Dissolve 0.150 g in a mixture of 10 mL of *water* and 50 mL of *ethanol*. Titrate with *0.1M sodium hydroxide*, determining the end-point potentiometrically, Appendix VIII B.

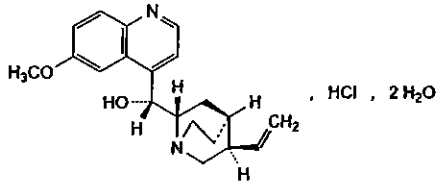
Each mL of *0.1M sodium hydroxide* is equivalent to 39.73 mg of $C_{20}H_{24}N_2O_2,2HCl$.

STORAGE

Quinine Dihydrochloride should be protected from light.

Quinine Hydrochloride

(Ph. Eur. monograph 0018)



$C_{20}H_{25}ClN_2O_2,2H_2O$ 396.9 *6119-47-7*

Action and use

Antiprotozoal (malaria).

Ph Eur

DEFINITION

Alkaloid monohydrochlorides, expressed as (*R*)-[(2*S*,4*S*,5*R*)-5-ethenyl-1-azabicyclo[2.2.2]oct-2-yl](6-methoxyquinolin-4-yl)methanol hydrochloride dihydrate.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white or colourless, fine, silky needles, often in clusters.

Solubility
Soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.10 g of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 0.10 g of *quinine sulfate CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *diethylamine R, ether R, toluene R* (10:24:40 *V/V/V*).

Application 5 µL.

Development Twice over a path of 15 cm; dry in a current of air for 15 min between the 2 developments.

Drying At 105 °C for 30 min and allow to cool.

Detection Spray with *iodoplatinate reagent R.*

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

B. Dissolve about 10 mg in *water R* and dilute to 10 mL with the same solvent. To 5 mL of this solution add 0.2 mL of *bromine water R* and 1 mL of *dilute ammonia R2.* A green colour develops.

C. Dissolve 0.1 g in 3 mL of *dilute sulfuric acid R* and dilute to 100 mL with *water R.* When examined in ultraviolet light at 366 nm, an intense blue fluorescence appears which disappears almost completely on the addition of 1 mL of *hydrochloric acid R.*

D. It gives the reactions of chlorides (*2.3.1*).

E. pH (see Tests).

TESTS

Solution S
Dissolve 1.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

pH (*2.2.3*)
6.0 to 6.8.

Dilute 10 mL of solution S to 20 mL with *carbon dioxide-free water R.*

Specific optical rotation (*2.2.7*)
−245 to −258 (dried substance).

Dissolve 0.500 g in *0.1 M hydrochloric acid* and dilute to 25.0 mL with the same acid.

Other cinchona alkaloids
Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution Dissolve 20 mg of the substance to be examined in 5 mL of the mobile phase, with gentle heating if necessary, and dilute to 10 mL with the mobile phase.

Reference solution (a) Dissolve 20 mg of *quinine sulfate CRS* in 5 mL of the mobile phase, with gentle heating if necessary, and dilute to 10 mL with the mobile phase.

Reference solution (b) Dissolve 20 mg of *quinidine sulfate CRS* (impurity A) in 5 mL of the mobile phase, with gentle heating if necessary, and dilute to 10 mL with the mobile phase.

Reference solution (c) To 1 mL of reference solution (a) add 1 mL of reference solution (b).

Reference solution (d) Dilute 1.0 mL of reference solution (a) to 10.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution (e) Dissolve 10 mg of *thiourea R* in the mobile phase and dilute to 10 mL with the mobile phase.

Column:
— *size:* l = 0.15-0.25 m, Ø = 4.6 mm;
— *stationary phase = octadecylsilyl silica gel for chromatography R* (5-10 µm).

Mobile phase Dissolve 6.8 g of *potassium dihydrogen phosphate R* and 3.0 g of *hexylamine R* in 700 mL of *water R,* adjust to pH 2.8 with *dilute phosphoric acid R,* add 60 mL of *acetonitrile R* and dilute to 1000 mL with *water R.*

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 250 nm for reference solution (e) and at 316 nm for the other solutions.

Injection 10 µL.

Run time 2.5 times the retention time of quinine.

Identification of peaks Use the chromatogram obtained with reference solution (a) to identify the peaks due to quinine and impurity C; use the chromatogram obtained with reference solution (b) to identify the peaks due to impurity A and dihydroquinidine; the chromatogram obtained with reference solution (c) shows 4 peaks due to impurity A, quinine, dihydroquinidine and impurity C, which are identified by comparison of their retention times with those of the corresponding peaks in the chromatograms obtained with reference solutions (a) and (b).

Relative retention With reference to quinine:
impurity C = about 1.4.

Relative retention With reference to impurity A:
dihydroquinidine = about 1.5.

System suitability:
— *resolution:* minimum 3.0 between the peaks due to quinine and impurity A and minimum 2.0 between the peaks due to dihydroquinidine and quinine in the chromatogram obtained with reference solution (c);
— *signal-to-noise ratio:* minimum 4 for the principal peak in the chromatogram obtained with reference solution (d);
— *mass distribution ratio:* 3.5 to 4.5 for the peak due to impurity A in the chromatogram obtained with reference solution (b), t_R, being calculated from the peak due to thiourea in the chromatogram obtained with reference solution (e); if necessary, adjust the concentration of acetonitrile in the mobile phase.

Limits:
— *impurity C:* maximum 10 per cent;
— *any impurity eluted before quinine:* for each impurity, maximum 5 per cent;
— *any other impurity:* for each impurity, maximum 2.5 per cent;
— *disregard limit:* the area of the principal peak in the chromatogram obtained with reference solution (d) (0.2 per cent).

Sulfates (*2.4.13*)
Maximum 500 ppm, determined on solution S.

Barium
To 15 mL of solution S add 1 mL of *dilute sulfuric acid R*. Allow to stand for 15 min. Any opalescence in the solution is not more intense than that in a mixture of 15 mL of solution S and 1 mL of *distilled water R*.

Loss on drying (*2.2.32*)
6.0 per cent to 10.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

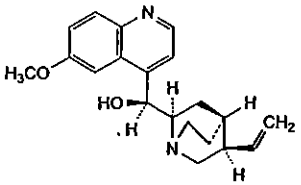
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.250 g in 50 mL of *ethanol (96 per cent) R* and add 5.0 mL of *0.01 M hydrochloric acid*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Read the volume added between the 2 inflexion points.

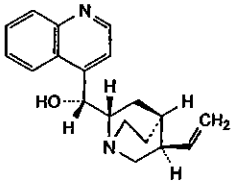
1 mL of *0.1 M sodium hydroxide* is equivalent to 36.09 mg of $C_{20}H_{25}ClN_2O_2$

STORAGE
Protected from light.

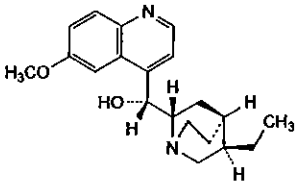
IMPURITIES



A. (*S*)-[(2*R*,4*S*,5*R*)-5-ethenyl-1-azabicyclo[2.2.2]oct-2-yl](6-methoxyquinolin-4-yl)methanol (quinidine),



B. (*R*)-[(2*S*,4*S*,5*R*)-5-ethenyl-1-azabicyclo[2.2.2]oct-2-yl](quinolin-4-yl)methanol (cinchonidine),

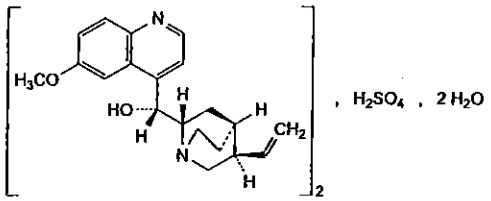


C. (*R*)-[(2*S*,4*S*,5*R*)-5-ethyl-1-azabicyclo[2.2.2]oct-2-yl](6-methoxyquinolin-4-yl)methanol (dihydroquinine).

Ph Eur

Quinine Sulfate

Quinine Sulphate
(Ph. Eur. monograph 0019)



$C_{40}H_{50}N_4O_8S,2H_2O$ 783 *6119-70-6*

Action and use
Antiprotozoal (malaria).

Preparation
Quinine Sulfate Tablets

Ph Eur

DEFINITION
Alkaloid monosulfates, expressed as bis[(*R*)-[(2*S*,4*S*,5*R*)-5-ethenyl-1-azabicyclo[2.2.2]oct-2-yl](6-methoxyquinolin-4-yl) methanol] sulfate dihydrate.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder or fine, colourless needles.

Solubility
Slightly soluble in water, sparingly soluble in boiling water and in ethanol (96 per cent).

IDENTIFICATION
A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.10 g of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 0.10 g of *quinine sulfate CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *diethylamine R, ether R, toluene R* (10:24:40 *V/V/V*).

Application 5 µL.

Development Twice over a path of 15 cm; dry in a current of air for 15 min between the 2 developments.

Drying At 105 °C for 30 min and allow to cool.

Detection Spray with *iodoplatinate reagent R.*

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

B. Dissolve about 5 mg in 5 mL of *water R*. Add 0.2 mL of *bromine water R* and 1 mL of *dilute ammonia R2*. A green colour develops.

C. Dissolve 0.1 g in 3 mL of *dilute sulfuric acid R* and dilute to 100 mL with *water R*. When examined in ultraviolet light at 366 nm, an intense blue fluorescence appears which disappears almost completely on the addition of 1 mL of *hydrochloric acid R*.

D. Dissolve about 45 mg in 5 mL of *dilute hydrochloric acid R*. The solution gives reaction (a) of sulfates (*2.3.1*).

E. pH (see Tests).

TESTS

Solution S

Dissolve 0.500 g in *0.1 M hydrochloric acid* and dilute to 25.0 mL with the same acid.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution GY_6 (*2.2.2, Method II*).

pH (*2.2.3*)

5.7 to 6.6 for a 10 g/L suspension in *water R*.

Specific optical rotation (*2.2.7*)

−237 to −245 (dried substance), determined on solution S.

Other cinchona alkaloids

Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution Dissolve 20 mg of the substance to be examined in 5 mL of the mobile phase, with gentle heating if necessary, and dilute to 10 mL with the mobile phase.

Reference solution (a) Dissolve 20 mg of *quinine sulfate CRS* in 5 mL of the mobile phase, with gentle heating if necessary, and dilute to 10 mL with the mobile phase.

Reference solution (b) Dissolve 20 mg of *quinidine sulfate CRS* (impurity A) in 5 mL of the mobile phase, with gentle heating if necessary, and dilute to 10 mL with the mobile phase.

Reference solution (c) To 1 mL of reference solution (a) add 1 mL of reference solution (b).

Reference solution (d) Dilute 1.0 mL of reference solution (a) to 10.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution (e) Dissolve 10 mg of *thiourea R* in the mobile phase and dilute to 10 mL with the mobile phase.

Column:

— *size*: *l* = 0.15-0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5-10 µm).

Mobile phase Dissolve 6.8 g of *potassium dihydrogen phosphate R* and 3.0 g of *hexylamine R* in 700 mL of *water R*, adjust to pH 2.8 with *dilute phosphoric acid R*, add 60 mL of *acetonitrile R* and dilute to 1000 mL with *water R*.

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 250 nm for reference solution (e) and at 316 nm for the other solutions.

Injection 10 µL.

Run time 2.5 times the retention time of quinine.

Identification of peaks Use the chromatogram obtained with reference solution (a) to identify the peaks due to quinine and impurity C; use the chromatogram obtained with reference solution (b) to identify the peaks due to impurity A and dihydroquinidine; the chromatogram obtained with reference solution (c) shows 4 peaks due to impurity A, quinine, dihydroquinidine and impurity C which are identified by comparison of their retention times with those of the corresponding peaks in the chromatograms obtained with reference solutions (a) and (b).

Relative retention With reference to quinine: impurity C = about 1.4.

Relative retention With reference to impurity A: dihydroquinidine = about 1.5.

System suitability:

— *resolution*: minimum 3.0 between the peaks due to quinine and impurity A and minimum 2.0 between the peaks due to dihydroquinidine and quinine in the chromatogram obtained with reference solution (c);

— *signal-to-noise ratio*: minimum 4 for the principal peak in the chromatogram obtained with reference solution (d);

— *mass distribution ratio*: 3.5 to 4.5 for the peak due to impurity A in the chromatogram obtained with reference solution (b), $t_{R'}$ being calculated from the peak due to thiourea in the chromatogram obtained with reference solution (e); if necessary, adjust the concentration of acetonitrile in the mobile phase.

Limits:

— *impurity C*: maximum 10 per cent;

— *any impurity eluted before quinine*: for each impurity, maximum 5 per cent;

— *any other impurity*: for each impurity, maximum 2.5 per cent;

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (d) (0.2 per cent).

Loss on drying (*2.2.32*)

3.0 per cent to 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

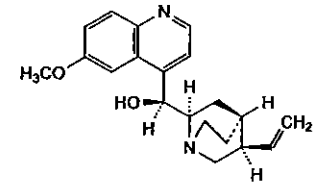
Dissolve 0.300 g in a mixture of 10 mL of *chloroform R* and 20 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 24.90 mg of $C_{40}H_{50}N_4O_8S$.

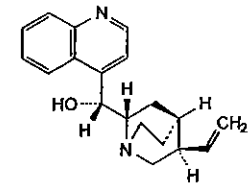
STORAGE

Protected from light.

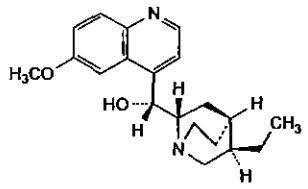
IMPURITIES



A. (*S*)-[(2*R*,4*S*,5*R*)-5-ethenyl-1-azabicyclo[2.2.2]oct-2-yl](6-methoxyquinolin-4-yl)methanol (quinidine),



B. (*R*)-[(2*S*,4*S*,5*R*)-5-ethenyl-1-azabicyclo[2.2.2]oct-2-yl](quinolin-4-yl)methanol (cinchonidine),

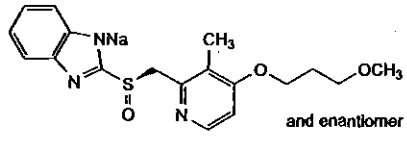


C. (*R*)-[(2*S*,4*S*,5*R*)-5-ethyl-1-azabicyclo[2.2.2]oct-2-yl](6-methoxyquinolin-4-yl)methanol (dihydroquinine).

Ph Eur

Rabeprazole Sodium

(Ph. Eur. monograph 2868)



$C_{18}H_{20}N_3NaO_3S$ 381.4 *117976-90-6*

Action and use
Proton pump inhibitor; treatment of peptic ulcer disease.

Ph Eur

DEFINITION
Sodium 2-[(*RS*)-[[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methyl]sulfinyl]benzimidazol-1-ide.

Content
97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
White or slightly yellowish-white, hygroscopic powder.

Solubility
Very soluble to freely soluble in water, freely soluble in anhydrous ethanol, practically insoluble in heptane.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Dissolve the substance to be examined in *methanol R*, evaporate to dryness and record the spectrum using the residue.

Comparison Repeat the operations using *rabeprazole sodium hydrate CRS*.

B. Loss on drying (see Tests).

C. It gives reaction (a) of sodium (*2.3.1*).

TESTS

pH (*2.2.3*)
9.5 to 11.5.

Dissolve 0.100 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Solvent mixture *methanol R, 0.1 M phosphate buffer solution pH 11.3 R* (20:80 *V/V*).

Test solution (a) Dissolve 20.0 mg of the substance to be examined in the solvent mixture and dilute to 20.0 mL with the solvent mixture.

Test solution (b) Dilute 5.0 mL of test solution (a) to 50.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 5 mg of *rabeprazole for system suitability CRS* (containing impurities A and H) in the solvent mixture and dilute to 5.0 mL with the solvent mixture.

Reference solution (c) Dissolve 20.0 mg of *rabeprazole sodium hydrate CRS* in the solvent mixture and dilute to 20.0 mL with the solvent mixture. Dilute 5.0 mL of the solution to 50.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 45 °C.

Mobile phase:
— *mobile phase A*: mix 5 volumes of *acetonitrile R* and 95 volumes of a 4.35 g/L solution of *dipotassium hydrogen phosphate R* previously adjusted to pH 7.0 with *phosphoric acid R*;
— *mobile phase B*: *methanol R*;
— *mobile phase C*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Mobile phase C (per cent *V/V*)
0 - 2	100	0	0
2 - 7	100 → 85	0	0 → 15
7 - 27	85 → 30	0 → 40	15 → 30
27 - 32	30 → 15	40 → 55	30

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 280 nm.

Autosampler Set at 6 °C.

Injection 5 µL of test solution (a) and reference solutions (a) and (b).

Identification of impurities Use the chromatogram supplied with *rabeprazole for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and H.

Relative retention With reference to rabeprazole (retention time = about 19 min): impurity A = about 0.9; impurity H = about 0.98.

System suitability Reference solution (b):
— *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity H and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to rabeprazole.

Calculation of percentage contents:
— for each impurity, use the concentration of rabeprazole sodium in reference solution (a).

Limits:
— *impurity A*: maximum 0.8 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 1.0 per cent;
— *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (c).

Calculate the percentage content of $C_{18}H_{20}N_3NaO_3S$ taking into account the assigned content of *rabeprazole sodium hydrate CRS*.

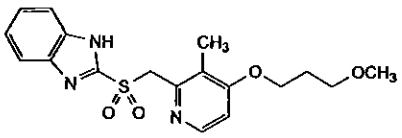
STORAGE

In an airtight container, protected from light.

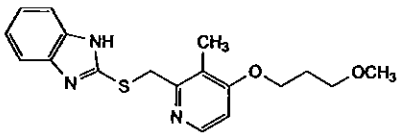
IMPURITIES

Specified impurities *A.*

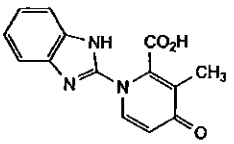
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D, E, F, G, H, I, K.*



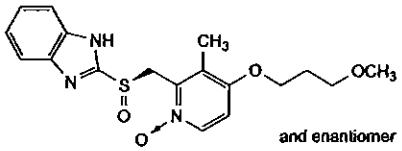
A. 2-[[[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methyl] sulfonyl]-1*H*-benzimidazole,



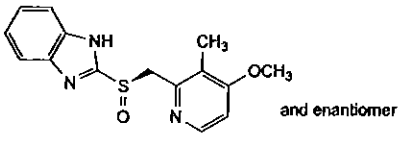
B. 2-[[[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methyl] sulfanyl]-1*H*-benzimidazole,



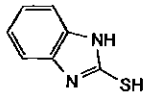
C. 1-(1*H*-benzimidazol-2-yl)-3-methyl-4-oxo-1,4-dihydropyridine-2-carboxylic acid,



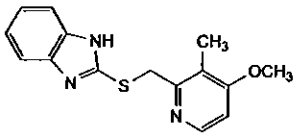
D. 2-[[(*RS*)-(1*H*-benzimidazol-2-yl)sulfinyl]methyl]-4-(3-methoxypropoxy)-3-methylpyridine 1-oxide,



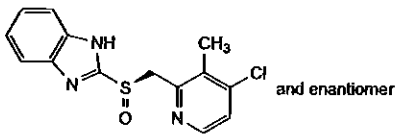
E. 2-[(*RS*)-[(4-methoxy-3-methylpyridin-2-yl)methyl] sulfinyl]-1*H*-benzimidazole,



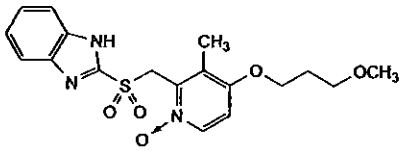
F. 1*H*-benzimidazole-2-thiol,



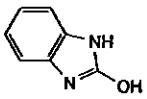
G. 2-[[(4-methoxy-3-methylpyridin-2-yl)methyl]sulfanyl]-1*H*-benzimidazole,



H. 2-[(*RS*)-[(4-chloro-3-methylpyridin-2-yl)methyl]sulfinyl]-1*H*-benzimidazole,



I. 2-[[(1*H*-benzimidazol-2-yl)sulfonyl]methyl]-4-(3-methoxypropoxy)-3-methylpyridine 1-oxide,

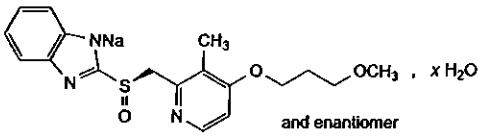


K. 1*H*-benzimidazol-2-ol.

Ph Eur

Rabeprazole Sodium Hydrate

(Ph. Eur. monograph 2331)



$C_{18}H_{20}N_3NaO_3S,xH_2O$ 381.4 (anhydrous substance)

891191-56-3

Action and use

Proton pump inhibitor; treatment of peptic ulcer disease.

Ph Eur

DEFINITION

Sodium 2-[(*RS*)-[[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methyl]sulfinyl]benzimidazol-1-ide hydrate.

Content

97.5 per cent to 102.0 per cent (anhydrous substance).

It contains a variable quantity of water.

CHARACTERS

Appearance

White or slightly yellowish-white, hygroscopic, amorphous or crystalline powder.

Solubility

Very soluble to freely soluble in water, freely soluble in anhydrous ethanol, practically insoluble in heptane.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *rabeprazole sodium hydrate CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

B. Water (see Tests).

C. It gives reaction (a) of sodium (*2.3.1*).

TESTS

pH (*2.2.3*)

9.5 to 11.5.

Dissolve 0.100 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Solvent mixture *methanol R, 0.1 M phosphate buffer solution pH 11.3 R* (20:80 *V/V*).

Test solution (a) Dissolve 20.0 mg of the substance to be examined in the solvent mixture and dilute to 20.0 mL with the solvent mixture.

Test solution (b) Dilute 5.0 mL of test solution (a) to 50.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 5 mg of *rabeprazole for system suitability CRS* (containing impurities A and H) in the solvent mixture and dilute to 5 mL with the solvent mixture.

Reference solution (c) Dissolve 20.0 mg of *rabeprazole sodium hydrate CRS* in the solvent mixture and dilute to 20.0 mL with the solvent mixture. Dilute 5.0 mL of the solution to 50.0 mL with the solvent mixture.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 45 °C.

Mobile phase:
- *mobile phase A*: mix 5 volumes of *acetonitrile R* and 95 volumes of a 4.35 g/L solution of *dipotassium hydrogen phosphate R* previously adjusted to pH 7.0 with *phosphoric acid R*;
- *mobile phase B*: *methanol R*;
- *mobile phase C*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Mobile phase C (per cent *V/V*)
0 - 2	100	0	0
2 - 7	100 → 85	0	0 → 15
7 - 27	85 → 30	0 → 40	15 → 30
27 - 32	30 → 15	40 → 55	30

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 280 nm.

Autosampler Set at 6 °C.

Injection 5 µL of test solution (a) and reference solutions (a) and (b).

Identification of impurities Use the chromatogram supplied with *rabeprazole for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and H.

Relative retention With reference to rabeprazole (retention time = about 19 min): impurity A = about 0.9; impurity H = about 0.98.

System suitability Reference solution (b):
- *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity H and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to rabeprazole.

Calculation of percentage contents:
- for each impurity, use the concentration of rabeprazole sodium hydrate in reference solution (a).

Limits:
- *impurity A*: maximum 0.15 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.5 per cent;
- *reporting threshold*: 0.05 per cent.

Water (*2.5.12*)

1.5 per cent to 7.0 per cent, determined on 0.200 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (c).

Calculate the percentage content of $C_{18}H_{20}N_3NaO_3S$ taking into account the assigned content of *rabeprazole sodium hydrate CRS*.

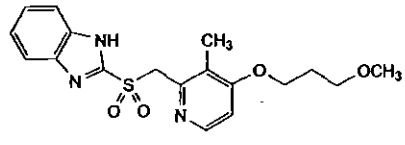
STORAGE

In an airtight container, protected from light.

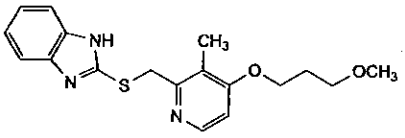
IMPURITIES

Specified impurities *A.*

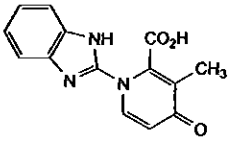
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D, E, F, G, H, I, K.*



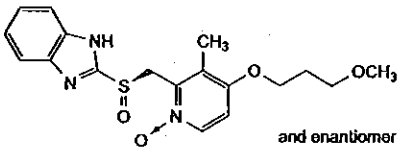
A. 2-[[[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methyl] sulfonyl]-1*H*-benzimidazole,



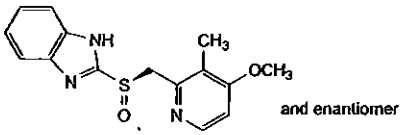
B. 2-[[[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methyl] sulfanyl]-1*H*-benzimidazole,



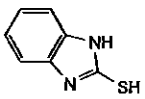
C. 1-(1*H*-benzimidazol-2-yl)-3-methyl-4-oxo-1,4-dihydropyridine-2-carboxylic acid,



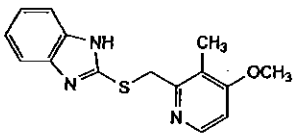
D. 2-[[(*RS*)-(1*H*-benzimidazol-2-yl)sulfinyl]methyl]-4-(3-methoxypropoxy)-3-methylpyridine 1-oxide,



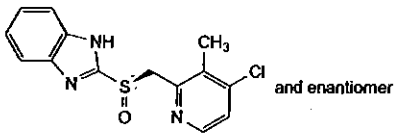
E. 2-[(*RS*)-[(4-methoxy-3-methylpyridin-2-yl)methyl] sulfinyl]-1*H*-benzimidazole,



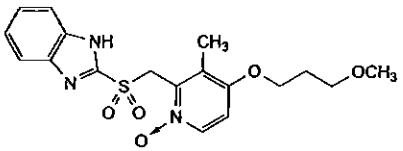
F. 1*H*-benzimidazole-2-thiol,



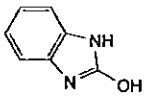
G. 2-[[(4-methoxy-3-methylpyridin-2-yl)methyl]sulfanyl]-1*H*-benzimidazole,



H. 2-[(*RS*)-[(4-chloro-3-methylpyridin-2-yl)methyl]sulfinyl]-1*H*-benzimidazole,



I. 2-[[(1*H*-benzimidazol-2-yl)sulfonyl]methyl]-4-(3-methoxypropoxy)-3-methylpyridine 1-oxide,

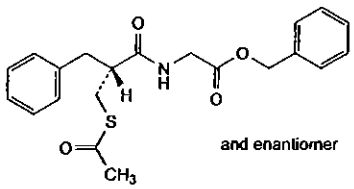


K. 1*H*-benzimidazol-2-ol.

Ph Eur

Racecadotril

(Ph. Eur. monograph 2171)



$C_{21}H_{23}NO_4S$ 385.5 *81110-73-8*

Ph Eur

DEFINITION

Benzyl [[(2*RS*)-2-[(acetylsulfanyl)methyl]-3-phenylpropanoyl] amino]acetate.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, freely soluble in methanol and in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *racecadotril CRS.*

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Dissolve 5.0 g in 10 mL of *acetone R.*

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture Mobile phase A, mobile phase B (50:50 *V/V*).

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

Test solution (b) Dilute 5.0 mL of test solution (a) to 25.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) *Prepare immediately before use.* Dilute 500 µL of *racecadotril impurity A CRS* in *acetonitrile R* and dilute to 250.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 100.0 mL with the solvent mixture.

Reference solution (c) Dissolve 5 mg of *racecadotril impurity G CRS* in the solvent mixture and dilute to 50 mL

with the solvent mixture. To 5 mL of this solution add 1 mL of test solution (b) and dilute to 100 mL with the solvent mixture.

Reference solution (d) Dissolve 50.0 mg of *racecadotril CRS* in the solvent mixture and dilute to 25.0 mL with the solvent mixture. Dilute 5.0 mL of this solution to 25.0 mL with the solvent mixture.

Reference solution (e) Dissolve 2 mg of *racecadotril for peak identification CRS* (containing impurities C, E and F) in 1.0 mL of the solvent mixture.

Column:
- *size*: *l* = 0.25 m, Ø = 4.0 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 30 °C.

Mobile phase:
- *mobile phase A*: dissolve 1.0 g of *potassium dihydrogen phosphate R* in *water R*, adjust to pH 2.5 with *phosphoric acid R* and dilute to 1000 mL with *water R*;
- *mobile phase B*: *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	60	40
5 - 25	60 → 20	40 → 80
25 - 35	20	80

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µL of the solvent mixture, test solution (a) and reference solutions (a), (b), (c) and (e).

Identification of impurities Use the chromatogram supplied with *racecadotril for peak identification CRS* and the chromatogram obtained with reference solution (e) to identify the peaks due to impurities C, E and F.

Relative retention With reference to racecadotril (retention time = about 16 min): impurity A = about 0.2; impurity C = about 0.3; impurity E = about 0.5; impurity F = about 0.9.

System suitability Reference solution (c):
- *resolution*: minimum 1.5 between the peaks due to impurity G and racecadotril.

Limits:
- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity C = 1.4; impurity E = 0.6; impurity F = 0.7;
- *impurities C, E, F*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 4 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

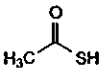
Injection Test solution (b) and reference solution (d).

Calculate the percentage content of $C_{21}H_{23}NO_4S$ from the declared content of *racecadotril CRS*.

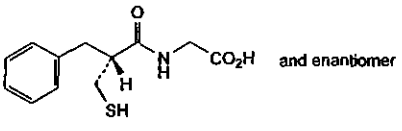
IMPURITIES

Specified impurities *A, C, E, F.*

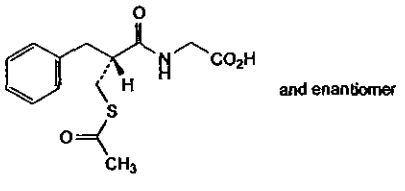
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, D, G, H.*



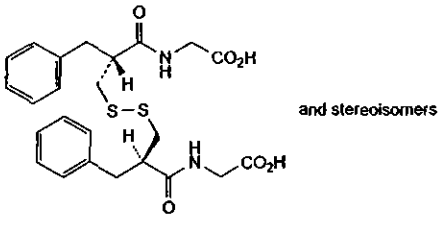
A. ethanethioic acid (thioacetic acid),



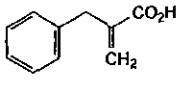
B. [[(2*RS*)-2-benzyl-3-sulfanylpropanoyl]amino]acetic acid,



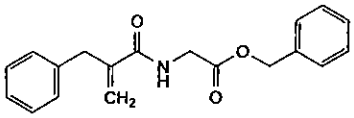
C. [[(2*RS*)-2-[(acetylsulfanyl)methyl]-3-phenylpropanoyl]amino]acetic acid,



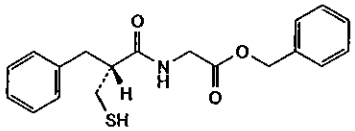
D. 5,10-dibenzyl-4,11-dioxo-7,8-dithia-3,12-diazatetradecanedioic acid,



E. 2-benzylprop-2-enoic acid (2-benzylacrylic acid),

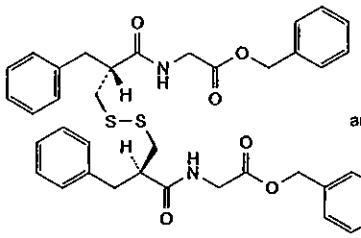


F. benzyl [(2-benzylprop-2-enoyl)amino]acetate,



and enantiomer

G. benzyl [[(2*RS*)-2-benzyl-3-sulfanylpropanoyl] amino]acetate,



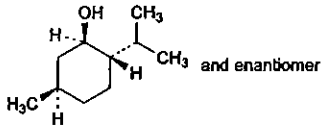
and stereoisomers

H. dibenzyl 5,10-dibenzyl-4,11-dioxo-7,8-dithia-3,12-diazatetradecanedioate.

Ph Eur

Racementhol

(Racemic Menthol, Ph. Eur. monograph 0623)



and enantiomer

$C_{10}H_{20}O$ 156.3 *89-78-1*

Preparation
Menthol and Benzoin Inhalation

Ph Eur

DEFINITION
Mixture of equal parts of (1*RS*,2*SR*,5*RS*)-5-methyl-2-(1-methylethyl)cyclohexanol.

CHARACTERS
Appearance
Free-flowing or agglomerated, crystalline powder or prismatic or acicular, colourless, shiny crystals.

Solubility
Practically insoluble in water, very soluble in ethanol (96 per cent) and in light petroleum, freely soluble in fatty oils and in liquid paraffin, very slightly soluble in glycerol.

mp
About 34 °C.

IDENTIFICATION
First identification: A, C.

Second identification: B, D.

A. Optical rotation (see Tests).

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 5 mL with the same solvent.

Reference solution Dissolve 25 mg of *menthol CRS* in *methanol R* and dilute to 5 mL with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *ethyl acetate R, toluene R* (5:95 *V/V*).

Application 2 µL.

Development Over a path of 15 cm.

Drying In air, until the solvents have evaporated.

Detection Spray with *anisaldehyde solution R* and heat at 100-105 °C for 5-10 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. Examine the chromatograms obtained in the test for related substances.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in position and approximate dimensions to the principal peak in the chromatogram obtained with reference solution (c).

D. Dissolve 0.20 g in 0.5 mL of *anhydrous pyridine R*. Add 3 mL of a 150 g/L solution of *dinitrobenzoyl chloride R* in *anhydrous pyridine R*. Heat on a water-bath for 10 min. Add 7.0 mL of *water R* in small quantities with stirring and allow to stand in iced water for 30 min. A precipitate is formed. Allow to stand and decant the supernatant. Wash the precipitate with 2 quantities, each of 5 mL, of iced *water R*, recrystallise from 10 mL of *acetone R*, wash with iced *acetone R* and dry at 75 °C at a pressure not exceeding 2.7 kPa for 30 min. The crystals melt (*2.2.14*) at 130 °C to 131 °C.

TESTS
Solution S
Dissolve 2.50 g in 10 mL of *ethanol (96 per cent) R* and dilute to 25.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity
Dissolve 1.0 g in *ethanol (96 per cent) R* and dilute to 10 mL with the same solvent. Add 0.1 mL of *phenolphthalein solution R*. The solution is colourless. Not more than 0.5 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink.

Optical rotation (*2.2.7*)
−0.2° to + 0.2°, determined on solution S.

Related substances
Gas chromatography (*2.2.28*).

Test solution (a) Dissolve 0.20 g of the substance to be examined in *methylene chloride R* and dilute to 50.0 mL with the same solvent.

Test solution (b) Dilute 1.0 mL of test solution (a) to 10.0 mL with *methylene chloride R*.

Reference solution (a) Dissolve 40.0 mg of the substance to be examined and 40.0 mg of *isomenthol R* in *methylene chloride R* and dilute to 100.0 mL with the same solvent.

Reference solution (b) Dilute 0.10 mL of test solution (a) to 100.0 mL with *methylene chloride R*.

Reference solution (c) Dissolve 40.0 mg of *menthol CRS* in *methylene chloride R* and dilute to 100.0 mL with the same solvent.

Column:
— *material:* glass;
— *size:* *l* = 2.0 m, Ø = 2 mm;
— *stationary phase:* diatomaceous earth for gas chromatography R impregnated with 15 per cent *m/m* of *macrogol 1500 R.*

Carrier gas *nitrogen for chromatography R.*

Flow rate 30 mL/min.

Temperature:
— *column:* 120 °C;
— *injection port:* 150 °C;
— *detector:* 200 °C.

Detection Flame ionisation.

Injection 1 µL.

Run time Twice the retention time of menthol.

System suitability:
— *resolution:* minimum 1.4 between the peaks due to menthol and isomenthol in the chromatogram obtained with reference solution (a);
— *signal-to-noise ratio:* minimum 5 for the principal peak in the chromatogram obtained with reference solution (b).

Limits Test solution (a):
— *total:* not more than 1 per cent of the area of the principal peak;
— *disregard limit:* 0.05 per cent of the area of the principal peak.

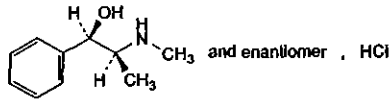
Residue on evaporation
Maximum 0.05 per cent.

Evaporate 2.00 g on a water-bath and heat in an oven at 100-105 °C for 1 h. The residue weighs not more than 1.0 mg.

Ph Eur

Racephedrine Hydrochloride

(Racemic Ephedrine Hydrochloride, Ph. Eur. monograph 0715)



$C_{10}H_{16}ClNO$ 201.7 *134-71-4*

Action and use
Adrenoceptor agonist.

Ph Eur

DEFINITION
Racemic ephedrine hydrochloride contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (1*RS*,2*SR*)-2-(methylamino)-1-phenylpropan-1-ol hydrochloride, calculated with reference to the dried substance.

CHARACTERS
A white or almost white, crystalline powder or colourless crystals, freely soluble in water, soluble in ethanol (96 per cent).

It melts at about 188 °C.

IDENTIFICATION
First identification: B, E.

Second identification: A, C, D, E.

A. Optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *racemic ephedrine hydrochloride CRS.* Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To 0.1 mL of solution S (see Tests) add 1 mL of *water R,* 0.2 mL of *copper sulfate solution R* and 1 mL of *strong sodium hydroxide solution R.* A violet colour is produced. Add 2 mL of *ether R* and shake. The ether layer is purple and the aqueous layer is blue.

E. To 5 mL of solution S add 5 mL of *water R.* The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S
Dissolve 5.00 g in *distilled water R* and dilute to 50.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity
To 10 mL of solution S add 0.1 mL of *methyl red solution R* and 0.1 mL of *0.01 M sodium hydroxide;* the solution is yellow. Add 0.2 mL of *0.01 M hydrochloric acid;* the solution is red.

Optical rotation (*2.2.7*)
+ 0.2° to −0.2°, determined on solution S.

Related substances
Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution (a) Dissolve 0.20 g of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Test solution (b) Dilute 1 mL of test solution (a) to 10 mL with *methanol R.*

Reference solution (a) Dissolve 20 mg of *racemic ephedrine hydrochloride CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dilute 1 mL of test solution (a) to 200 mL with *methanol R.*

Apply separately to the plate 10 µL of each solution. Develop over a path of 15 cm using a mixture of 5 volumes of *chloroform R,* 15 volumes of *concentrated ammonia R* and 80 volumes of *2-propanol R.* Allow the plate to dry in air. Spray with *ninhydrin solution R* and heat at 110 °C for 5 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). Disregard any spot of lighter colour than the background.

Sulfates (*2.4.13*)
15 mL of solution S complies with the limit test for sulfates (100 ppm).

Loss on drying (*2.2.32*)
Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.170 g in 30 mL of *ethanol (96 per cent) R*. Add 5.0 mL of *0.01 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the two points of inflexion.

1 mL of *0.1 M sodium hydroxide* corresponds to 20.17 mg of $C_{10}H_{16}ClNO$.

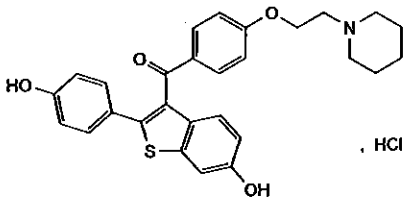
STORAGE

Store protected from light.

Ph Eur

Raloxifene Hydrochloride

(Ph. Eur. monograph 2375)



$C_{28}H_{28}ClNO_4S$ 510.0 *82640-04-8*

Action and use

Selective oestrogen receptor modulator.

Ph Eur

DEFINITION

[6-Hydroxy-2-(4-hydroxyphenyl)-1-benzothiophen-3-yl][4-[2-(piperidin-1-yl)ethoxy]phenyl]methanone hydrochloride.

Content

97.5 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

Almost white or pale-yellow powder.

Solubility

Very slightly soluble or practically insoluble in water and in acetone, slightly soluble in ethanol (96 per cent *V/V*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *raloxifene hydrochloride CRS*.

B. Dissolve 20 mg of the substance to be examined in 2 mL of *methanol R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R*, mobile phase A (30:70 *V/V*).

Test solution Dissolve 30 mg of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) In order to produce impurity C *in situ*, to 6 mg of the substance to be examined add 15 mL of *acetonitrile R*, 3 mL of *water R* and 5 mL of stabilised *strong hydrogen peroxide solution R*. Store at 30 °C for at least 6 h then dilute to 50.0 mL with mobile phase A. To 1.0 mL of this solution add 3 mg of the substance to be examined dissolved in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 3 mg of *raloxifene for peak identification CRS* (containing impurity A) in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *base-deactivated octylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 35 °C.

Mobile phase:

— *mobile phase A*: 9.0 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*;

— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 9	75	25
9 - 40	75 → 50	25 → 50

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 10 µL.

Identification of impurity A Use the chromatogram supplied with *raloxifene for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peak due to impurity A.

Relative retention With reference to raloxifene (retention time = about 18 min): impurity A = about 0.7; impurity C = about 1.2.

System suitability:

— *resolution*: minimum 3.0 between the peaks due to raloxifene and impurity C in the chromatogram obtained with reference solution (b);

— *symmetry factor*: maximum 1.8 for the principal peak in the chromatogram obtained with reference solution (a).

Limits:

— *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*).

Buffer solution pH 2.5 7.2 g/L Solution of *potassium dihydrogen phosphate R* adjusted to pH 2.5 with *phosphoric acid R*.

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *raloxifene hydrochloride CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution (b) In order to produce impurity C *in situ*, to 6 mg of the substance to be examined add 15 mL of *acetonitrile R*, 3 mL of *water R* and 5 mL of stabilised *strong hydrogen peroxide solution R*. Store at 30 °C for at least 6 h, then dilute to 50.0 mL with buffer solution pH 2.5.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *base-deactivated octylsilyl silica gel for chromatography R* (3.5 µm);
- *temperature*: 35 °C.

Mobile phase *acetonitrile R*, buffer solution pH 2.5 (33:67 *V/V*).

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 10 µL.

Run time Twice the retention time of raloxifene.

Relative retention With reference to raloxifene (retention time = about 3 min): impurity C = about 1.2.

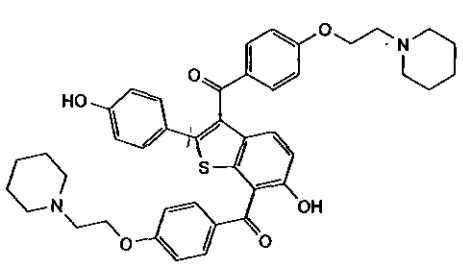
System suitability:
- *resolution*: minimum 2.0 between the peaks due to raloxifene and impurity C in the chromatogram obtained with reference solution (b); if necessary, adjust the concentration of acetonitrile in the mobile phase;
- *symmetry factor*: maximum 1.8 for the principal peak in the chromatogram obtained with reference solution (a).

Calculate the percentage content of $C_{28}H_{28}ClNO_4S$ from the declared content of *raloxifene hydrochloride CRS*.

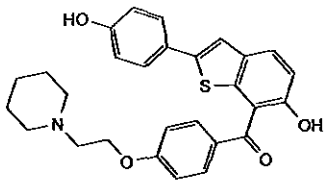
IMPURITIES

Specified impurities *A.*

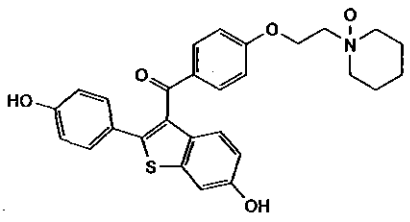
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C.*



A. [6-hydroxy-2-(4-hydroxyphenyl)-7-[4-[2-(piperidin-1-yl)ethoxy]benzoyl]-1-benzothiophen-3-yl][4-[2-(piperidin-1-yl)ethoxy]phenyl]methanone,



B. [6-hydroxy-2-(4-hydroxyphenyl)-1-benzothiophen-7-yl][4-[2-(piperidin-1-yl)ethoxy]phenyl]methanone,

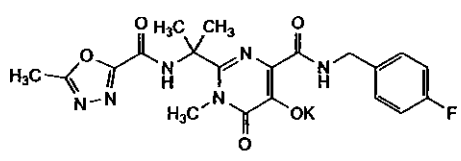


C. [6-hydroxy-2-(4-hydroxyphenyl)-1-benzothiophen-3-yl][4-[2-(piperidin-1-yl)ethoxy]phenyl]methanone *N*-oxide.

Ph Eur

Raltegravir Potassium

(Ph. Eur. monograph 2887)



$C_{20}H_{20}FKN_6O_5$ 482.5 *871038-72-1*

Action and use
Antiviral (HIV).

Preparations
Raltegravir Chewable Tablets
Raltegravir Tablets

Ph Eur

DEFINITION

Potassium 4-[[(4-fluorophenyl)methyl]carbamoyl]-1-methyl-2-[2-(5-methyl-1,3,4-oxadiazole-2-carboxamido)propan-2-yl]-6-oxo-1,6-dihydropyrimidin-5-olate.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Soluble in water, very slightly soluble in ethanol (96 per cent), practically insoluble in heptane.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *raltegravir potassium CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

B. It gives reaction (b) of potassium (*2.3.1*).

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R, water R* (25:75 *V/V*).

Test solution Dissolve 25.0 mg of the substance to be examined in 100 mL of the solvent mixture using sonication for 5 min. Add about 140 mL of the solvent mixture then dilute to 250.0 mL with the solvent mixture.

Reference solution (a) Dissolve 25.0 mg of *raltegravir potassium CRS* in 100 mL of the solvent mixture using sonication for 5 min. Add about 140 mL of the solvent mixture then dilute to 250.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 2 mg of *raltegravir impurity E CRS* in the solvent mixture and dilute to 20.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 50.0 mL with reference solution (a).

Reference solution (d) In order to prepare impurity C *in situ*, dissolve 20 mg of the substance to be examined in a 40 g/L solution of *sodium hydroxide R* and dilute to 10 mL with the same solvent. Stir the solution for 30 min. To 5 mL of the solution add 5 mL of a 103 g/L solution of *hydrochloric acid R* and dilute to 50 mL with the solvent mixture.

Reference solution (e) Dissolve 5 mg of *raltegravir for peak identification CRS* (containing impurities F and G) in 20 mL of the solvent mixture using sonication for 5 min. Add about 25 mL of the solvent mixture then dilute to 50 mL with the solvent mixture.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *phenylsilyl silica gel for chromatography R* (3.5 µm);
- *temperature*: 15 °C.

Mobile phase:
- *mobile phase A*: 0.1 per cent *V/V* solution of *phosphoric acid R*;
- *mobile phase B*: *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	75	25
2 - 5	75 → 60	25 → 40
5 - 10	60 → 55	40 → 45
10 - 19	55 → 10	45 → 90
19 - 22	10 → 5	90 → 95

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µL of the test solution and reference solutions (b), (c), (d) and (e).

Identification of impurities Use the chromatogram obtained with reference solution (d) to identify the peak due to impurity C; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity E; use the chromatogram supplied with *raltegravir for peak identification CRS* and the chromatogram obtained with reference solution (e) to identify the peaks due to impurities F and G.

Relative retention With reference to raltegravir (retention time = about 10 min): impurity C = about 0.7; impurity E = about 0.95; impurity G = about 1.1; impurity F = about 1.15.

System suitability Reference solution (c):
- *resolution*: minimum 1.5 between the peaks due to impurity E and raltegravir.

Calculation of percentage contents:
- *correction factor*: multiply the peak area of impurity C by 1.6;
- for each impurity, use the concentration of raltegravir potassium in reference solution (b).

Limits:
- *impurity C*: maximum 0.3 per cent;
- *impurities E, F, G*: for each impurity, maximum 0.15 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.5 per cent;
- *reporting threshold*: 0.05 per cent.

Water (*2.5.12*)

Maximum 0.6 per cent, determined on 0.500 g. Use as the solvent a mixture of equal volumes of *methanol R* and *formamide R*.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

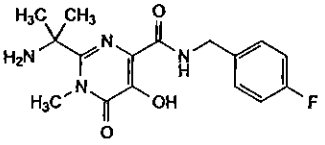
Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{20}H_{20}FKN_6O_5$ taking into account the assigned content of *raltegravir potassium CRS*.

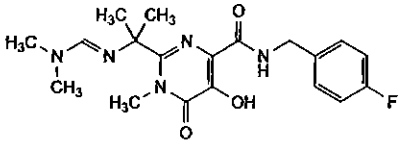
IMPURITIES

Specified impurities *C, E, F, G.*

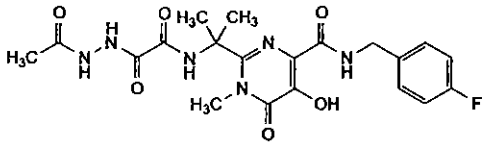
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, D, H.*



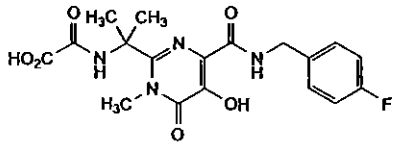
A. 2-(2-aminopropan-2-yl)-*N*-[(4-fluorophenyl)methyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide,



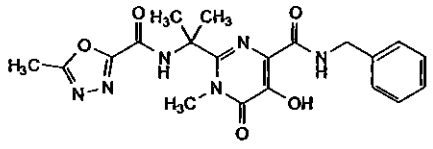
B. 2-[2-[(*E*)-[(dimethylamino)methylidene]amino]propan-2-yl]-*N*-[(4-fluorophenyl)methyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide,



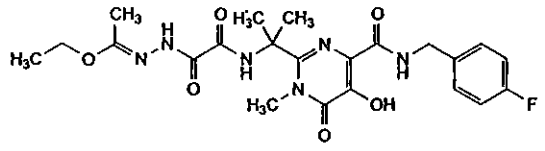
C. 2-[2-[2-(2-acetylhydrazin-1-yl)-2-oxoacetamido]propan-2-yl]-*N*-[(4-fluorophenyl)methyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide,



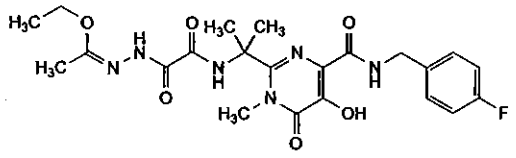
D. {[2-[4-[[(4-fluorophenyl)methyl]carbamoyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl]propan-2-yl]amino}oxoacetic acid,



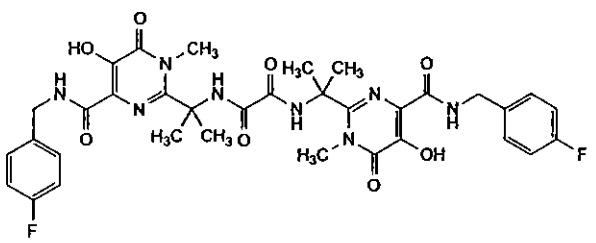
E. *N*-benzyl-5-hydroxy-1-methyl-2-[2-(5-methyl-1,3,4-oxadiazole-2-carboxamido)propan-2-yl]-6-oxo-1,6-dihydropyrimidine-4-carboxamide,



F. ethyl (1*E*)-*N*-[[2-[4-[[(4-fluorophenyl)methyl]carbamoyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl]propan-2-yl]oxamoyl]ethanehydrazonate,



G. ethyl (1*Z*)-*N*-[[2-[4-[[(4-fluorophenyl)methyl]carbamoyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl]propan-2-yl]oxamoyl]ethanehydrazonate,

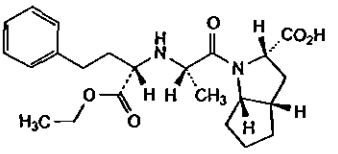


H. N^1,N^2-bis[2-[4-[[(4-fluorophenyl)methyl]carbamoyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl]propan-2-yl]oxamide.

Ph Eur

Ramipril

(Ph. Eur. monograph 1368)



$C_{23}H_{32}N_2O_5$ 416.5 *87333-19-5*

Action and use
Angiotensin converting enzyme inhibitor.

Preparations
Ramipril Capsules
Ramipril Tablets

Ph Eur

DEFINITION

(2*S*,3a*S*,6a*S*)-1-[(2*S*)-2-[[(2*S*)-1-Ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid.

Content
98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Sparingly soluble in water, freely soluble in methanol.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *ramipril CRS.*

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 0.1 g in *methanol R* and dilute to 10.0 mL with the same solvent.

Specific optical rotation (*2.2.7*)
+ 32.0 to + 38.0 (dried substance).

Dissolve 0.250 g in a mixture of 14 volumes of *hydrochloric acid R1* and 86 volumes of *methanol R* and dilute to 25.0 mL with the same mixture of solvents.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 20 mg of the substance to be examined in mobile phase A and dilute to 20.0 mL with mobile phase A.

Reference solution (a) Dissolve 2 mg of *ramipril impurity A CRS*, 2 mg of *ramipril impurity B CRS*, 2 mg of *ramipril impurity C CRS* and 2 mg of *ramipril impurity D CRS* in mobile phase A and dilute to 25 mL with mobile phase A. To 1 mL of this solution, add 5 mL of the test solution and dilute to 10 mL with mobile phase B.

Reference solution (b) Dilute 5.0 mL of the test solution to 100.0 mL with mobile phase B. Dilute 5.0 mL of this solution to 50.0 mL with mobile phase B.

Reference solution (c) Dilute 1.0 mL of reference solution (b) to 10.0 mL with mobile phase B.

Column:
— *size*: *l* = 0.25 m, Ø = 4.0 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm);
— *temperature*: 65 °C.

Mobile phase:
— *mobile phase A*: dissolve 2.0 g of *sodium perchlorate R* in a mixture of 0.5 mL of *triethylamine R* and 800 mL of *water for chromatography R*; adjust to pH 3.6 with *phosphoric acid R* and add 200 mL of *acetonitrile R1*;
— *mobile phase B*: dissolve 2.0 g of *sodium perchlorate R* in a mixture of 0.5 mL of *triethylamine R* and 300 mL of *water for chromatography R*; adjust to pH 2.6 with *phosphoric acid R* and add 700 mL of *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 6	90	10
6 - 7	90 → 75	10 → 25
7 - 20	75 → 65	25 → 35
20 - 30	65 → 25	35 → 75
30 - 50	25	75

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 210 nm.

Equilibration With the mobile phase at the initial composition for at least 35 min; if a suitable baseline cannot be obtained, use another grade of triethylamine.

Injection 10 µL.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C and D.

Relative retention With reference to ramipril (retention time = about 18 min): impurity A = about 0.8; impurity B = about 1.3; impurity C = about 1.5; impurity D = about 1.7.

System suitability:
— *resolution*: minimum 3.0 between the peaks due to impurity A and ramipril in the chromatogram obtained with reference solution (a);
— *signal-to-noise ratio*: minimum 3 for the principal peak in the chromatogram obtained with reference solution (c);
— *symmetry factor*: 0.8 to 2.0 for the peak due to ramipril in the chromatogram obtained with the test solution.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity C by 2.4;
— *impurities A, B, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
— *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.2 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C at a pressure not exceeding 0.1 kPa for 4 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 25 mL of *methanol R* and add 25 mL of *water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 mL of *0.1 M sodium hydroxide* is equivalent to 41.65 mg of $C_{23}H_{32}N_2O_5$.

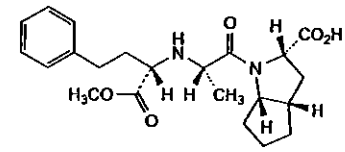
STORAGE

Protected from light.

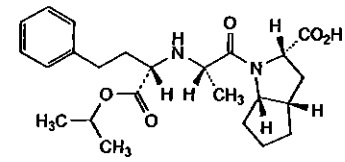
IMPURITIES

Specified impurities *A, B, C, D.*

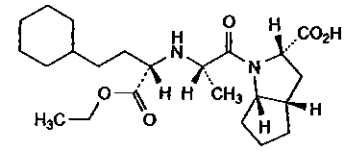
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *E, F, G, H, I, J, K, L, M, N, O.*



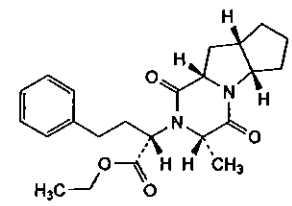
A. (2*S*,3a*S*,6a*S*)-1-[(2*S*)-2-[[(2*S*)-1-methoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid (ramipril methyl ester),



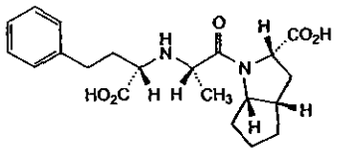
B. (2*S*,3a*S*,6a*S*)-1-[(2*S*)-2-[[(2*S*)-1-oxo-4-phenyl-1-[(propan2-yl)oxy]butan-2-yl]amino]propanoyl] octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid (ramipril isopropyl ester),



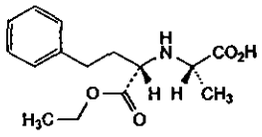
C. (2*S*,3a*S*,6a*S*)-1-[(2*S*)-2-[[(2*S*)-4-cyclohexyl-1-ethoxy-1oxobutan-2-yl]amino]propanoyl]octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid (hexahydroramipril),



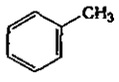
D. ethyl (2*S*)-2-[(3*S*,5a*S*,8a*S*,9a*S*)-3-methyl-1,4-dioxodecahydro-2*H*-cyclopenta[4,5]pyrrolo[1,2-*a*]pyrazin-2-yl]-4-phenylbutanoate (ramipril diketopiperazine),



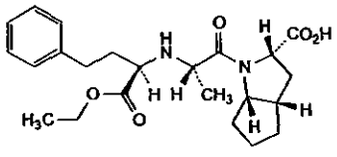
E. (2*S*,3a*S*,6a*S*)-1-[(2*S*)-2-[[(1*S*)-1-carboxy-3-phenylpropyl]amino]propanoyl]octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid (ramiprilat),



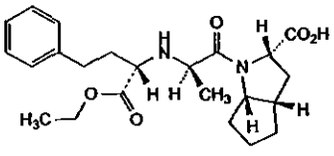
F. (2*S*)-2-[[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoic acid,



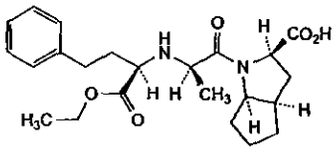
G. methylbenzene (toluene),



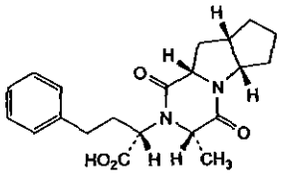
H. (2*S*,3a*S*,6a*S*)-1-[(2*S*)-2-[[(2*R*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid ((*R*,*S*,*S*,*S*,*S*)-epimer of ramipril),



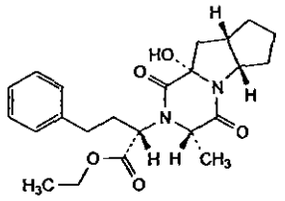
I. (2*S*,3a*S*,6a*S*)-1-[(2*R*)-2-[[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid ((*S*,*R*,*S*,*S*,*S*)-epimer of ramipril),



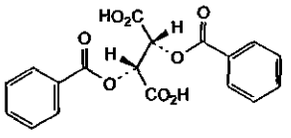
J. (2*R*,3a*R*,6a*R*)-1-[(2*R*)-2-[[(2*R*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid (enantiomer of ramipril),



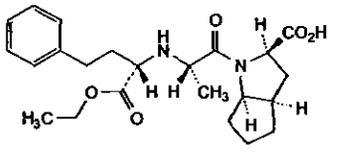
K. (2*S*)-2-[(3*S*,5a*S*,8a*S*,9a*S*)-3-methyl-1,4-dioxodecahydro-2*H*-cyclopenta[4,5]pyrrolo[1,2-*a*]pyrazin-2-yl]-4-phenylbutanoic acid (ramiprilate diketopiperazine),



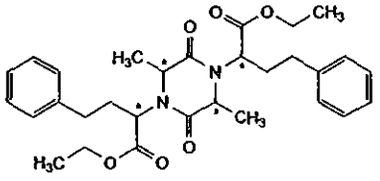
L. ethyl (2*S*)-2-[(3*S*,5a*S*,8a*S*,9a*S*)-9a-hydroxy-3-methyl-1,4-dioxodecahydro-2*H*-cyclopenta[4,5]pyrrolo[1,2-*a*]pyrazin-2-yl]-4-phenylbutanoate (ramipril hydroxydiketopiperazine),



M. (2*R*,3*R*)-2,3-bis(benzoyloxy)butanedioic acid (dibenzoyl tartric acid),



N. (2*R*,3a*R*,6a*R*)-1-[(2*S*)-2-[[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid ((*S*,*S*,*R*,*R*,*R*)-isomer of ramipril),

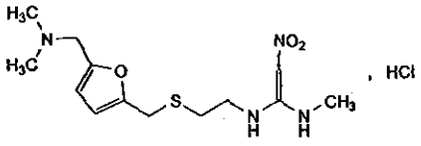


O. diethyl (2*E*,2′*E*)-2,2′-[(2*E*,5*E*)-2,5-dimethyl-3,6-dioxopiperazine-1,4-diyl]bis(4-phenylbutanoate).

Ph Eur

Ranitidine Hydrochloride

(Ph. Eur. monograph 0946)



$C_{13}H_{23}ClN_4O_3S$ 350.9 *66357-59-3*

Action and use
Histamine H_2 receptor antagonist; treatment of peptic ulcer disease.

Preparations
Ranitidine Injection
Ranitidine Oral Solution
Ranitidine Tablets
Ranitidine Effervescent Tablets

Ph Eur

DEFINITION

N-[2-[[[5-[(Dimethylamino)methyl]furan-2-yl]methyl]sulfanyl]ethyl]-*N'*-methyl-2-nitroeth-1-ene-1,1-diamine hydrochloride.

Content

98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance

White or pale yellow, crystalline powder, hygroscopic.

Solubility

Freely soluble in water, sparingly soluble or slightly soluble in anhydrous ethanol, very slightly soluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *ranitidine hydrochloride CRS.*

If the spectra obtained in the solid state show differences, dissolve 10 mg of the substance to be examined and 10 mg of the reference substance separately in 0.5 mL of *methanol R* in an agate mortar. Evaporate to dryness under a stream of *nitrogen R*. Dry the residues under vacuum for 30 min. Add 3 drops of *liquid paraffin R* to the residues and triturate until the mull shows a milky appearance. Compress the mulls between 2 plates transparent to infrared radiation and record new spectra.

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 100.0 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

pH (*2.2.3*)

4.5 to 6.0 for solution S.

Related substances

Liquid chromatography (*2.2.29*).

Buffer solution Dissolve 6.8 g of *potassium dihydrogen phosphate R* in 950 mL of *water for chromatography R*. Adjust to pH 7.1 with *strong sodium hydroxide solution R* and dilute to 1000 mL with *water for chromatography R*.

Test solution Dissolve 13 mg of the substance to be examined in mobile phase A and dilute to 100.0 mL with mobile phase A.

Reference solution (a) Dissolve 6.5 mg of *ranitidine for impurity A identification CRS* in mobile phase A and dilute to 50 mL with mobile phase A.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

Reference solution (c) Dissolve the contents of a vial of *ranitidine impurity J CRS* in 1 mL of the test solution.

Blank solution Mobile phase A.

Column:
- *size*: *l* = 0.10 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl amorphous organosilica polymer for chromatography R* (3.5 µm);
- *temperature*: 35 °C.

Mobile phase:
- *mobile phase A*: *acetonitrile for chromatography R*, buffer solution (2:98 *V/V*);
- *mobile phase B*: *acetonitrile for chromatography R*, buffer solution (22:78 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	100 → 0	0 → 100
10 - 15	0	100

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 10 µL.

Identification of impurities Use the chromatogram supplied with *ranitidine for impurity A identification CRS* and the chromatogram obtained with reference solution (a) to identify the peak due to impurity A; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity J.

Relative retention With reference to ranitidine (retention time = about 7 min): impurity J = about 0.9; impurity A = about 1.7.

System suitability:
- *resolution*: minimum 1.5 between the peaks due to impurity J and ranitidine in the chromatogram obtained with reference solution (c);
- the chromatogram obtained with the blank solution does not show any peak with the same relative retention as the peak due to impurity A in the chromatogram obtained with reference solution (a).

Calculation of percentage contents:
- *correction factor*: multiply the peak area of impurity J by 2.0;
- for each impurity, use the concentration of ranitidine hydrochloride in reference solution (b).

Limits:
- *impurity A*: maximum 0.3 per cent;
- *impurity J*: maximum 0.15 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.5 per cent;
- *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)

Maximum 0.75 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C at a pressure not exceeding 0.1 kPa.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.280 g in 35 mL of *water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M sodium hydroxide* is equivalent to 35.09 mg of $C_{13}H_{23}ClN_4O_3S$.

STORAGE

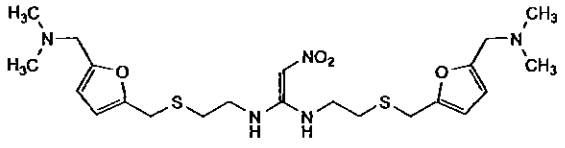
In airtight container, protected from light.

IMPURITIES

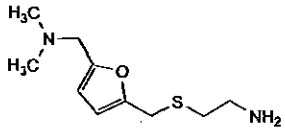
Specified impurities *A, J.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general

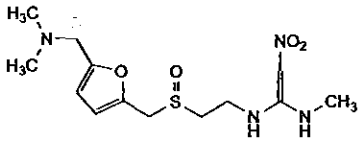
monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) B, C, D, E, F, G, H, I, K.



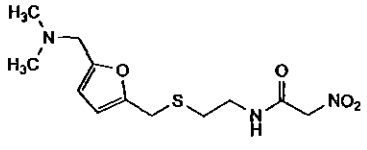
A. *N*,*N*′-bis[2-[[[5-[(dimethylamino)methyl]furan-2-yl]methyl]sulfanyl]ethyl]-2-nitroeth-1-ene-1,1-diamine,



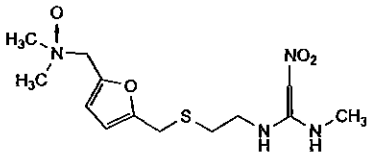
B. 2-[[[5-[(dimethylamino)methyl]furan-2-yl]methyl]sulfanyl]ethan-1-amine,



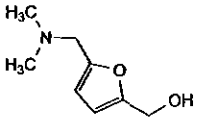
C. *N*-[2-[[[5-[(dimethylamino)methyl]furan-2-yl]methyl]sulfinyl]ethyl]-*N*′-methyl-2-nitroeth-1-ene-1,1-diamine,



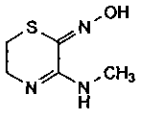
D. *N*-[2-[[[5-[(dimethylamino)methyl]furan-2-yl]methyl]sulfanyl]ethyl]-2-nitroacetamide,



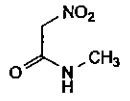
E. [5-[[[2-[[1-(methylamino)-2-nitroeth-1-en-1-yl]amino]ethyl]sulfanyl]methyl]furan-2-yl]-*N*,*N*-dimethylmethanamine *N*-oxide,



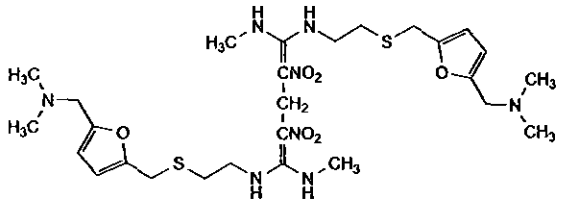
F. [5-[(dimethylamino)methyl]furan-2-yl]methanol,



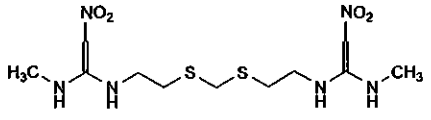
G. [3-(methylamino)-5,6-dihydro-2*H*-1,4-thiazin-2-ylidene]hydroxylamine,



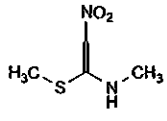
H. *N*-methyl-2-nitroacetamide,



I. 2,2′-methylenebis[*N*-[2-[[[5-[(dimethylamino)methyl]furan-2-yl]methyl]sulfanyl]ethyl]-*N*′-methyl-2-nitroeth-1-ene-1,1-diamine],



J. *N*,*N*′′-[methylenebis(sulfanediylethan-2,1-diyl)]bis(*N*′-methyl-2-nitroeth-1-ene-1,1-diamine),



K. *N*-methyl-1-(methylsulfanyl)-2-nitroeth-1-en-1-amine.

Ph Eur

Refined Rapeseed Oil

(Ph. Eur. monograph 1369)

Ph Eur

DEFINITION

Fatty oil obtained from the seeds of *Brassica napus* L. and *Brassica campestris* L. by mechanical expression or by extraction. It is then refined. A suitable antioxidant may be added.

CHARACTERS

Appearance
Clear, light yellow liquid.

Solubility
Practically insoluble in water and in ethanol (96 per cent), miscible with light petroleum (bp: 40-60 °C).

Relative density
About 0.917.

Refractive index
About 1.473.

IDENTIFICATION

Identification of fatty oils by thin-layer chromatography (*2.3.2*).

Results The chromatogram obtained is similar to the corresponding chromatogram shown in Figure 2.3.2.-1.

TESTS

Acid value (*2.5.1*)
Maximum 0.5, determined on 10.0 g.

Peroxide value (*2.5.5, Method A*)
Maximum 10.0.

Unsaponifiable matter (*2.5.7*)
Maximum 1.5 per cent, determined on 5.0 g.

Alkaline impurities (*2.4.19*)
It complies with the test.

Composition of fatty acids (*2.4.22, Method A*)
Use the mixture of calibrating substances in Table 2.4.22.-3.

Composition of the fatty-acid fraction of the oil:
— *palmitic acid*: 2.5 per cent to 6.0 per cent,
— *stearic acid*: maximum 3.0 per cent,
— *oleic acid*: 50.0 per cent to 67.0 per cent,
— *linoleic acid*: 16.0 per cent to 30.0 per cent,
— *linolenic acid*: 6.0 per cent to 14.0 per cent,
— *eicosenoic acid*: maximum 5.0 per cent,
— *erucic acid*: maximum 2.0 per cent.

Water (*2.5.32*)
Maximum 0.1 per cent, determined on 1.00 g.

STORAGE
In an airtight, well-filled container, protected from light.

LABELLING
The label states whether the oil is obtained by mechanical expression or by extraction.

Ph Eur

Products of Recombinant DNA Technology

(Ph. Eur. monograph 0784)

Ph Eur

This monograph provides general requirements for the manufacture and control of finished products derived from recombinant DNA (rDNA) technology, and includes requirements for the active substance in these products. Active substances produced by rDNA technology are expected to meet the requirements given in the corresponding section of the present monograph. These requirements are not necessarily comprehensive in a given case and complementary or additional requirements may be prescribed in an individual monograph or imposed by the competent authority.

The monograph is also applicable to vaccine antigens produced by rDNA technology, with more detailed requirements provided in the general monograph Vaccines for human use (0153) and in individual vaccine monographs.

Certain aspects of the monograph may apply to products produced in transgenic animals and plants.

The monograph is not applicable to recombinant organisms that are intended to be used directly in man and animals, for example as live recombinant vectors or vaccines.

DEFINITION

Products of rDNA technology are produced by genetic modification in which DNA coding for the required product is introduced, usually by means of a plasmid or a viral vector, into suitable micro-organisms such as bacteria and yeast, or a suitable cell line of mammalian (including human), insect or plant origin. The DNA introduced can then be expressed as a protein.

The desired product is then recovered by extraction and purification. The cell or micro-organism used to harbour the vector is referred to as the host cell, and the stable association of the two used in the manufacturing process is referred to as the host-vector system.

Products of rDNA technology can also undergo intentional modifications such as pegylation or conjugation.

PRODUCTION

GENERAL PROVISIONS

Production of rDNA products is based on a validated seed-lot system using a host-vector combination that has been shown suitable.

The seed-lot system typically uses a master cell bank and a working cell bank. The host cell, the vector, the host-vector system, the master cell bank and the working cell bank, as well as their establishment, maintenance and cultivation are to be described in detail.

Where products of rDNA technology are manufactured using materials of human or animal origin, the requirements of general chapter *5.1.7. Viral safety* apply.

The production process is validated for the following aspects:
— process consistency, including cell-culture/fermentation, purification and any subsequent intentional modification of the active substance where applicable;
— removal or inactivation of extraneous agents;
— removal of product- and process-related impurities (e.g. unwanted variants, host-cell proteins and DNA, antibiotics, cell-culture components);
— removal of pyrogenic substances where applicable.

A batch shown to be stable and representative of batches tested clinically is used as a reference preparation for identification, tests and assay. The reference preparation is appropriately characterised.

EXPRESSION VECTOR AND HOST CELL

The starting materials to be used for the production of the rDNA product, including the expression vector, the host-cell and the pool of transformed or transfected cells from which the initial clone will be derived, are documented.

Information on the source and history of the host cell and of the expression vector is recorded. The gene coding for the protein of interest as well as other functional sequences of the vector should be characterised (e.g. nucleotide sequence of the gene of interest and its flanking regions, restriction map).

The suitability of the host-vector system, particularly with regard to microbiological purity, is demonstrated by:
— phenotypic and genotypic characterisation of the host cell;
— characterisation of the expression vector within the host cell (e.g. integration, copy number, nucleotide sequence);
— documentation on the raw materials, the transformation/transfection of the host cell by the expression vector and the strategy for deriving the clone used to establish the cell bank system.

CELL BANKS

The master cell bank (MCB) is a homogeneous suspension of the original cells already transformed or transfected by the expression vector containing the desired gene, distributed as equal volumes in a single operation into individual containers for storage (e.g. in liquid nitrogen). In some cases, it may be necessary to establish separate master cell banks for the expression vector and the host cells.

The working cell bank (WCB) is a homogeneous suspension of cells derived from the MCB(s) at a finite passage level, distributed as equal volumes in a single operation into individual containers for storage (e.g. in liquid nitrogen).

For each type of cell bank, all containers are treated identically during storage and once removed from storage, are not returned to the cell stock.

The characterisation and testing of eukaryotic and prokaryotic cell banks are critical components of the control of rDNA-derived biological products. Cell banks are tested to confirm identity, purity and suitability of the cell substrate for the intended manufacturing use. The strategy for testing the cell banks may vary according to the nature and biological properties of the cells (e.g. growth properties) and their cultivation history (e.g. use of animal-derived raw material). Molecular methods are used to analyse the expression vector for copy number, insertions or deletions, and the number of integration sites. The nucleotide sequences of the coding region, flanking regions and promoters are shown to be identical to those determined for the expression vector. The coding region corresponds to that expected for the protein sequence. If any differences in nucleic acid sequences are identified, these must be clearly defined and the expression vector shown to be stable and capable of expressing the expected product consistently.

Cell banks must be characterised and tested at different stages including the MCB, the WCB and cells at or beyond the maximum population doubling level used for production.

For prokaryotic and yeast cell banks, characterisation and testing includes molecular identity of the gene being expressed, identity and purity of the cells including strain identification (e.g. by biochemical, genetic, or proteomic methods), phenotypic and genotypic strain characterisation, viability, presence of the plasmid (e.g. sequence, copy number, restriction map, percentage of cells retaining the plasmid), microbiological purity and, if appropriate, a test for bacteriophage. Additional tests may be relevant and shall be addressed on a case-by-case basis.

For animal cell banks, characterisation and testing includes morphology, identity, viability, genetic stability of the cells (e.g. copy number, integrity of the expression cassette) and testing for extraneous agents. Animal cell substrates have the capacity to propagate extraneous agents such as mycoplasma and viruses. In addition, animal cells may contain endogenous agents such as endogenous retroviruses. Consequently, a testing strategy with regard to extraneous agents should be developed based on a risk assessment taking into account the nature and the history of the cell line.

CULTURE MEDIA AND OTHER RAW MATERIALS

The quality of media and other raw materials used in the production of rDNA proteins is controlled, with consideration given to the impact of the raw material on the quality, safety and efficacy of the final medicinal product. In particular, the origin of the materials must be known and their traceability documented.

CULTURE AND HARVEST

The cell bank (e.g. 1 or more vials(s) of the WCB) is used to start the culture process. In-process controls (e.g. for population doubling levels, cell concentration, volumes, pH, cultivation times, temperature, microbial tests) are established to ensure suitable performance and consistency with regard to the culture process.

Criteria for harvesting and terminating the production must be defined by the manufacturer, and take into account the limit for *in vitro* cell age (e.g. limited number of passages or population doublings) for which the cell substrate has been demonstrated as stable and capable of producing the desired product/intermediate. Each harvest is tested to ensure absence of contamination.

When prokaryotic or other microbial cells are used to manufacture the product, absence of contamination of the harvest is demonstrated primarily by establishing microbial purity. When animal cells are used, the absence of extraneous agents is verified using appropriate *in vitro* cell culture methods and/or molecular methods.

PURIFICATION

The extraction and purification procedure must be shown to yield the desired intermediates at a suitable purity. In this respect, an analysis of the step(s) taken to control the product-related impurities, and to remove or inactivate process-related impurities or contaminants is conducted.

In-process controls (e.g. for yield, volume, pH, processing time, temperature, elution profile and fraction selection, microbial tests) are established to ensure suitable performance and consistency with regard to the purification process.

Holding times and storage conditions of intermediates are to be defined where appropriate.

CHARACTERISATION

During development studies, the active substance is characterised extensively in order to determine its structure, physico-chemical properties, biological activity, immunochemical properties and purity.

Characterisation is necessary to identify the quality attributes that may be important for the safety and efficacy of the product. It provides the basis for release specifications, stability evaluation and for any testing that may have to be performed in support of process changes.

Structure

The characterisation should determine, as far as possible, the primary and higher-order structure of the protein, any post-translational modifications such as glycosylation, and any other intentional modification.

The amino-acid sequence is deduced from the DNA sequence of the expression vector and confirmed by analysis of the produced protein. The amino-acid sequence and the disulfide bridges of the substance are determined using a combination of techniques such as peptide mapping (*2.2.55*) and mass spectrometry (*2.2.43*).

The analysis of pegylated proteins should include, but not be limited to, the location of modification and the degree of site occupancy.

Glycosylation is characterised, and covers overall monosaccharide composition (neutral sugars, amino sugars and sialic acids), glycosylation site(s) and type (e.g. N- or O-linked), degree of site occupancy, and oligosaccharide structures of glycan chains (extensions, branching and linkage) using, for example, the principles and techniques described in general chapter *2.2.59. Glycan analysis of glycoproteins.*

Due to their possible immunogenicity, particular attention is paid to glycan structures that are not known to be present in natural human proteins.

The higher-order structure of the substance is examined using physico-chemical methods such as circular dichroism spectroscopy, Fourier transform infrared spectroscopy, fluorescence, differential scanning calorimetry, proton nuclear magnetic resonance spectrometry and/or hydrogen-deuterium exchange mass spectrometry.

Biological assays based on functional activity may also serve as additional confirmation of the higher-order structure.

Biological activity

The biological activity (i.e. the specific ability of a product to achieve a defined biological effect) is assessed by biological,

biochemical (including immunochemical assays) or physico-chemical assays, as appropriate.

The mechanism of action is investigated and preferably reflected in the potency assay.

Immunochemical properties

Where relevant for the mechanism of action, e.g. in the case of antibodies, immunochemical properties (such as Fc-effector functions) are characterised extensively.

Product-related substances and impurities

Products of rDNA technology commonly display several sources of heterogeneity (e.g. N- or C-terminal processing, N-terminal pyroglutamation, deamidation, oxidation, isomerisation, fragmentation, disulfide bond mismatch, N-linked and O-linked glycosylation, glycation, aggregation), which leads to a complex product profile consisting of several molecular entities or variants. When the activity, safety and efficacy of these variants are comparable to those of the desired product, the variants are regarded as product-related substances. Other variants are considered as product-related impurities.

The methods used to assess product-related substances and impurities should be capable of detecting structural variants with different physico-chemical properties, e.g. charge, size and hydrophobicity. Typically, a combination of orthogonal methods is applied, e.g. chromatographic, electrophoretic and spectroscopic techniques.

Characterisation of charged variants, such as differentially sialylated or deamidated variants, is performed using appropriate methods (e.g. capillary electrophoresis, isoelectric focusing, ion-exchange chromatography), which may be coupled to other techniques such as mass spectrometry.

High-molecular-mass forms such as dimers and higher oligomers can be resolved and quantified by size-based separation procedures (e.g. size-exclusion chromatography, field flow fractionation, analytical ultracentrifugation) coupled with suitable detection methods (e.g. ultraviolet, fluorescence, light scattering).

Process-related impurities and contaminants

Process-related impurities derived from the upstream process may include host-cell proteins, host-cell DNA, or other media components (e.g. inducers, antibiotics, serum). They are to be evaluated qualitatively and quantitatively.

Host-cell proteins are investigated using a sensitive assay capable of detecting a wide range of protein impurities, and taking into account the recommendations given in general chapter *2.6.34. Host-cell protein assays.*

Residual host-cell DNA is determined using a suitably sensitive assay.

Process-related impurities derived from the downstream process may include enzymes, processing reagents (e.g. guanidine, dyes, oxidising and reducing agents), salts (e.g. heavy metals, non-metallic ions), solvents, carriers, ligands (e.g. protein A) and other leachables.

Contaminants include all adventitiously introduced materials not intended to be part of the manufacturing process (e.g. micro-organisms, microbial proteases, bacterial endotoxins).

Process-related impurities and contaminants are controlled using appropriate strategies based on the principles of risk management.

Content

Content is determined by use of an appropriate physico-chemical or immunochemical assay. The total protein content (expressed in mass units) can be determined as described in general chapter *2.5.33. Total protein.* The protein content is determined using several methods, including absolute method(s).

CONTROL STRATEGY

Based on the understanding of the product and process, a planned set of controls that ensures process performance and product quality (i.e. a control strategy) is established. This control strategy includes the control of process parameters, in-process controls, control of raw materials, intermediates, active substance and finished product, as well as the methods used and the frequency of control.

The control strategy should ensure that the quality attributes relevant to the safety and efficacy of the product are within the appropriate range, limit or distribution to ensure the desired product quality.

The selection of tests and the stages at which they are performed are based on knowledge of the manufacturing process and extensive characterisation of the active substance and finished product. Some characterisation tests may be selected to become part of the specifications.
The specifications for the active substance and finished product are only 1 part of the overall control strategy.

ACTIVE SUBSTANCE

The active substance is tested for appearance, identity, microbial quality and bacterial endotoxins, product-related substances, product- and process-related impurities, structural integrity, protein content and biological activity, with comparison to suitable reference standards where appropriate.

When the active substance is a conjugated or chemically modified protein, e.g. a pegylated protein, appropriate tests must be performed on both the modified and unmodified protein. Tests for product-related variants (e.g. proportion of modified and unmodified protein) and process-related impurities derived from the modification procedure (e.g. by-products of the modification reaction, reagents) are carried out and acceptance criteria should be established.

FINISHED PRODUCT

One or more batches of active substance may be combined to obtain the finished product. Suitable stabilisers and excipients may be added.

The finished product complies with the relevant dosage form monographs, with the relevant individual monograph as appropriate, and with the following considerations.

IDENTIFICATION

The identity test(s) must be specific and must be based on unique aspects of the product's molecular structure or other specific properties such as the size of the molecule, its primary sequence, its isoelectric profile, its chromatographic properties and its functional conformation, with comparison of the product to a suitable reference standard where appropriate. Methods used in the determination of potency or purity may also contribute to the identification.

TESTS

Methods capable of determining process-related impurities and product-related substances and impurities (e.g. resulting from product truncation, fragmentation, aggregation, oxidation, deamidation) are applied and acceptance criteria established, as appropriate. Where impurities are controlled at a suitable earlier stage, the tests may be omitted at the finished product stage.

Tests applied to modified proteins
Suitable tests are carried out depending on the type of modification and the product complies with the approved limits.

ASSAY

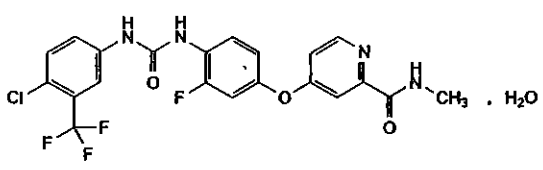
Content
It complies with the limits approved for the particular product. It is usually based on protein content and expressed in mass units. The procedures described in general chapter *2.5.33. Total protein* may be applied. When appropriate, other methods using a suitable reference standard such as liquid chromatography (*2.2.29*) may also be used. For modified proteins, the content refers to the protein part of the molecule.

Potency
A potency assay is established using a suitable reference standard and carried out against this reference standard. General chapter *5.3. Statistical analysis of results of biological assays and tests* may be used to design the assay and calculate the results.

Ph Eur

Regorafenib Monohydrate

(Ph. Eur. monograph 3012)



$C_{21}H_{15}ClF_4N_4O_3,H_2O$ 500.8 *1019206-88-2*

Action and use
Tyrosine kinase inhibitor; treatment of metastatic colorectal cancer and unresectable or metastatic gastrointestinal stromal tumours.

Preparation
Regorafenib Tablets

Ph Eur

DEFINITION
4-[4-[[[4-Chloro-3-(trifluoromethyl)phenyl] carbamoyl]amino]-3-fluorophenoxy]-*N*-methylpyridine-2-carboxamide monohydrate.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white or pinkish or brownish powder.

Solubility
Practically insoluble in water, sparingly soluble in acetone, slightly soluble in ethanol (96 per cent) and in methanol, practically insoluble in heptane.

It shows polymorphism (*5.9*).

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *regorafenib monohydrate CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *acetone R*, evaporate to dryness and record new spectra using the residues.

B. Water (see Tests).

TESTS

Impurity A
Liquid chromatography (*2.2.29*). *Carry out the test protected from light and store the solutions at 2-8 °C.*

Test solution Dissolve 0.250 g of the substance to be examined in *tetrahydrofuran R* and dilute to 5.0 mL with the same solvent.

Reference solution Dissolve 5.0 mg of *regorafenib impurity A CRS* in *tetrahydrofuran R* and dilute to 50.0 mL with the same solvent. Dilute 2.5 mL of the solution to 50.0 mL with *tetrahydrofuran R.*

Column:
— *size*: *l* = 0.15 m, Ø = 3.0 mm;
— *stationary phase*: *end-capped ethylene-bridged polar-embedded octadecylsilyl silica gel for chromatography (hybrid material) R* (3.5 µm);
— *temperature*: 50 °C.

Mobile phase:
— *mobile phase A*: mix 8 volumes of *acetonitrile for chromatography R* and 92 volumes of a solution containing 0.5 g/L of *dipotassium hydrogen phosphate R* and 1.5 g/L of *potassium dihydrogen phosphate R*;
— *mobile phase B*: *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	100	0
2 - 17	100 → 22	0 → 78

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 228 nm.

Autosampler Set at 8 °C.

Injection 3.0 µL.

Relative retention With reference to regorafenib (retention time = about 14 min): impurity A = about 0.6.

System suitability Reference solution:
— *repeatability*: maximum relative standard deviation of 10.0 per cent determined on 6 injections.

Calculation of content:
— for impurity A, use the concentration of impurity A in the reference solution.

Limit:
— *impurity A*: maximum 100 ppm.

Related substances
Liquid chromatography (*2.2.29*). *Carry out the test protected from light and store the solutions at 2-8 °C.*

Test solution (a) Dissolve 30.0 mg of the substance to be examined in *methanol R* and dilute to 20.0 mL with the same solvent.

Test solution (b) Dilute 15.0 mL of test solution (a) to 50.0 mL with *methanol R.*

Reference solution (a) Dissolve 30.0 mg of *regorafenib monohydrate CRS* in *methanol R* and dilute to 20.0 mL with the same solvent. Dilute 15.0 mL of the solution to 50.0 mL with *methanol R.*

Reference solution (b) Dilute 1.0 mL of test solution (a) to 100.0 mL with *methanol R.* Dilute 1.0 mL of this solution to 10.0 mL with *methanol R.*

Reference solution (c) Dissolve 2 mg of *sorafenib CRS* in *methanol R* and dilute to 50 mL with the same solvent. Dilute 0.2 mL of the solution to 2 mL with test solution (a).

Reference solution (d) Dissolve 3 mg of *regorafenib impurity C CRS* in *methanol R* and dilute to 100 mL with the same solvent.

Reference solution (e) Dissolve 3 mg of *regorafenib impurity D CRS* in *dimethyl sulfoxide R* and dilute to 100 mL with the same solvent.

Reference solution (f) Mix 1 mL of reference solution (d) and 1 mL of reference solution (e) and dilute to 20 mL with *methanol R*.

Column:
— *size*: *l* = 0.15 m, Ø = 2.1 mm;
— *stationary phase*: *end-capped ethylene-bridged phenylsilyl silica gel for chromatography (hybrid material) R* (3.5 µm);
— *temperature*: 63 °C.

Mobile phase:
— *mobile phase A*: *acetonitrile for chromatography R*, 0.1 per cent *V/V* solution of *trifluoroacetic acid R* (3:97 *V/V*);
— *mobile phase B*: *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	100	0
2 - 15	100 → 78	0 → 22
15 - 25	78 → 60	22 → 40
25 - 33	60 → 36	40 → 64
33 - 37	36	64

Flow rate 0.9 mL/min.

Detection Spectrophotometer at 232 nm.

Autosampler Set at 8 °C.

Injection 5 µL of test solution (a) and reference solutions (b), (c) and (f).

Identification of peaks Use the chromatogram obtained with reference solution (f) to identify the peaks due to impurities C and D; use the chromatogram obtained with reference solution (c) to identify the peak due to sorafenib.

Relative retention With reference to regorafenib (retention time = about 25 min): impurity C = about 0.4; impurity D = about 0.8; sorafenib = about 0.95.

System suitability Reference solution (c):
— *resolution*: minimum 3.0 between the peaks due to sorafenib and regorafenib.

Calculation of percentage contents:
— *correction factors*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity C = 0.5; impurity D = 0.6;
— for each impurity, use the concentration of regorafenib monohydrate in reference solution (b).

Limits:
— *impurity D*: maximum 0.2 per cent;
— *impurity C*: maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.5 per cent;
— *reporting threshold*: 0.05 per cent.

Water (*2.5.32*)
3.2 per cent to 4.0 per cent, determined on 50.0 mg using the evaporation technique at 150 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

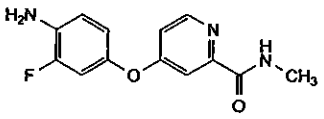
Injection Test solution (b) and reference solution (a).

Calculate the percentage content of $C_{21}H_{15}ClF_4N_4O_3$ taking into account the assigned content of *regorafenib monohydrate CRS*.

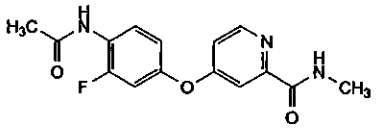
IMPURITIES

Specified impurities *A, C, D.*

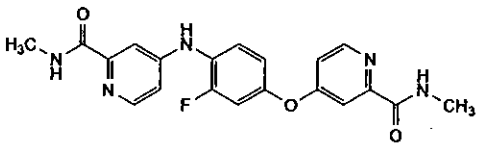
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, E.*



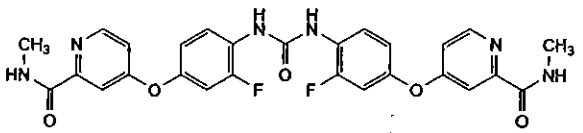
A. 4-(4-amino-3-fluorophenoxy)-*N*-methylpyridine-2-carboxamide,



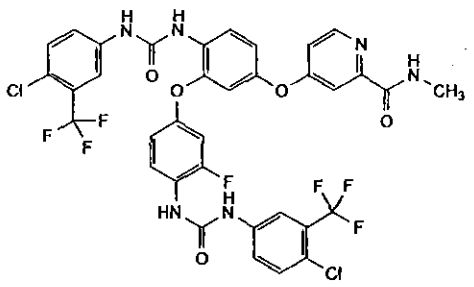
B. 4-(4-acetamido-3-fluorophenoxy)-*N*-methylpyridine-2-carboxamide,



C. 4-[3-fluoro-4-[[2-(methylcarbamoyl)pyridin-4-yl]amino] phenoxy]-*N*-methylpyridine-2-carboxamide,



D. 3^3,7^2-difluoro-*N*,*N*′-dimethyl-5-oxo-2,8-dioxa-4,6-diaza-1(4),9(4)-dipyridina-3(1,4),7(1,4)-dibenzenanonaphane-1^2,9^2-dicarboxamide,

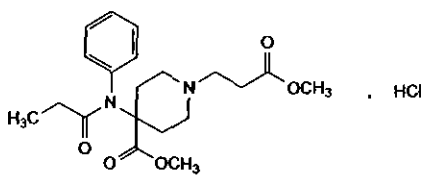


E. 9[4]-chloro-3[4]-[[[4-chloro-3-(trifluoromethyl) phenyl]carbamoyl]amino]-5[3]-fluoro-*N*-methyl-7-oxo-9[3]-(trifluoromethyl)-2,4-dioxa-6,8-diaza-1(4)-pyridina-3 (1,3),5(1,4),9(1)-tribenzenanonaphane-1[2]-carboxamide.

Ph Eur

Remifentanil Hydrochloride

(Ph. Eur. monograph 2644)



$C_{20}H_{29}ClN_2O_5$ 412.9 *132539-07-2*

Action and use
Opioid receptor agonist; analgesic.

Ph Eur

DEFINITION
Methyl 1-(3-methoxy-3-oxopropyl)-4-[phenyl (propanoyl)amino]piperidine-4-carboxylate hydrochloride.

Content
97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water, soluble in acetonitrile and in methanol, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *remifentanil hydrochloride CRS*.
B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).
Dissolve 0.5 g in *water R* and dilute to 50.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use and keep them at not more than 5 °C.*
Buffer solution Dissolve 6.0 g of *anhydrous sodium dihydrogen phosphate R* in 950 mL of *water for chromatography R*, adjust to pH 3.0 with *phosphoric acid R* and dilute to 1000 mL with *water for chromatography R*.
Test solution (a) Dissolve 30.0 mg of the substance to be examined in mobile phase B and dilute to 20.0 mL with mobile phase B.
Test solution (b) Dilute 1.0 mL of test solution (a) to 20.0 mL with mobile phase B.
Reference solution (a) Dissolve 3 mg of *remifentanil impurity mixture CRS* (containing impurities A, B, C, E, L, N and O) in mobile phase B and dilute to 2 mL with mobile phase B.
Reference solution (b) Dilute 1.0 mL of test solution (a) to 100.0 mL with mobile phase B. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase B.
Reference solution (c) Dissolve 30.0 mg of *remifentanil hydrochloride CRS* in mobile phase B and dilute to 20.0 mL with mobile phase B. Dilute 1.0 mL of the solution to 20.0 mL with mobile phase B.
Column:
— *size*: *l* = 0.10 m, Ø = 2.1 mm;
— *stationary phase*: *end-capped octadecylphenylsilyl silica gel for chromatography R* (2 µm);
— *temperature*: 40 °C.
Mobile phase:
— *mobile phase A*: *acetonitrile R1, methanol R2*, buffer solution (1:9:90 *V/V/V*);
— *mobile phase B*: *acetonitrile R1*, buffer solution, *methanol R2* (12:40:48 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 8	85	15
8 - 40	85 → 45	15 → 55

Flow rate 0.65 mL/min.
Detection Spectrophotometer at 210 nm.
Injection 5.0 µL of test solution (a) and reference solutions (a) and (b).
Identification of impurities Use the chromatogram supplied with *remifentanil impurity mixture CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, E, L, N and O.
Relative retention With reference to remifentanil (retention time = about 10 min): impurity L = about 0.2; impurity O = about 0.40; impurity B = about 0.44; impurity A = about 0.7; impurity C = about 0.80; impurity N = about 0.85; impurity E = about 1.4.
System suitability Reference solution (a):
— *resolution*: minimum 1.5 between the peaks due to impurities O and B; minimum 2.0 between the peaks due to impurities C and N.
Calculation of percentage contents:
— *correction factor*: multiply the peak area of impurity L by 1.5;
— for each impurity, use the concentration of remifentanil hydrochloride in reference solution (b).
Limits:
— *impurity C*: maximum 0.5 per cent;
— *impurity A*: maximum 0.2 per cent;
— *impurities B, E, L*: for each impurity, maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;

— *total*: maximum 1.0 per cent;
— *reporting threshold*: 0.05 per cent.

Methyl acrylate

Head-space gas chromatography (*2.2.28*).

Internal standard solution Dilute 25.0 µL of *propanol R* to 100.0 mL with *water for chromatography R*. Dilute 50.0 mL of the solution to 1000.0 mL with *water for chromatography R*.

Test solution Dissolve 0.100 g of the substance to be examined in the internal standard solution and dilute to 5.0 mL with the internal standard solution.

Reference solution Dilute 75.0 µL of *methyl acrylate R* to 100.0 mL with the internal standard solution. Dilute 10.0 mL of the solution to 100.0 mL with the internal standard solution. Dilute 7.0 mL of this solution to 100.0 mL with the internal standard solution.

Precolumn:
— *material*: deactivated fused silica;
— *size*: *l* = 5 m, Ø = 0.25 mm.

Column:
— *material*: fused silica;
— *size*: *l* = 60 m, Ø = 0.53 mm;
— *stationary phase*: *cyanopropyl(7)phenyl(7)methyl(86) polysiloxane R* (film thickness 3 µm).

Carrier gas *helium for chromatography R*.

Flow rate 8.3 mL/min.

Pressure 68.9 kPa.

Split ratio 1:10.

Static head-space conditions that may be used:
— *equilibration temperature*: 90 °C;
— *equilibration time*: 5 min;
— *transfer-line temperature*: 180 °C;
— *pressurisation time*: 2 min;
— *injection*: 6 s or 1 mL;
— *withdrawal time*: 12 s;
— *shaker*: on.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 35	35
	35 - 40	35 → 210
	40 - 50	210
Injection port		180
Detector		250

Detection Flame ionisation.

Retention time Propanol = about 25 min; methyl acrylate = about 27 min.

System suitability Reference solution:
— *resolution*: minimum 5.0 between the peaks due to propanol and methyl acrylate.

Calculate the content of methyl acrylate, taking its relative density to be 0.955 at 20 °C.

Limit:
— *methyl acrylate*: maximum 250 ppm.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Mobile phase:

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 8	85	15
8 - 12	85 → 83	15 → 17
12 - 15	83 → 15	17 → 85
15 - 25	15	85

Injection Test solution (b) and reference solution (c).

Retention time Remifentanil = about 11 min.

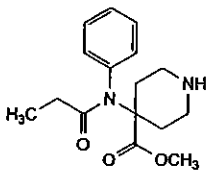
System suitability Reference solution (c):
— *symmetry factor*: maximum 2.2 for the peak due to remifentanil.

Calculate the percentage content of $C_{20}H_{29}ClN_2O_5$ taking into account the assigned content of *remifentanil hydrochloride CRS*.

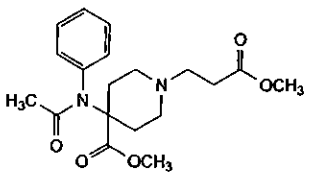
IMPURITIES

Specified impurities *A, B, C, E, L.*

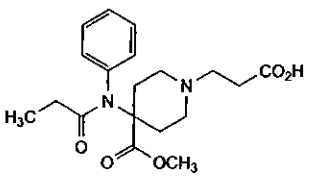
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *D, G, H, I, J, K, M, N, O.*



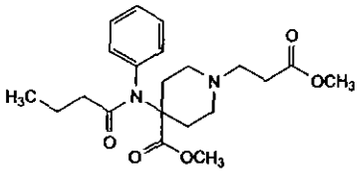
A. methyl 4-[phenyl(propanoyl)amino]piperidine-4-carboxylate,



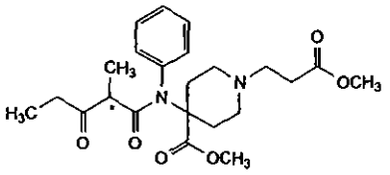
B. methyl 4-[acetyl(phenyl)amino]-1-(3-methoxy-3-oxopropyl)piperidine-4-carboxylate,



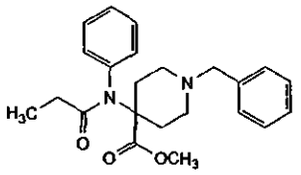
C. 3-[4-(methoxycarbonyl)-4-[phenyl(propanoyl) amino]piperidin-1-yl]propanoic acid,



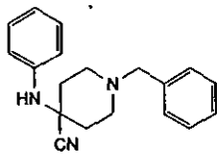
D. methyl 4-[butanoyl(phenyl)amino]-1-(3-methoxy-3-oxopropyl)piperidine-4-carboxylate,



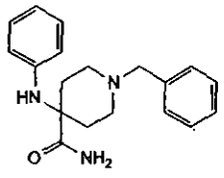
E. methyl 1-(3-methoxy-3-oxopropyl)-4-[(2-methyl-3-oxopentanoyl)(phenyl)amino]piperidine-4-carboxylate,



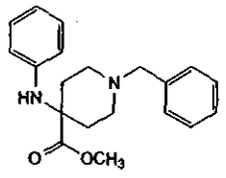
G. methyl 1-benzyl-4-[phenyl(propanoyl)amino]piperidine-4-carboxylate,



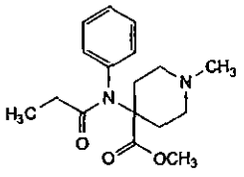
H. 1-benzyl-4-(phenylamino)piperidine-4-carbonitrile,



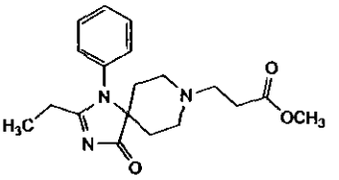
I. 1-benzyl-4-(phenylamino)piperidine-4-carboxamide,



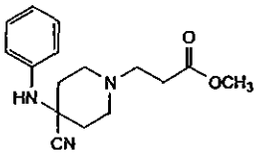
J. methyl 1-benzyl-4-(phenylamino)piperidine-4-carboxylate,



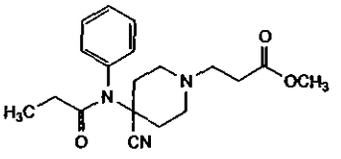
K. methyl 1-methyl-4-[phenyl(propanoyl)amino]piperidine-4-carboxylate,



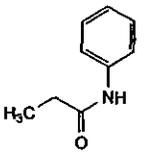
L. methyl 3-(2-ethyl-4-oxo-1-phenyl-1,3,8-triazaspiro[4.5]dec-2-en-8-yl)propanoate,



M. methyl 3-[4-cyano-4-(phenylamino)piperidin-1-yl] propanoate,



N. methyl 3-[4-cyano-4-[phenyl(propanoyl)amino]piperidin-1-yl]propanoate,

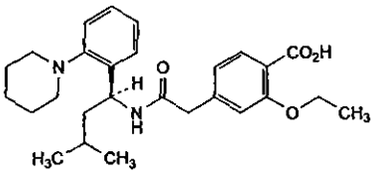


O. *N*-phenylpropanamide.

Ph Eur

Repaglinide

(Ph. Eur. monograph 2135)



$C_{27}H_{36}N_2O_4$ 452.6 *135062-02-1*

Action and use
Stimulates insulin release; treatment of diabetes mellitus.

Preparation
Repaglinide Tablets

Ph Eur

DEFINITION
2-Ethoxy-4-[2-[[(1*S*)-3-methyl-1-[2-(piperidin-1-yl) phenyl]butyl]amino]-2-oxoethyl]benzoic acid.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, freely soluble in methanol and in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Specific optical rotation (*2.2.7*): + 6.3 to + 7.7.

Dissolve 1.00 g in *methanol R* and dilute to 20.0 mL with the same solvent.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *repaglinide CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *anhydrous ethanol R*, evaporate to dryness and record new spectra using the residues.

TESTS

Enantiomeric purity

Liquid chromatography (*2.2.29*). *Prepare the solutions in amber flasks and vials.*

Test solution Dissolve 10.0 mg of the substance to be examined in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dissolve 5.0 mg of *repaglinide impurity E CRS* in *methanol R* and dilute to 50.0 mL with the same of solvent.

Reference solution (b) Dilute 2.0 mL of reference solution (a) to 100.0 mL with *methanol R.*

Reference solution (c) Mix 1.0 mL of the test solution and 10 mL of reference solution (a) and dilute to 50.0 mL with *methanol R.*

Column:
- *size: l* = 0.1 m, Ø = 4.0 mm,
- *stationary phase: silica gel AGP for chiral chromatography R* (5 µm).

Mobile phase:
- *mobile phase A*: 1.0 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 4.7 with *dilute sodium hydroxide solution R;*
- *mobile phase B: acetonitrile R;*

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 4	80 → 60	20 → 40
4 - 6	60	40

Equilibration after installation of the column for use Using *water R*, slowly increase the flow rate from 0.2 mL/min to 0.5 mL/min. Maintain the flow rate at 0.5 mL/min for 5 min. The column must be washed for 1 h at a flow rate of 1 mL/min with *water R* and for 1 h with the mobile phase at the initial composition prior to the 1st analysis.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 240 nm.

Injection 10 µL of the test solution and reference solutions (b) and (c).

Retention time Repaglinide = about 3.3 min; impurity E = about 5.0 min.

System suitability Reference solution (c):
- *resolution*: minimum 1.5 between the peaks due to repaglinide and impurity E.

Limit:
- *impurity E*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 30.0 mg of the substance to be examined in *acetonitrile R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dilute 5.0 mL of the test solution to 100.0 mL with *acetonitrile R.* Dilute 2.0 mL of this solution to 100.0 mL with *acetonitrile R.*

Reference solution (b) With the aid of an ultrasonic bath, dissolve the contents of 1 vial of *repaglinide for system suitability CRS* in 2.0 mL of *acetonitrile R.*

Column:
- *size: l* = 0.15 m, Ø = 4.6 mm,
- *stationary phase: silica gel for chromatography, alkyl-bonded for use with highly aqueous mobile phases R* (5 µm),
- *temperature*: 45 °C.

Mobile phase:
- *mobile phase A*: 4.0 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 3.2 with *dilute phosphoric acid R;*
- *mobile phase B*: mobile phase A, *acetonitrile R* (300:700 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	50 → 7	50 → 93
20 - 30	7	93

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 240 nm.

Injection 10 µL.

Relative retention With reference to repaglinide (retention time = about 10 min): impurity A = about 0.2; impurity B = about 0.3; impurity C = about 0.4; impurity D = about 1.5.

System suitability Reference solution (b):
- *resolution*: minimum 5.0 between the peaks due to impurity B and impurity C,
- the chromatogram obtained is similar to the chromatogram supplied with *repaglinide for system suitability CRS.*

Limits:
- *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.6; impurity B = 0.7; impurity C = 3.1;
- *impurities A, B, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

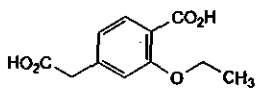
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.320 g in 10 mL *methanol R* and add 60 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

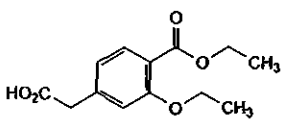
1 mL of *0.1 M perchloric acid* is equivalent to 45.26 mg of $C_{27}H_{36}N_2O_4$.

STORAGE
Protected from light.

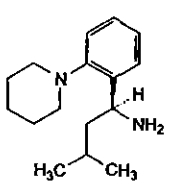
IMPURITIES
Specified impurities *A, B, C, D, E.*



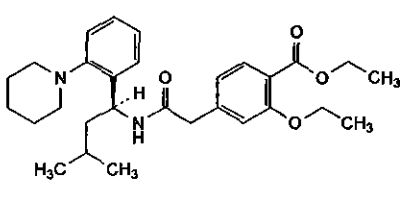
A. 4-(carboxymethyl)-2-ethoxybenzoic acid,



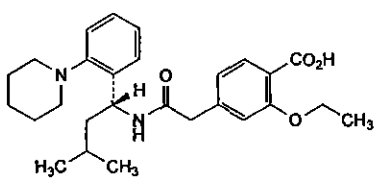
B. [3-ethoxy-4-(ethoxycarbonyl)phenyl]acetic acid,



C. (1*S*)-3-methyl-1-[2-(piperidin-1-yl)phenyl]butan-1-amine,



D. ethyl 2-ethoxy-4-[2-[[(1*S*)-3-methyl-1-[2-(piperidin-1-yl)phenyl]butyl]amino]-2-oxoethyl]benzoate,

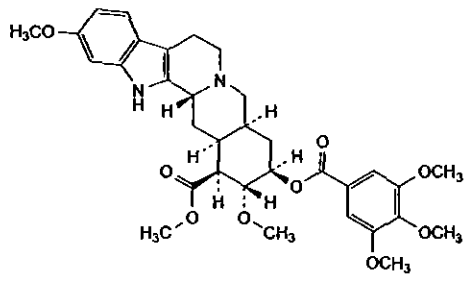


E. 2-ethoxy-4-[2-[[(1*R*)-3-methyl-1-[2-(piperidin-1-yl)phenyl]butyl]amino]-2-oxoethyl]benzoic acid.

Ph Eur

Reserpine

(Ph. Eur. monograph 0528)



$C_{33}H_{40}N_2O_9$ 609 *50-55-5*

Action and use
Rauwolfia alkaloid; treatment of hypertension.

Ph Eur

DEFINITION
Methyl 11,17α-dimethoxy-18β-[(3,4,5-trimethoxybenzoyl)oxy]-3β,20α-yohimban-16β-carboxylate.

Content
— *reserpine*: 98.0 per cent to 102.0 per cent (dried substance),
— *total alkaloids*: 99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or slightly yellow, small crystals or crystalline powder, darkening slowly on exposure to light.

Solubility
Practically insoluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION
First identification: B.
Second identification: A, C, D, E.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 20.0 mg in *chloroform R* and dilute to 10.0 mL with the same solvent. Dilute 1.0 mL of this solution to 100.0 mL with *ethanol (96 per cent) R*. Examine immediately.

Spectral range 230-350 nm.

Absorption maximum At 268 nm.

Specific absorbance at the absorption maximum 265 to 285.

Over the range 288-295 nm, the curve shows a slight absorption minimum followed by a shoulder or a slight absorption maximum; over this range, the specific absorbance is about 170.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *reserpine CRS*.

C. To about 1 mg add 0.1 mL of a 1 g/L solution of *sodium molybdate R* in *sulfuric acid R*. A yellow colour is produced which becomes blue within 2 min.

D. To about 1 mg add 0.2 mL of a freshly prepared 10 g/L solution of *vanillin R* in *hydrochloric acid R*. A pink colour develops within 2 min.

E. Mix about 0.5 mg with 5 mg of *dimethylaminobenzaldehyde R* and 0.2 mL of *glacial acetic*

acid R and add 0.2 mL of *sulfuric acid R*. A green colour is produced. Add 1 mL of *glacial acetic acid R*. The colour becomes red.

TESTS

Specific optical rotation (*2.2.7*)
−116 to −128 (dried substance).

Dissolve 0.250 g in *chloroform R* and dilute to 25.0 mL with the same solvent. Examine immediately.

Oxidation products
Dissolve 20 mg in *glacial acetic acid R* and dilute to 100.0 mL with the same acid. The absorbance (*2.2.25*) measured immediately at the absorption maximum at 388 nm is not greater than 0.10.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 0.500 g by drying *in vacuo* at 60 °C at a pressure not exceeding 0.7 kPa for 3 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 0.5 g.

ASSAY

Total alkaloids
Dissolve 0.500 g in a mixture of 6 mL of *acetic anhydride R* and 40 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 60.9 mg of total alkaloids.

Reserpine
Protect the solutions from light Moisten 25.0 mg with 2 mL of *ethanol (96 per cent) R*, add 2 mL of *0.25 M sulfuric acid* and 10 mL of *ethanol (96 per cent) R*, and warm gently to dissolve. Cool and dilute to 100.0 mL with *ethanol (96 per cent) R*. Dilute 5.0 mL of this solution to 50.0 mL with *ethanol (96 per cent) R*. Prepare a reference solution in the same manner using 25.0 mg of *reserpine CRS*. Place 10.0 mL of each solution separately in 2 boiling-tubes, add 2.0 mL of *0.25 M sulfuric acid* and 2.0 mL of a freshly prepared 3 g/L solution of *sodium nitrite R*. Mix and heat in a water-bath at 55 °C for 35 min. Cool, add 1.0 mL of a freshly prepared 50 g/L solution of *sulfamic acid R* and dilute to 25.0 mL with *ethanol (96 per cent) R*. Measure the absorbance (*2.2.25*) of each solution at the absorption maximum at 388 nm, using as the compensation liquid 10.0 mL of the same solution prepared at the same time in the same manner, but omitting the sodium nitrite.

Calculate the content of $C_{33}H_{40}N_2O_9$ from the absorbances measured and the concentrations of the solutions.

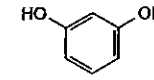
STORAGE

Protected from light.

Ph Eur

Resorcinol

(Ph. Eur. monograph 0290)



$C_6H_6O_2$ 110.1 *108-46-3*

Action and use
Keratolytic.

Ph Eur

DEFINITION

Resorcinol contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of benzene-1,3-diol, calculated with reference to the dried substance.

CHARACTERS

A colourless or slightly pinkish-grey, crystalline powder or crystals, turning red on exposure to light and air, very soluble in water and in alcohol.

IDENTIFICATION

A. Melting point (*2.2.14*): 109 °C to 112 °C.

B. Dissolve 0.1 g in 1 mL of *water R*, add 1 mL of *strong sodium hydroxide solution R* and 0.1 mL of *chloroform R*, heat and allow to cool. An intense, deep-red colour develops which becomes pale yellow on the addition of a slight excess of *hydrochloric acid R*.

C. Thoroughly mix about 10 mg with about 10 mg of *potassium hydrogen phthalate R*, both finely powdered. Heat over a naked flame until an orange-yellow colour is obtained. Cool and add 1 mL of *dilute sodium hydroxide solution R* and 10 mL of *water R* and shake to dissolve. The solution shows an intense green fluorescence.

TESTS

Solution S
Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_5 or R_5 (*2.2.2, Method II*) and remains so when heated in a water-bath for 5 min.

Acidity or alkalinity
To 10 mL of solution S add 0.05 mL of *bromophenol blue solution R2*. Not more than 0.05 mL of *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Related substances
Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution Dissolve 0.5 g of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution Dilute 0.1 mL of the test solution to 20 mL with *methanol R*.

Apply separately to the plate 2 µL of each solution. Develop over a path of 15 cm using a mixture of 40 volumes of *ethyl acetate R* and 60 volumes of *hexane R*. Allow the plate to dry in air for 15 min and expose it to iodine vapour. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Pyrocatechol
To 2 mL of solution S add 1 mL of *ammonium molybdate solution R2* and mix. Any yellow colour in the solution is not more intense than that in a standard prepared at the same time in the same manner using 2 mL of a 0.1 g/L solution of *pyrocatechol R*.

Loss on drying (*2.2.32*)
Not more than 1.0 per cent, determined on 1.00 g of powdered substance by drying in a desiccator for 4 h.

Sulfated ash (*2.4.14*)
Not more than 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.500 g in *water R* and dilute to 250.0 mL with the same solvent. To 25.0 mL of the solution in a ground-glass-stoppered flask add 1.0 g of *potassium bromide R*, 50.0 mL of *0.0167 M potassium bromate*, 15 mL of *chloroform R* and 15.0 mL of *hydrochloric acid R1*. Stopper the flask, shake and allow to stand in the dark for 15 min, shaking occasionally. Add 10 mL of a 100 g/L solution of *potassium iodide R*, shake thoroughly, allow to stand for 5 min and titrate with *0.1 M sodium thiosulfate*, using 1 mL of *starch solution R* as indicator.

1 mL of *0.0167 M potassium bromate* is equivalent to 1.835 mg of $C_6H_6O_2$.

STORAGE
Store protected from light.

Ph Eur

Synthetic Retinol Concentrate (Oily Form)

(Vitamin A Concentrate (Oily Form) Synthetic, Ph. Eur. monograph 0219)

Action and use
Vitamin A.

Ph Eur

DEFINITION
Oily concentrate prepared from synthetic retinol ester (*0217*) as is or by dilution with a suitable vegetable fatty oil.

Content
95.0 per cent to 110.0 per cent of the vitamin A content stated on the label, which is not less than 500 000 IU/g.

It may contain suitable stabilisers such as antioxidants.

CHARACTERS
Appearance
Yellow or brownish-yellow, oily liquid.

Solubility
Practically insoluble in water, soluble or partly soluble in anhydrous ethanol, miscible with organic solvents.

Partial crystallisation may occur in highly concentrated solutions.

IDENTIFICATION
Thin-layer chromatography (*2.2.27*).

Test solution Prepare a solution containing about 3.3 IU of vitamin A per microlitre in *cyclohexane R* containing 1 g/L of *butylhydroxytoluene R*.

Reference solution Prepare a 10 mg/mL solution of *retinol esters CRS* (i.e. 3.3 IU of each ester per microlitre) in *cyclohexane R* containing 1 g/L of *butylhydroxytoluene R*.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *ether R, cyclohexane R* (20:80 *V/V*).

Application 3 µL.

Development Immediately, over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution:
— the chromatogram shows the individual spots of the corresponding esters. The elution order from bottom to top is: retinol acetate, retinol propionate and retinol palmitate.

Results The composition of the test solution is confirmed by the correspondence of the principal spot or spots with those obtained with the reference solution.

TESTS
Acid value (*2.5.1*)
Maximum 2.0, determined on 2.0 g.

Peroxide value (*2.5.5, Method A*)
Maximum 10.0.

Use *carbon dioxide-free water R* instead of *water R* and titrate with *0.01 M sodium thiosulfate* at a rate of 5 mL/min, stirring with a magnetic stirrer. Determine the end-point potentiometrically (*2.2.20*).

Related substances
The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

ASSAY
Carry out the assay as rapidly as possible, avoiding exposure to actinic light, air, oxidising agents, oxidation catalysts (e.g. copper, iron), acids and prolonged heat; use freshly prepared solutions. If partial crystallisation has occurred, homogenise the material at about 65 °C, but avoid prolonged heating.

Carry out the assay according to Method A. If the assay is not shown to be valid, use Method B.

Method A
Ultraviolet absorption spectrophotometry (*2.2.25*).

Dissolve an appropriate quantity of the preparation to be examined (25.0-100.0 mg) in 5 mL of *pentane R* and dilute with *2-propanol R1* to a presumed concentration of 10-15 IU/mL.

Verify that the absorption maximum of the solution lies between 325 nm and 327 nm and measure the absorbances at 300 nm, 326 nm, 350 nm and 370 nm. Repeat the readings at each wavelength and take the mean values. Calculate the ratio A_λ/A_{326} for each wavelength.

If the ratios do not exceed: 0.60 at 300 nm, 0.54 at 350 nm, 0.14 at 370 nm, calculate the content of vitamin A in International Units per gram using the following expression:

$$\frac{A_{326} \times V \times 1900}{100 \times m}$$

A_{326} = absorbance at 326 nm,
m = mass of the preparation to be examined used, in grams,
V = total volume to which the preparation to be examined is diluted to give 10-15 IU/mL,
1900 = factor to convert the specific absorbance of esters of retinol into International Units per gram.

If one or more of the ratios A_λ/A_{326} exceeds the values given, or if the wavelength of the absorption maximum does not lie between 325 nm and 327 nm, use Method B.

Method B

Liquid chromatography (*2.2.29*).

Test solution Introduce 0.100 g of the preparation to be examined into a 100 mL volumetric flask and dissolve immediately in 5 mL of *pentane R*. Add 40 mL of *0.1 M tetrabutylammonium hydroxide in 2-propanol* and swirl gently. Let the mixture react for 10 min at 60-65 °C for hydrolysis, swirling occasionally. Allow to cool to room temperature, dilute to 100.0 mL with *2-propanol R* containing 1 g/L of *butylhydroxytoluene R*, and homogenise carefully to avoid air bubbles (solution A). Dilute this solution with *2-propanol R* to a final concentration of 100 IU/mL. Homogenise carefully to avoid air bubbles.

Reference solution Introduce about 0.100 g of *retinol acetate CRS* into a 100 mL volumetric flask and proceed as described for solution A. Dilute the solution with *2-propanol R* to a final concentration of 100 IU/mL. Homogenise carefully to avoid air bubbles.

Column:
- *size*: l = 0.125 m, Ø = 4 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *water for chromatography R, methanol R* (5:95 *V/V*).

Flow rate 1 mL/min.

Detection Spectrophotometer at 325 nm.

Injection 10 µL.

Run time 1.5 times the retention time of retinol.

Retention time Retinol = about 3 min.

Calculate the content of vitamin A in International Units per gram using the following expression:

$$\frac{A_1 \times C \times m_2}{A_2 \times m_1}$$

A_1 = area of the peak due to retinol in the chromatogram obtained with the test solution,

A_2 = area of the peak due to retinol in the chromatogram obtained with the reference solution,

C = concentration of *retinol acetate CRS*, in International Units per gram, determined by method A; the absorption ratios A_λ/A_{326} must conform,

m_1 = mass of the preparation to be examined used to prepare the test solution, in milligrams,

m_2 = mass of *retinol acetate CRS* used to prepare the reference solution , in milligrams.

STORAGE

In an airtight container, protected from light.

Once the container has been opened, its contents are to be used as soon as possible; any part of the contents not used at once must be protected under an inert gas.

LABELLING

The label states:
- the number of International Units per gram;
- the name of the ester or esters;
- the name of any added stabilisers;
- the method of restoring the solution if partial crystallisation has occurred.

Ph Eur

Synthetic Retinol Concentrate (Powder Form)

(Vitamin A Concentrate (Powder Form), Synthetic, Ph. Eur. monograph 0218)

Action and use

Vitamin A.

Ph Eur

DEFINITION

Powder concentrate obtained by dispersing a synthetic retinol ester (*0217*) in a matrix of *Gelatin (0330)* or *Acacia (0307)* or other suitable material.

Content

95.0 per cent to 115.0 per cent of the vitamin A content stated on the label, which is not less than 250 000 IU/g.

It may contain suitable stabilisers such as antioxidants.

CHARACTERS

Appearance

Yellowish powder usually in the form of particles of almost uniform size.

Solubility

Practically insoluble in water, swells or forms an emulsion, depending on the formulation.

IDENTIFICATION

Thin-layer chromatography (*2.2.27*).

Test solution Introduce a quantity of the preparation to be examined containing about the equivalent of 17 000 IU of vitamin A into a 20 mL glass-stoppered test tube. Add about 20 mg of *bromelains R*, 2 mL of *water R* and about 150 µL of *2-propanol R*, swirling gently for 2-5 min in a water-bath at 60-65 °C. Cool to below 30 °C and add 5 mL of *2-propanol R* containing 1 g/L of *butylhydroxytoluene R*. Shake vigorously for 1 min, allow to stand for a few minutes and use the supernatant solution.

Reference solution Prepare a 10 mg/mL solution of *retinol esters CRS* (i.e. 3.3 IU of each ester per microlitre) in *2-propanol R* containing 1 g/L of *butylhydroxytoluene R*.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *ether R, cyclohexane R* (20:80 *V/V*).

Application 3 µL.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution:
- the chromatogram shows the individual spots of the corresponding esters. The elution order from bottom to top is: retinol acetate, retinol propionate and retinol palmitate.

Results The composition of the test solution is confirmed by the correspondence of the principal spot or spots with those obtained with the reference solution.

TESTS

Related substances

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

ASSAY

Carry out the assay as rapidly as possible, avoiding exposure to actinic light, air, oxidising agents, oxidation catalysts (e.g. copper, iron), acids and prolonged heat.

Liquid chromatography (*2.2.29*).

Test solution (a) Introduce 0.200 g of the preparation to be examined into a 100 mL volumetric flask. Add 20-30 mg of *bromelains R*, 5.0 mL of *water R* and 0.15 mL of *2-propanol R*. Heat gently in a water-bath at 60 °C for about 5 min, swirling occasionally. Add 40 mL of *0.1 M tetrabutylammonium hydroxide in 2-propanol*. Swirl gently and let the mixture react for 10 min at 60-65 °C for hydrolysis, swirling occasionally. Ensure that all sample material is wetted. Allow to cool to room temperature, dilute to 100.0 mL with *2-propanol R* containing 1 g/L of *butylhydroxytoluene R*, and homogenise carefully to avoid air bubbles. The solution may be turbid.

Test solution (b) Dilute test solution (a) with *2-propanol R* to a final concentration of 100 IU/mL. Filter before injection.

Reference solution (a) Introduce about 0.100 g of *retinol acetate CRS* into a 100 mL volumetric flask and dissolve immediately in 5 mL of *pentane R*. Add 40 mL of *0.1 M tetrabutylammonium hydroxide in 2-propanol*. Swirl gently and let the mixture react for 10 min at 60-65 °C for hydrolysis, swirling occasionally. Allow to cool to room temperature, dilute to 100.0 mL with *2-propanol R* containing 1 g/L of *butylhydroxytoluene R*, and homogenise carefully to avoid air bubbles.

Reference solution (b) Dilute reference solution (a) with *2-propanol R* to a final concentration of 100 IU/mL. Homogenise carefully to avoid air bubbles.

Column:
- *size*: l = 0.125 m, Ø = 4 mm,
- *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *water R, methanol R* (5:95 *V/V*).

Flow rate 1 mL/min.

Detection Spectrophotometer at 325 nm.

Injection 10 µL of test solution (b) and reference solution (b).

Run time 1.5 times the retention time of retinol.

Retention time Retinol = about 3 min.

Calculate the content of vitamin A using the following expression:

$$\frac{A_1 \times C \times m_2}{A_2 \times m_1}$$

A_1 = area of the peak due to retinol in the chromatogram obtained with test solution (b),
A_2 = area of the peak due to retinol in the chromatogram obtained with reference solution (b),
C = concentration of *retinol acetate CRS* in International Units per gram, determined by the method below,
m_1 = mass of the substance to be examined in test solution (a), in milligrams,
m_2 = mass of *retinol acetate CRS* in reference solution (a), in milligrams.

The exact concentration of *retinol acetate CRS* is assessed by ultraviolet absorption spectrophotometry (*2.2.25*). Dissolve 25-100 mg of *retinol acetate CRS*, weighed with an accuracy of 0.1 per cent, in 5 mL of *pentane R* and dilute with *2-propanol R1* to a presumed concentration of 10-15 IU/mL.

Verify that the absorption maximum of the solution lies between 325 nm and 327 nm and measure the absorbances at 300 nm, 326 nm, 350 nm and 370 nm. Repeat the readings at each wavelength and take the mean values. Calculate the ratio A_λ/A_{326} for each wavelength.

If the ratios do not exceed: 0.60 at 300 nm, 0.54 at 350 nm, 0.14 at 370 nm, calculate the content of vitamin A in International Units per gram using the following expression:

$$\frac{A_{326} \times V \times 1900}{100 \times m}$$

A_{326} = absorbance at 326 nm,
m = mass of *retinol acetate CRS*, in grams,
V = total volume to which the *retinol acetate CRS* is diluted to give 10-15 IU/mL,
1900 = factor to convert the specific absorbance of esters of retinol into International Units per gram.

The absorbance ratios A_λ/A_{326} must conform.

STORAGE

In an airtight container, protected from light.

Once the container has been opened, its contents are to be used as soon as possible; any part of the contents not used at once should be protected by an atmosphere of inert gas.

LABELLING

The label states:
- the number of International Units per gram,
- the name of the ester or esters,
- the name of the principal excipient or excipients used and the name of any added stabilisers.

Ph Eur

Synthetic Retinol Concentrate, Solubilisate/Emulsion

Synthetic Retinol Concentrate (Water-dispersible Form)

(Vitamin A Concentrate, (Solubilisate/Emulsion), Synthetic, Ph. Eur. monograph 0220)

Ph Eur

DEFINITION

Liquid concentrate (water is generally used as solvent) of a synthetic retinol ester (*0217*) and a suitable solubiliser.

Content

95.0 per cent to 115.0 per cent of the vitamin A content stated on the label, which is not less than 100 000 IU/g.

It may contain suitable stabilisers such as antimicrobial preservatives and antioxidants.

CHARACTERS

Appearance

Yellow or yellowish liquid of variable opalescence and viscosity. Highly concentrated solutions may become cloudy at low temperature or take the form of a gel.

A mixture of 1 g with 10 mL of *water R* previously warmed to 50 °C gives, after cooling to 20 °C, a uniform, slightly opalescent and slightly yellow dispersion.

IDENTIFICATION

Thin-layer chromatography (*2.2.27*).

Test solution Introduce a quantity of the preparation to be examined containing about the equivalent of 17 000 IU of vitamin A into a 20 mL glass-stoppered test tube. Add 5 mL of *2-propanol R* containing 1 g/L of *butylhydroxytoluene R* and mix thoroughly.

Reference solution Prepare a 10 mg/mL solution of *retinol esters CRS* (i.e. 3.3 IU of each ester per microlitre) in *2-propanol R* containing 1 g/L of *butylhydroxytoluene R*.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *ether R, cyclohexane R* (20:80 *V/V*).

Application 3 µL.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution:

— the chromatogram shows the individual spots of the corresponding esters. The elution order from bottom to top is: retinol acetate, retinol propionate and retinol palmitate.

Results The composition of the test solution is confirmed by the correspondence of the principal spot or spots with those obtained with the reference solution.

TESTS

Related substances

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

ASSAY

Carry out the assay as rapidly as possible, avoiding exposure to actinic light, air, oxidising agents, oxidation catalysts (e.g. copper, iron), acids and prolonged heat.

Liquid chromatography (*2.2.29*).

Test solution (a) Introduce 0.200 g of the preparation to be examined into a 100 mL volumetric flask. Add 40 mL of *0.1 M tetrabutylammonium hydroxide in 2-propanol.* Swirl into dispersion and let the mixture react for 10 min at 60-65 °C for hydrolysis, swirling occasionally. Ensure that all sample material is wetted. Allow to cool to room temperature, dilute to 100.0 mL with *2-propanol R* containing 1 g/L of *butylhydroxytoluene R* and homogenise carefully to avoid air bubbles. Residue of the matrix may make the solution more or less cloudy.

Test solution (b) Dilute test solution (a) with *2-propanol R* to a final concentration of 100 IU/mL. Filter before injection.

Reference solution (a) Introduce about 0.100 g of *retinol acetate CRS* into a 100 mL volumetric flask and dissolve immediately in 5 mL of *pentane R.* Add 40 mL of *0.1 M tetrabutylammonium hydroxide in 2-propanol.* Swirl gently and let the mixture react for 10 min at 60-65 °C for hydrolysis, swirling occasionally. Allow to cool to room temperature, dilute to 100.0 mL with *2-propanol R* containing 1 g/L of *butylhydroxytoluene R,* and homogenise carefully to avoid air bubbles.

Reference solution (b) Dilute reference solution (a) with *2-propanol R* to a final concentration of 100 IU/mL. Homogenise carefully to avoid air bubbles.

Column:

— *size:* l = 0.125 m, Ø = 4 mm,

— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *water R, methanol R* (5:95 *V/V*).

Flow rate 1 mL/min.

Detection Spectrophotometer at 325 nm.

Injection 10 µL of test solution (b) and reference solution (b).

Run time 1.5 times the retention time of retinol.

Retention time Retinol = about 3 min.

Calculate the content of vitamin A using the following expression:

$$\frac{A_1 \times C \times m_2}{A_2 \times m_1}$$

A_1 = area of the peak due to retinol in the chromatogram obtained with test solution (b),

A_2 = area of the peak due to retinol in the chromatogram obtained with reference solution (b),

C = concentration of *retinol acetate CRS* in International Units per gram, determined by the method below,

m_1 = mass of the substance to be examined in test solution (a), in milligrams,

m_2 = mass of *retinol acetate CRS* in reference solution (a), in milligrams.

The exact concentration of *retinol acetate CRS* is assessed by ultraviolet absorption spectrophotometry (*2.2.25*). Dissolve 25-100 mg of *retinol acetate CRS,* weighed with an accuracy of 0.1 per cent, in 5 mL of *pentane R* and dilute with *2-propanol R1* to a presumed concentration of 10-15 IU/mL.

Verify that the absorption maximum of the solution lies between 325 nm and 327 nm and measure the absorbances at 300 nm, 326 nm, 350 nm and 370 nm. Repeat the readings at each wavelength and take the mean values. Calculate the ratio A_λ/A_{326} for each wavelength.

If the ratios do not exceed: 0.60 at 300 nm, 0.54 at 350 nm, 0.14 at 370 nm, calculate the content of vitamin A in International Units per gram using the following expression:

$$\frac{A_{326} \times V \times 1900}{100 \times m}$$

A_{326} = absorbance at 326 nm,

m = mass of *retinol acetate CRS,* in grams,

V = total volume to which the *retinol acetate CRS* is diluted to give 10-15 IU/mL,

1900 = factor to convert the specific absorbance of esters of retinol into International Units per gram.

The absorbance ratios A_λ/A_{326} must conform.

STORAGE

In an airtight container, protected from light, at the temperature stated on the label.

Once the container has been opened, its contents are to be used as soon as possible; any part of the contents not used at once should be protected by an atmosphere of inert gas.

LABELLING

The label states:

— the number of International Units per gram,

— the name of the ester or esters,

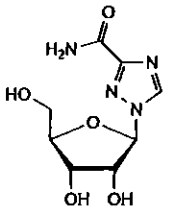
— the name of the principal solubiliser or solubilisers used and the name of any added stabilisers,

— the storage temperature.

Ph Eur

Ribavirin

(Ph. Eur. monograph 2109)



$C_8H_{12}N_4O_5$ 244.2 *36791-04-5*

Action and use
Antiviral (hepatitis C, respiratory syncytial virus).

Preparation
Ribavirin Powder for Nebuliser Solution

Ph Eur

DEFINITION
1-β-D-Ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamide.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water, slightly soluble in ethanol (96 per cent), slightly soluble or very slightly soluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *ribavirin CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methylene chloride R*, evaporate to dryness and record new spectra using the residues.

TESTS

pH (*2.2.3*)
4.0 to 6.5.

Dissolve 0.200 g in *carbon dioxide-free water R* and dilute to 10.0 mL with the same solvent.

Specific optical rotation (*2.2.7*)
−33 to −37 (dried substance).

Dissolve 0.250 g in *water R* and dilute to 25.0 mL with the same solvent. Determine the specific optical rotation within 10 min of preparing the solution.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in *water for chromatography R* and dilute to 100.0 mL with the same solvent.

Reference solution (a) In order to produce impurity A *in situ*, mix 5.0 mL of the test solution and 5.0 mL of a 42 g/L solution of *sodium hydroxide R* and allow to stand for 90 min. Neutralise with 5.0 mL of a 103 g/L solution of *hydrochloric acid R* and mix well.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *water for chromatography R*. Dilute 1.0 mL of this solution to 10.0 mL with *water for chromatography R*.

Reference solution (c) Dissolve 50.0 mg of *ribavirin CRS* in *water for chromatography R* and dilute to 100.0 mL with the same solvent.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (3 µm) suitable for use with highly aqueous mobile phases ;
— *temperature*: 25 °C.

Mobile phase:
— *mobile phase A*: dissolve 1.0 g of *anhydrous sodium sulfate R* in 950 mL of *water for chromatography R*, add 2.0 mL of a 5 per cent *V/V* solution of *phosphoric acid R*, adjust to pH 2.8 with a 5 per cent *V/V* solution of *phosphoric acid R* and dilute to 1000 mL with *water for chromatography R*;
— *mobile phase B*: *acetonitrile R1*, mobile phase A (5:95 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	100	0
15 - 25	100 → 0	0 → 100
25 - 35	0	100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 5 µL of the test solution and reference solutions (a) and (b).

Relative retention With reference to ribavirin (retention time = about 6 min): impurity A = about 0.8.

System suitability Reference solution (a):
— *resolution*: minimum 4.0 between the peaks due to impurity A and ribavirin.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity A by 2.3;
— *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 5 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (c).

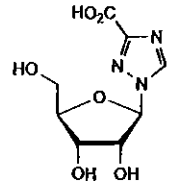
Calculate the percentage content of $C_8H_{12}N_4O_5$ from the declared content of *ribavirin CRS*.

STORAGE
Protected from light.

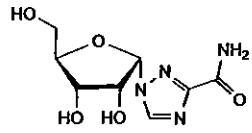
IMPURITIES

Specified impurities A.

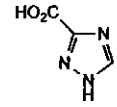
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) B, C, D, F, G.



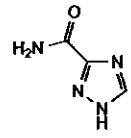
A. 1-β-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboxylic acid,



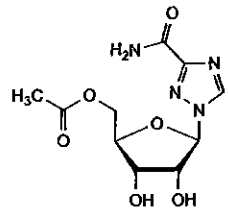
B. 1-α-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamide (anomer),



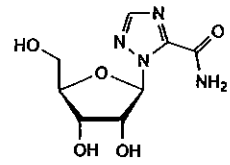
C. 1*H*-1,2,4-triazole-3-carboxylic acid,



D. 1*H*-1,2,4-triazole-3-carboxamide,



F. 1-(5-*O*-acetyl-β-D-ribofuranosyl)-1*H*-1,2,4-triazole-3-carboxamide (5′-*O*-acetylribavirin),

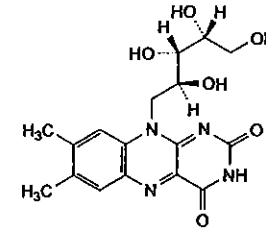


G. 1-β-D-ribofuranosyl-1*H*-1,2,4-triazole-5-carboxamide (*N*-isomer).

Ph Eur

Riboflavin

(Ph. Eur. monograph 0292)



$C_{17}H_{20}N_4O_6$ 376.4 *83-88-5*

Action and use
Vitamin B_2.

Ph Eur

DEFINITION

7,8-Dimethyl-10-[(2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl]benzo[*g*]pteridine-2,4(3*H*,10*H*)-dione.

This monograph applies to riboflavin produced by fermentation.

Content
97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance
Yellow or orange-yellow, crystalline powder.

Solubility
Very slightly soluble in water, practically insoluble in ethanol (96 per cent).

Solutions deteriorate on exposure to light, especially in the presence of alkali.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Thin-layer chromatography (*2.2.27*).

Test solution Suspend 25 mg of the substance to be examined in 10 mL of *water R*, shake for 5 min and filter the suspension to remove the undissolved material.

Reference solution Suspend 25 mg of *riboflavin CRS* in 10 mL of *water R*, shake for 5 min and filter the suspension to remove the undissolved material.

Plate *TLC silica gel plate R* (2-10 µm).

Mobile phase *water R*.

Application As follows, drying in a current of cold air after each individual application:

— *1st application*: 2 µL of *methylene chloride R* then 2 µL of the test solution;

— *2nd application*: 2 µL of *methylene chloride R* then 2 µL of the reference solution.

Development Over a path of 6 cm.

Drying In a current of cold air.

Detection Examine in ultraviolet light at 365 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. Dissolve about 1 mg in 100 mL of *water R*. The solution has, by transmitted light, a pale greenish-yellow colour, and,

by reflected light, an intense yellowish-green fluorescence which disappears on the addition of mineral acids or alkalis.

TESTS

Specific optical rotation (*2.2.7*)

−115 to −135 (dried substance).

Dissolve 50.0 mg in *0.05 M sodium hydroxide* free from carbonate and dilute to 10.0 mL with the same alkaline solution. Measure the optical rotation within 30 min of dissolution.

Absorbance (*2.2.25*)

Test solution Dilute the final solution prepared for the assay with an equal volume of *water R*.

Absorption maxima At 223 nm, 267 nm, 373 nm and 444 nm.

Absorbance ratios:

— A_{373}/A_{267} = 0.31 to 0.33;

— A_{444}/A_{267} = 0.36 to 0.39.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use and protect from light.*

Solution A 13.6 g/L solution of *sodium acetate R*.

Test solution With the aid of ultrasound, dissolve 0.120 g of the substance to be examined in 10 mL of *0.1 M sodium hydroxide* and dilute to 100 mL with solution A.

Reference solution (a) Dilute 1.0 mL of the test solution to 10.0 mL with solution A. Dilute 1.0 mL of this solution to 100.0 mL with solution A.

Reference solution (b) With the aid of ultrasound, dissolve the contents of a vial of *riboflavin for peak identification CRS* (containing impurities C and D) in 1.0 mL of a mixture of 1 volume of mobile phase B and 9 volumes of mobile phase A.

Reference solution (c) In order to prepare *in situ* impurities A and B, dissolve 10 mg of the substance to be examined in 1 mL of *0.5 M sodium hydroxide*. Expose to daylight for 1.5 h. Add 0.5 mL of *acetic acid R* and dilute to 100 mL with *water R*.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: *phosphoric acid R, water R* (1:1000 *V/V*);

— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	90	10
5 - 20	90 → 80	10 → 20
20 - 25	80	20
25 - 35	80 → 50	20 → 50
35 - 45	50	50

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 267 nm.

Injection 10 µL.

Identification of impurities Use the chromatogram supplied with *riboflavin for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities C and D.

Relative retention With reference to riboflavin (retention time = about 16 min): impurity C = about 0.2; impurity D = about 0.5; impurity A = about 1.4; impurity B = about 1.9.

System suitability:

— *resolution*: minimum 5 between the peaks due to impurities A and B in the chromatogram obtained with reference solution (c);

— the chromatogram obtained with reference solution (b) is similar to the chromatogram supplied with *riboflavin for peak identification CRS*.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.7; impurity B = 1.4; impurity C = 2.3; impurity D = 1.4;

— *impurity A*: not more than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.025 per cent);

— *impurities B, C, D*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit for peaks other than those due to impurity A*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 1.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on the residue obtained in the test for loss on drying.

ASSAY

Carry out the assay protected from light.

In a brown-glass 500 mL volumetric flask, suspend 65.0 mg in 5 mL of *water R* ensuring that it is completely wetted and dissolve in 5 mL of *dilute sodium hydroxide solution R*. As soon as dissolution is complete, add 100 mL of *water R* and 2.5 mL of *glacial acetic acid R* and dilute to 500.0 mL with *water R*. Place 20.0 mL of this solution in a 200 mL brown-glass volumetric flask, add 3.5 mL of a 14 g/L solution of *sodium acetate R* and dilute to 200.0 mL with *water R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 444 nm.

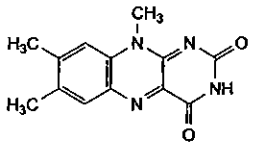
Calculate the content of $C_{17}H_{20}N_4O_6$ taking the specific absorbance to be 328.

STORAGE

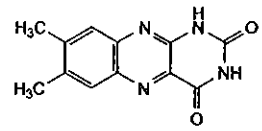
In an airtight container, protected from light.

IMPURITIES

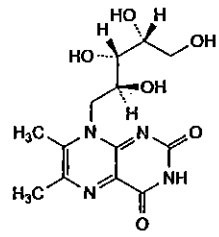
Specified impurities *A, B, C, D.*



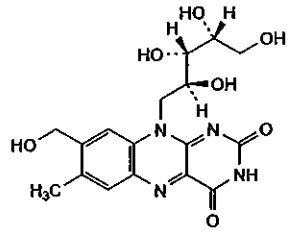
A. 7,8,10-trimethylbenzo[*g*]pteridine-2,4(3*H*,10*H*)-dione (lumiflavine),



B. 7,8-dimethylbenzo[*g*]pteridine-2,4(1*H*,3*H*)-dione,



C. 6,7-dimethyl-8-[(2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl]pteridine-2,4(3*H*,8*H*)-dione,

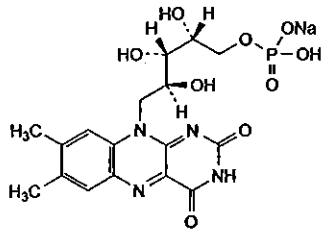


D. 8-(hydroxymethyl)-7-methyl-10-[(2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl]benzo[*g*]pteridine-2,4(3*H*,10*H*)-dione.

Ph Eur

Riboflavin Sodium Phosphate

(Ph. Eur. monograph 0786)



$C_{17}H_{20}N_4NaO_9P$ 478.3 *130-40-5*

Action and use
Vitamin B2.

Preparation
Vitamins B and C Injection

Ph Eur

DEFINITION
Mixture containing riboflavin 5′-(sodium hydrogen phosphate) as the main component and other riboflavin sodium monophosphates.

Content
73.0 per cent to 79.0 per cent of riboflavin ($C_{17}H_{20}N_4O_6$; M_r 376.4) (dried substance).

It contains a variable quantity of water.

CHARACTERS

Appearance
Yellow or orange-yellow, crystalline, hygroscopic powder.

Solubility
Soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 50.0 mg in *phosphate buffer solution pH 7.0 R* and dilute to 100.0 mL with the same buffer solution. Dilute 2.0 mL of this solution to 100.0 mL with *phosphate buffer solution pH 7.0 R*.

Spectral range 230-350 nm.

Absorption maximum At 266 nm.

Specific absorbance at the absorption maximum 580 to 640.

B. Examine the chromatograms obtained in the test for related substances.

Results The principal peak in the chromatogram obtained with the test solution is similar in position and approximate size to the principal peak in the chromatogram obtained with reference solution (b).

C. Dissolve about 10 mg in *dilute sodium hydroxide solution R* and dilute to 100 mL with the same solution. Expose 1 mL of this solution to ultraviolet light at 254 nm for 5 min, add sufficient *acetic acid R* to make the solution acidic to *blue litmus paper R* and shake with 2 mL of *methylene chloride R*. The lower layer shows yellow fluorescence.

D. To 0.5 g add 10 mL of *nitric acid R* and evaporate the mixture to dryness on a water-bath. Ignite the residue until it becomes white, dissolve the residue in 5 mL of *water R* and filter. The filtrate gives reaction (a) of sodium and reaction (b) of phosphates (*2.3.1*).

TESTS

pH (*2.2.3*)
5.0 to 6.5.

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Specific optical rotation (*2.2.7*)
+ 38.0 to + 43.0 (dried substance).

Dissolve 0.300 g in 18.2 mL of *hydrochloric acid R1* and dilute to 25.0 mL with *water R*.

Impurity E
To about 35 mg add 10 mL of *methylene chloride R*, shake for 5 min and filter. The filtrate is not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Related substances
Liquid chromatography (*2.2.29*). *Carry out the test protected from actinic light.*

Test solution Dissolve 0.100 g of the substance to be examined in 50 mL of *water R* and dilute to 100.0 mL with the mobile phase. Dilute 8.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 60 mg of *riboflavin CRS* (impurity D) in 1 mL of *hydrochloric acid R* and dilute to 250.0 mL with *water R*. Dilute 4.0 mL of this solution to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve 0.100 g of *riboflavin sodium phosphate CRS* in 50 mL of *water R* and dilute to 100.0 mL with the mobile phase. Dilute 8.0 mL of this solution to 50.0 mL with the mobile phase.

Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *methanol R*, 7.35 g/L solution of *potassium dihydrogen phosphate R* (150:850 *V/V*).

Flow rate 2 mL/min.

Detection Spectrophotometer at 266 nm.

Injection 100 µL.

Run time Until the peak due to riboflavin can be clearly evaluated.

Relative retention With reference to riboflavin 5′-monophosphate (retention time = about 20 min): impurity A = about 0.2; impurity B = about 0.3; impurity C = about 0.5; riboflavin 3′-monophosphate = about 0.7; riboflavin 4′-monophosphate = about 0.9; impurity D = about 2.

System suitability Reference solution (b):
— *resolution*: minimum 1.5 between the peaks due to riboflavin 4′-monophosphate and riboflavin 5′-monophosphate.

Calculate the percentage content of free riboflavin (impurity D) and of riboflavin in the form of the diphosphates of riboflavin (impurities A, B, C) from the areas of the peaks in the chromatogram obtained with the test solution and the amount of free riboflavin in reference solution (a).

Limits:
— *impurity D*: maximum 6.0 per cent (dried substance);
— *sum of impurities A, B and C*: maximum 6.0 per cent (dried substance).

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Inorganic phosphate
Maximum 1.5 per cent.

Dissolve 0.10 g in *water R* and dilute to 100 mL with the same solvent. To 5 mL of this solution, add 10 mL of *water R*, 5 mL of *buffered copper sulfate solution pH 4.0 R*, 2 mL of a 30 g/L solution of *ammonium molybdate R*, 1 mL of a freshly prepared solution containing 20 g/L of *4-methylaminophenol sulfate R* and 50 g/L of *sodium metabisulfite R*, and 1 mL of a 3 per cent *V/V* solution of *perchloric acid R*. Dilute to 25.0 mL with *water R* and measure, within 15 min of its preparation, the absorbance (*2.2.25*) of the solution at 800 nm, using as the compensation liquid a solution prepared in the same manner but without the substance to be examined. The absorbance is not greater than that of a solution prepared as follows: to 15 mL of *phosphate standard solution (5 ppm PO_4) R*, add 5 mL of *buffered copper sulfate solution pH 4.0 R*, 2 mL of a 30 g/L solution of *ammonium molybdate R*, 1 mL of a freshly prepared solution containing 20 g/L of *4-methylaminophenol sulfate R* and 50 g/L of *sodium metabisulfite R*, and 1 mL of a 3 per cent *V/V* solution of *perchloric acid R*; dilute to 25.0 mL with *water R*.

Loss on drying (*2.2.32*)
Maximum 8.0 per cent, determined on 1.000 g by drying in an oven at 105 °C at a pressure not exceeding 0.7 kPa for 5 h.

ASSAY

Carry out the assay protected from light.

Dissolve 0.100 g in 150 mL of *water R*, add 2 mL of *glacial acetic acid R* and dilute to 1000.0 mL with *water R*. To 10.0 mL of this solution add 3.5 mL of a 14 g/L solution of *sodium acetate R* and dilute to 50.0 mL with *water R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 444 nm.

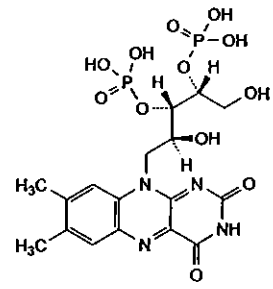
Calculate the content of $C_{17}H_{20}N_4O_6$ taking the specific absorbance to be 328.

STORAGE

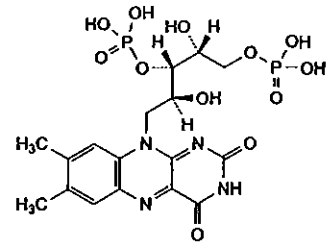
In an airtight container, protected from light.

IMPURITIES

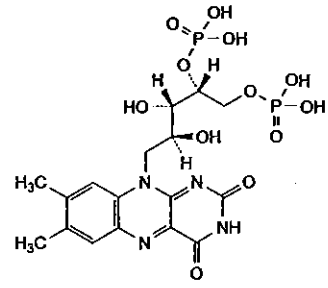
Specified impurities *A, B, C, D, E.*



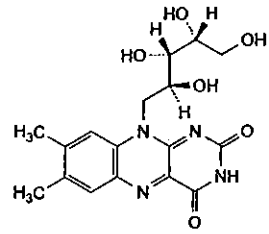
A. riboflavin 3′,4′-diphosphate,



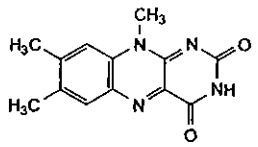
B. riboflavin 3′,5′-diphosphate,



C. riboflavin 4′,5′-diphosphate,



D. riboflavin,

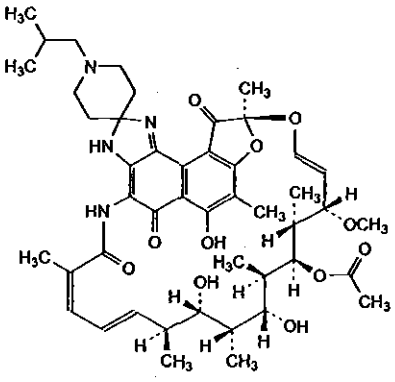


E. 7,8,10-trimethylbenzo[g]pteridine-2,4(3*H*,10*H*)-dione (lumiflavin).

Ph Eur

Rifabutin

(Ph. Eur. monograph 1657)



$C_{46}H_{62}N_4O_{11}$ 847 *72559-06-9*

Action and use
Rifamycin antimycobacterial drug.

Ph Eur

DEFINITION
(9*S*,12*E*,14*S*,15*R*,16*S*,17*R*,18*R*,19*R*,20*S*,21*S*,22*E*,24*Z*)-6,18,20-Trihydroxy-14-methoxy-7,9,15,17,19,21,25-heptamethyl-1′-(2-methylpropyl)-5,10,26-trioxo-3,5,9,10-tetrahydrospiro[9,4-(epoxypentadeca[1,11,13]trienimino)-2*H*-furo[2′,3′:7,8]naphtho[1,2-*d*]imidazole-2,4′-piperidine]-16-yl acetate.

Semi-synthetic product derived from a fermentation product.

Content
96.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
Reddish-violet amorphous powder.

Solubility
Slightly soluble in water, soluble in methanol, slightly soluble in alcohol.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *rifabutin CRS*.

B. Examine the chromatograms obtained in the test for related substances.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Impurity A
Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.100 g of the substance to be examined in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 10 mL with the same mixture of solvents.

Reference solution Dissolve 10 mg of *rifabutin impurity A CRS* in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 10 mL with the same mixture of solvents. Dilute 3 mL of the solution to 100 mL with a mixture of equal volumes of *methanol R* and *methylene chloride R*.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *acetone R, light petroleum R* (23:77 *V/V*).

Application 10 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Expose the plate to iodine vapour for about 5 min, then spray with *potassium iodide and starch solution R* and allow to stand for 5 min.

Limit:
— *impurity A*: any spot corresponding to impurity A is not more intense than the spot in the chromatogram obtained with the reference solution (0.3 per cent).

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *rifabutin CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 100.0 mL with the mobile phase.

Reference solution (c) Dissolve about 10 mg of *rifabutin CRS* in 2 mL of *methanol R*, add 1 mL of *dilute sodium hydroxide solution R* and allow to stand for about 4 min. Add 1 mL of *dilute hydrochloric acid R* and dilute to 50 mL with the mobile phase.

Column:
— *size*: *l* = 0.110 m, Ø = 4.6 mm,
— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix equal volumes of *acetonitrile R* and a 13.6 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 6.5 with *dilute sodium hydroxide solution R*.

Flow rate 1 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL.

Run time 2.5 times the retention time of rifabutin.

Relative retention With reference to rifabutin (retention time = about 9 min): impurity E = about 0.5; impurity B = about 0.6; impurity D = about 0.9; impurity C = about 1.3.

System suitability Reference solution (c):
— *resolution*: minimum 2.0 between the second peak of the 3 peaks due to degradation products and the peak due to rifabutin.

Limits:
— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b)

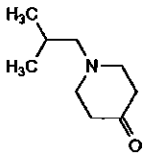
(1.0 per cent); not more than 1 such peak has an area greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),
— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent),
— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.12*)
Maximum 2.5 per cent, determined on 0.200 g.

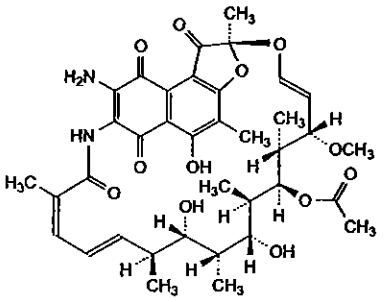
Sulfated ash (*2.4.14*)
Maximum 0.3 per cent, determined on 1.0 g.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.
Injection Test solution and reference solution (a).
Calculate the percentage content of ribabutin.

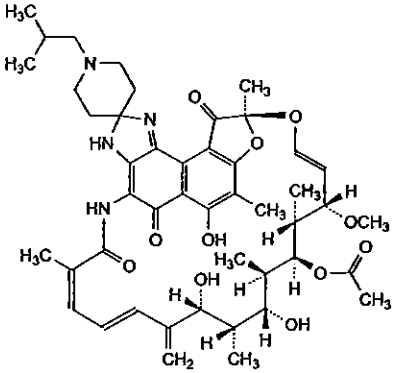
IMPURITIES



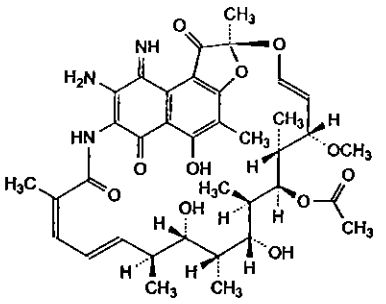
A. 1-(2-methylpropyl)piperidin-4-one,



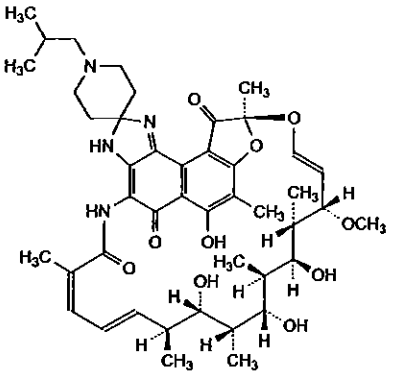
B. 3-aminorifamycin S,



C. 21,31-didehydrorifabutin,



D. 3-amino-4-imidorifamycin S,

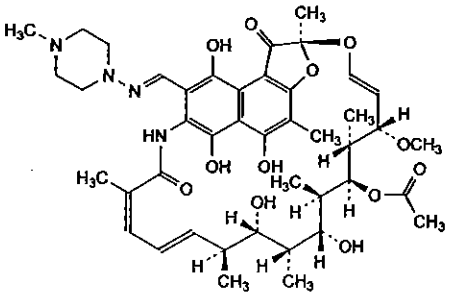


E. 16-deacetylrifabutin.

Ph Eur

Rifampicin

(Ph. Eur. monograph 0052)



$C_{43}H_{58}N_4O_{12}$ 823 *13292-46-1*

Action and use
Rifamycin antituberculosis drug.

Preparations
Rifampicin Capsules
Rifampicin Oral Suspension

Ph Eur

DEFINITION
(2*S*,12*Z*,14*E*,16*S*,17*S*,18*R*,19*R*,20*R*,21*S*,22*R*,23*S*,24*E*)-5,6,9,17,19-Pentahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-8-[[(4-methylpiperazin-1-yl)imino]methyl]-1,11-dioxo-1,2-dihydro-2,7-(epoxypentadeca[1,11,13]trienimino) naphto[2,1-*b*]furan-21-yl acetate.

Semi-synthetic antibiotic obtained from rifamycin SV.

Content
97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

Reddish-brown or brownish-red, crystalline powder.

Solubility

Slightly soluble in water, soluble in methanol, slightly soluble in acetone and in ethanol (96 per cent).

IDENTIFICATION

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 50 mg in 50 mL of *methanol R*. Dilute 1 mL of this solution to 50 mL with *phosphate buffer solution pH 7.4 R*.

Spectral range 220-500 nm.

Absorption maxima At 237 nm, 254 nm, 334 nm and 475 nm.

Absorbance ratio A_{334}/A_{475} = about 1.75.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Mulls in *liquid paraffin R*.

Comparison *rifampicin CRS*.

C. Suspend about 25 mg in 25 mL of *water R*, shake for 5 min and filter. To 5 mL of the filtrate add 1 mL of a 100 g/L solution of *ammonium persulfate R* in *phosphate buffer solution pH 7.4 R* and shake for a few minutes. The colour changes from orange-yellow to violet-red and no precipitate is formed.

TESTS

pH (*2.2.3*)

4.5 to 6.5 for a 10 g/L suspension in *carbon dioxide-free water R*.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the test solution and the reference solution immediately before use.*

Solvent mixture To 10 volumes of a 210.1 g/L solution of *citric acid monohydrate R* add 23 volumes of a 136.1 g/L solution of *potassium dihydrogen phosphate R*, 77 volumes of a 174.2 g/L solution of *dipotassium hydrogen phosphate R*, 250 volumes of *acetonitrile R* and 640 volumes of *water R*.

Test solution Dissolve 20.0 mg of the substance to be examined in *acetonitrile R* and dilute to 10.0 mL with the same solvent. Dilute 5.0 mL of this solution to 50.0 mL with the solvent mixure.

Reference solution Dissolve 20.0 mg of *rifampicin quinone CRS* (impurity A) in *acetonitrile R* and dilute to 100.0 mL with the same solvent. To 1.0 mL of this solution add 1.0 mL of the test solution and dilute to 100.0 mL with the solvent mixture.

Column:

— *size*: *l* = 0.12 m, Ø = 4.6 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 35 volumes of *acetonitrile R* and 65 volumes of a solution containing 0.1 per cent *V/V* of *phosphoric acid R*, 1.9 g/L of *sodium perchlorate R*, 5.9 g/L of *citric acid monohydrate R* and 20.9 g/L of *potassium dihydrogen phosphate R*.

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL.

Run time Twice the retention time of rifampicin.

System suitability Reference solution:

— *resolution*: minimum 4.0 between the peaks due to rifampicin and impurity A; if necessary, adjust the concentration of acetonitrile in the mobile phase.

Limits:

— *impurity A*: not more than 1.5 times the area of the corresponding peak in the chromatogram obtained with the reference solution (1.5 per cent);

— *any other impurity*: for each impurity, not more than the area of the peak due to rifampicin in the chromatogram obtained with the reference solution (1.0 per cent);

— *sum of impurities other than A*: not more than 3.5 times the area of the peak due to rifampicin in the chromatogram obtained with the reference solution (3.5 per cent);

— *disregard limit*: 0.05 times the area of the peak due to rifampicin in the chromatogram obtained with the reference solution (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying at 80 °C at a pressure not exceeding 0.67 kPa for 4 h.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 2.0 g.

ASSAY

Dissolve 0.100 g in *methanol R* and dilute to 100.0 mL with the same solvent. Dilute 2.0 mL of this solution to 100.0 mL with *phosphate buffer solution pH 7.4 R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 475 nm, using *phosphate buffer solution pH 7.4 R* as the compensation liquid.

Calculate the content of $C_{43}H_{58}N_4O_{12}$, taking the specific absorbance to be 187.

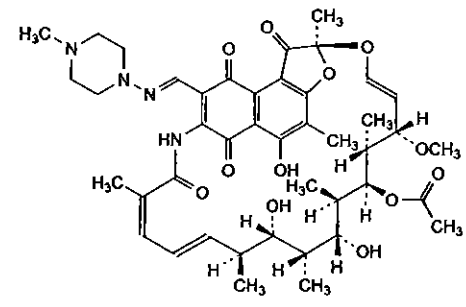
STORAGE

Under nitrogen in an airtight container, protected from light, at a temperature not exceeding 25 °C.

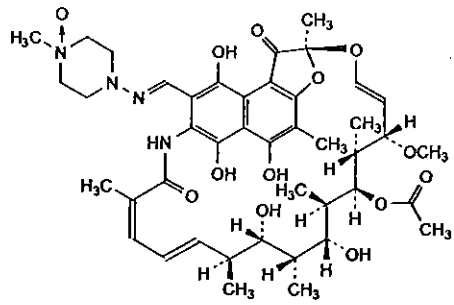
IMPURITIES

Specified impurities *A*.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B*.



A. rifampicin quinone,

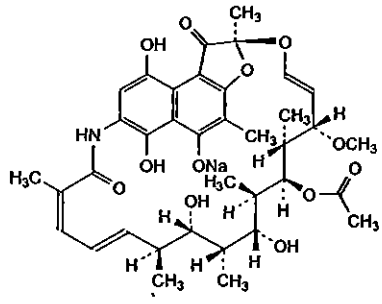


B. rifampicin *N*-oxide.

Ph Eur

Rifamycin Sodium

(Ph. Eur. monograph 0432)



$C_{37}H_{46}NNaO_{12}$ 720 *14897-39-3*

Action and use
Rifamycin antituberculosis drug.

Ph Eur

DEFINITION
Sodium
(2*S*,12*Z*,14*E*,16*S*,17*S*,18*R*,19*R*,20*R*,21*S*,22*R*,23*S*,24*E*)-21-(acetyloxy)-6,9,17,19-tetrahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-1,11-dioxo-1,2-dihydro-2,7-(epoxypentadeca[1,11,13]trienazano)naphtho[2,1-*b*]furan-5-olate.

Monosodium salt of rifamycin SV, obtained by chemical transformation of rifamycin B, which is produced during the growth of certain strains of *Amycolatopsis mediterranei*. Rifamycin SV may also be obtained directly from certain *A. mediterranei* mutants.

Potency
Minimum 900 IU/mg (anhydrous substance).

PRODUCTION
It is produced by methods of manufacture designed to minimise or eliminate substances lowering blood pressure.

CHARACTERS
Appearance
Fine or slightly granular, red powder.

Solubility
Soluble in water, freely soluble in anhydrous ethanol.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Preparation Discs of *potassium bromide R*.
Comparison *rifamycin sodium CRS*.

B. Suspend 70 mg of the substance to be examined in 0.5 mL of *water R*. Add 1.5 mL of *methoxyphenylacetic reagent R* to obtain a clear red solution. Cool in ice-water for 30 min. A precipitate is formed. Place in water at 20 °C and stir for 5 min. The precipitate does not disappear. Add 1 mL of *dilute ammonia R1*. The precipitate dissolves completely. Add 1 mL of *ammonium carbonate solution R*. No precipitate is formed.

TESTS
pH (*2.2.3*)
6.5 to 8.0.
Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Absorbance (*2.2.25*)
Dissolve 20.0 mg in 5 mL of *methanol R* and dilute to 100.0 mL with freshly prepared *phosphate buffer solution pH 7.0 R1* to which 1 g/L of *ascorbic acid R* has been added immediately before use. Dilute 5.0 mL of this solution to 50.0 mL with the same phosphate buffer solution containing ascorbic acid. Allow to stand for 30 min. The solution shows an absorption maximum at 445 nm. The specific absorbance at this absorption maximum is 190 to 210 (anhydrous substance).

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*
Solvent mixture Mix 50 volumes of *acetonitrile R* and 50 volumes of a 3.9 g/L solution of *sodium dihydrogen phosphate R* previously adjusted to pH 3.0 with *phosphoric acid R*.
Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.
Reference solution (a) Dissolve 10.0 mg of *rifamycin B CRS* (impurity A) and 40.0 mg of *rifamycin S CRS* (impurity B) in the solvent mixture and dilute to 200.0 mL with the solvent mixture. Dilute 5.0 mL of this solution to 50.0 mL with the solvent mixture.
Reference solution (b) Dissolve 25 mg of the substance to be examined and 8 mg of *rifamycin S CRS* in the solvent mixture and dilute to 250.0 mL with the solvent mixture.
Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).
Mobile phase:
— *mobile phase A*: mix 10 volumes of *acetonitrile R* and 90 volumes of a 3.9 g/L solution of *sodium dihydrogen phosphate R* adjusted to pH 7.5 with *dilute sodium hydroxide solution R*;
— *mobile phase B*: mix 30 volumes of a 3.9 g/L solution of *sodium dihydrogen phosphate R* adjusted to pH 7.5 with *dilute sodium hydroxide solution R* and 70 volumes of *acetonitrile R*;
— *temperature*: minimum 20 °C;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 40	80 → 20	20 → 80
40 - 45	20	80
45 - 47	20 → 80	80 → 20

Flow rate 1 mL/min.
Detection Spectrophotometer at 254 nm.

Injection 20 µL.

Elution order Impurity A, rifamycin SV, impurity B.

System suitability Reference solution (b):
— *resolution*: minimum 5.0 between the peaks due to rifamycin SV and impurity B.

Limits:
— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (2 per cent);
— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *sum of impurities other than A and B*: not more than the area of the peak due to impurity B in the chromatogram obtained with reference solution (a) (2 per cent);
— *disregard limit*: 0.05 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (a) (0.1 per cent).

Water (*2.5.12*)
12.0 per cent to 17.0 per cent, determined on 0.200 g.

Bacterial endotoxins (*2.6.14*)
Less than 0.50 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for removal of bacterial endotoxins.

ASSAY

Carry out the microbiological assay of antibiotics (*2.7.2*). Use *rifamycin sodium CRS* as the chemical reference substance.

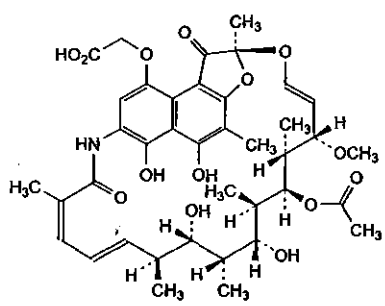
STORAGE

In an airtight container, protected from light at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-evident container.

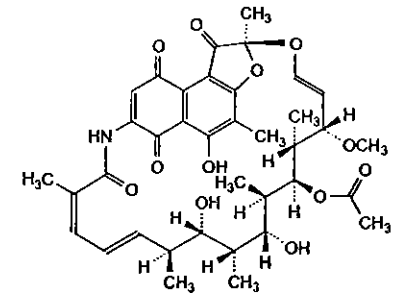
IMPURITIES

Specified impurities *A, B.*

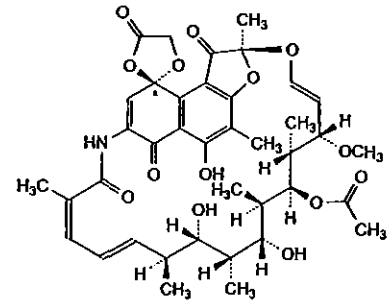
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C.*



A. [(2*S*,12*Z*,14*E*,16*S*,17*S*,18*R*,19*R*,20*R*,21*S*,22*R*,23*S*,24*E*)-21-(acetyloxy)-5,6,17,19-tetrahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-1,11-dioxo-1,2-dihydro-2,7-(epoxypentadeca[1,11,13]trienazano)naphtho[2,1-*b*]furan-9-yl]acetic acid (rifamycin B),



B. [(2*S*,12*Z*,14*E*,16*S*,17*S*,18*R*,19*R*,20*R*,21*S*,22*R*,23*S*,24*E*)-5,17,19-trihydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-1,6,9,11-tetraoxo-1,2-dihydro-2,7-(epoxypentadeca[1,11,13]trienazano)naphtho[2,1-*b*]furan-21-yl acetate (rifamycin S),



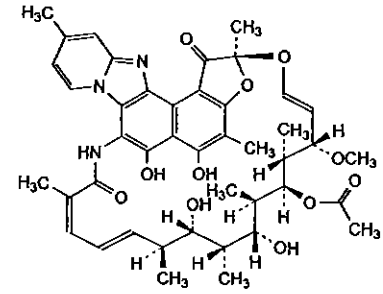
and epimer at C

C. [(2*RS*,2′*S*,12′*Z*,14′*E*,16′*S*,17′*S*,18′*R*,19′*R*,20′*R*,21′*S*,22′*R*,23′*S*,24′*E*)-5′,17′,19′-trihydroxy-23′-methoxy-2′,4′,12′,16′,18′,20′,22′-heptamethyl-1′,4,6′,11′-tetraoxo-1′,2′-dihydrospiro[1,3-dioxolane-2,9′(6′*H*)-[2,7](epoxypentadeca[1,11,13]trienazano)naphtho[2,1-*b*]furan]-21′-yl acetate (rifamycin O).

Ph Eur

Rifaximin

(Ph. Eur. monograph 2362)



$C_{43}H_{51}N_3O_{11}$ 786 *80621-81-4*

Action and use
Antibacterial; treatment of infective diarrhoea.

Preparation
Rifaximin Tablets

Ph Eur

DEFINITION

(2*S*,16*Z*,18*E*,20*S*,21*S*,22*R*,23*R*,24*R*,25*S*,26*R*,27*S*,28*E*)-5,6,21,23-Tetrahydroxy-27-methoxy-2,4,11,16,20,22,24,26-octamethyl-1,15-dioxo-1,2-dihydro-2,7-(epoxypentadeca

[1,11,13]trienoimino)[1]benzofuro[4,5-*e*]pyrido[1,2-*a*] benzimidazol-25-yl acetate.

Semi-synthetic product derived from a fermentation product.

Content
97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
Red-orange, hygroscopic, crystalline powder.

Solubility
Practically insoluble in water, soluble in acetone and in methanol.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *rifaximin CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *ethanol R*, evaporate to dryness and record new spectra using the residues.

TESTS

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R, water R* (40:60 *V/V*).

Test solution (a) Dissolve 0.100 g of the substance to be examined in 8 mL of *acetonitrile R* and dilute to 20 mL with *water R*.

Test solution (b) Dissolve 40.0 mg of the substance to be examined in the solvent mixture and dilute to 100.0 mL with the solvent mixture. Dilute 5.0 mL of the solution to 50.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of test solution (a) to 50.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 5 mg of *rifaximin for system suitability CRS* (containing impurity H) in 4 mL of the solvent mixture.

Reference solution (c) Dissolve 40.0 mg of *rifaximin CRS* in the solvent mixture and dilute to 100.0 mL with the solvent mixture. Dilute 5.0 mL of the solution to 50.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 40 °C.

Mobile phase Mix 37 volumes of a 3.16 g/L solution of *ammonium formate R* adjusted to pH 7.2 with *dilute ammonia R1* and 63 volumes of a mixture of equal volumes of *acetonitrile R* and *methanol R*.

Flow rate 1.4 mL/min.

Detection Spectrophotometer at 276 nm.

Injection 20 µL of test solution (a) and reference solutions (a) and (b).

Run time 3 times the retention time of rifaximin.

Relative retention With reference to rifaximin (retention time = about 12 min): impurities D and H = about 0.7.

System suitability Reference solution (b):
— *resolution*: minimum 3.0 between the peaks due to impurities D + H and rifaximin.

Limits:
— *sum of impurities D and H*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)
Maximum 4.5 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (c).

Calculate the percentage content of $C_{43}H_{51}N_3O_{11}$ using the chromatogram obtained with reference solution (c) and the declared content of *rifaximin CRS*.

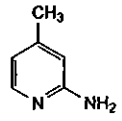
STORAGE

In an airtight container, protected from light.

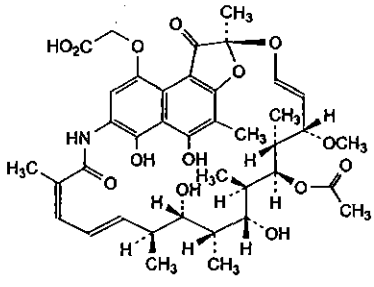
IMPURITIES

Specified impurities *D, H.*

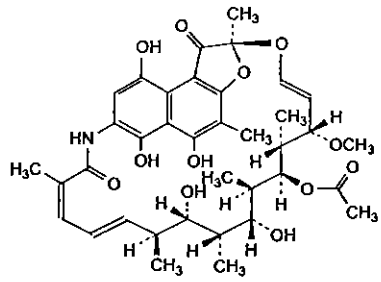
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, E, F, G.*



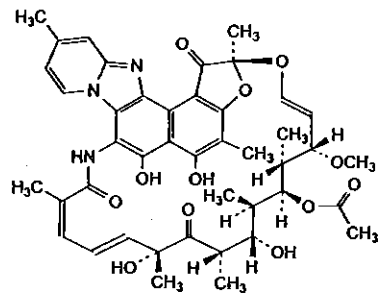
A. 4-methylpyridin-2-amine,



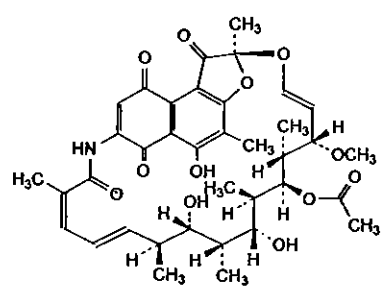
B. rifamycin B,



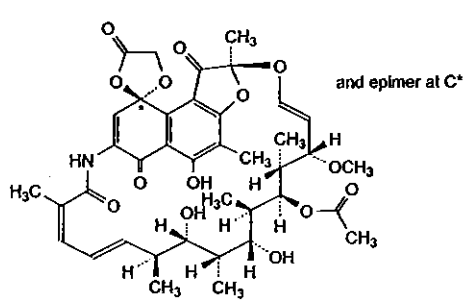
C. rifamycin SV,



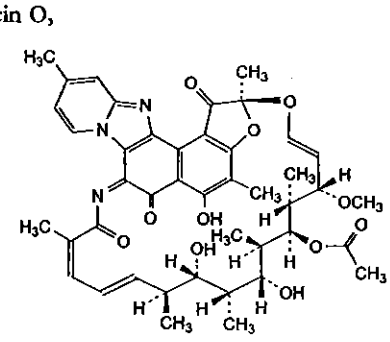
D. rifaximin Y,



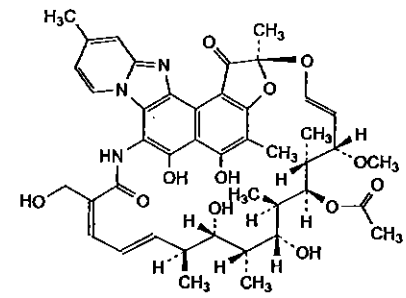
E. rifamycin S,



F. rifamycin O,



G. (2*S*,7*Z*,16*Z*,18*E*,20*S*,21*S*,22*R*,23*R*,24*R*,25*S*,26*R*,27*S*,28*E*)-5,21,23-trihydroxy-27-methoxy-2,4,11,16,20,22,24,26-octamethyl-1,6,15-trioxo-1,2,6,7-tetrahydro-2,7-(epoxypentadeca[1,11,13]trienonitrilo)[1]benzofuro[4,5-*e*]pyrido[1,2-*a*]benzimidazol-25-yl acetate (6-*O*,14-didehydrorifaximin),

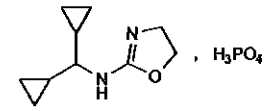


H. (2*S*,16*Z*,18*E*,20*S*,21*S*,22*R*,23*R*,24*R*,25*S*,26*R*,27*S*,28*E*)-5,6,21,23-tetrahydroxy-16-(hydroxymethyl)-27-methoxy-2,4,11,20,22,24,26-heptamethyl-1,15-dioxo-1,2-dihydro2,7-(epoxypentadeca[1,11,13]trienoimino)[1]benzofuro[4,5-*e*]pyrido[1,2-*a*]benzimidazol-25-yl acetate (16-desmethyl-16-(hydroxymethyl)rifaximin).

Ph Eur

Rilmenidine Dihydrogen Phosphate

(Ph. Eur. monograph 2020)



$C_{10}H_{19}N_2O_5P$ 278.2 *85409-38-7*

Action and use
Antihypertensive.

Ph Eur

DEFINITION

N-(Dicyclopropylmethyl)-4,5-dihydro-oxazol-2-amine dihydrogen phosphate.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Freely soluble in water, slightly soluble in alcohol, practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of rilmenidine dihydrogen phosphate.*

B. Dissolve 10 mg in *water R* and dilute to 1 mL with the same solvent. The solution gives reaction (b) of phosphates (*2.3.1*).

TESTS

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 60.0 mg of the substance to be examined in *water R* and dilute to 20.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *water R* and dilute 10.0 mL of this solution to 50.0 mL with the same solvent.

Reference solution (b) Dilute 5.0 mL of reference solution (a) to 20.0 mL with *water R*.

Reference solution (c) Dissolve 15.0 mg of *rilmenidine for system suitability CRS* in *water R* and dilute to 5.0 mL with the same solvent.

Column:
- *size*: *l* = 0.15 m, Ø = 3 mm,
- *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (5 µm) with a pore size of 10 nm and a carbon loading of 25 per cent,
- *temperature*: 40 °C.

Mobile phase:
- *mobile phase A*: dissolve 3 g of *sodium heptanesulfonate R* in *water R* and dilute to 860 mL with the same solvent; add 130 mL of *methanol R2*, 10 mL of *tetrahydrofuran for chromatography R* and 1.0 mL of *phosphoric acid R*,
- *mobile phase B*: dissolve 3 g of *sodium heptanesulfonate R* in *water R* and dilute to 600 mL with the same solvent; add 350 mL of *acetonitrile for chromatography R*, 50 mL of *tetrahydrofuran for chromatography R* and 1.0 mL of *phosphoric acid R*,

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 14	100 → 0	0 → 100
14 - 15	0 → 100	100 → 0
15 - 30	100	0

Flow rate 1 mL/min.

Detection Spectrophotometer at 205 nm.

Injection 20 µL.

Relative retention With reference to rilmenidine (retention time = about 13 min): impurity A = about 0.6; impurity B = about 0.9; impurity C = about 1.4.

With these conditions the inflexion of the baseline, corresponding to the beginning of the gradient, appears on the recorder after a minimum time *t* of 5 min. If this is not the case (*t* < 5 min) modify the chromatographic sequence by adding an isocratic elution with 100 per cent of mobile phase A for a time corresponding to (5-*t*) min before the linear gradient.

System suitability Reference solution (c):
- *peak-to-valley ratio*: minimum 3, where H_p = height above the baseline of the peak due to impurity B and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to rilmenidine.

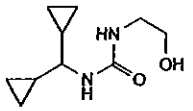
Limits:
- *any impurity*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
- *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent),
- *disregard limit*: area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 50 °C for 2 h.

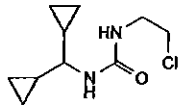
ASSAY
Dissolve 0.200 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 27.82 mg of $C_{10}H_{19}N_2O_5P$.

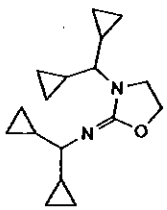
IMPURITIES
Specified impurities *A, B, C.*



A. 1-(dicyclopropylmethyl)-3-(2-hydroxyethyl)urea,



B. 1-(2-chloroethyl)-3-(dicyclopropylmethyl)urea,

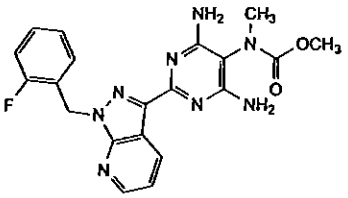


C. *N*,3-bis(dicyclopropylmethyl)oxazolidin-2-imine.

Ph Eur

Riociguat

(Ph. Eur. monograph 3078)



$C_{20}H_{19}FN_8O_2$ 422.4 *625115-55-1*

Action and use
Guanylate cyclase stimulator; treatment of pulmonary hypertension.

Preparation
Riociguat Tablets

Ph Eur

DEFINITION
Methyl [4,6-diamino-2-[1-[(2-fluorophenyl)methyl]-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]pyrimidin-5-yl](methyl) carbamate.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or yellowish powder.

Solubility
Practically insoluble in water, very slightly soluble in anhydrous ethanol, practically insoluble in heptane.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *riociguat CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

TESTS

Protect the solutions from light throughout the tests.

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture Mobile phase A, *acetonitrile R* (20:80 *V/V*).

Test solution Dissolve 20.0 mg of the substance to be examined in the solvent mixture using sonication and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 4 mg of *riociguat for system suitability CRS* (containing impurities B and C) in the solvent mixture using sonication and dilute to 10 mL with the solvent mixture.

Reference solution (c) Dissolve 20.0 mg of *riociguat CRS* in the solvent mixture using sonication and dilute to 50.0 mL with the solvent mixture.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase:
- *mobile phase A*: *perchloric acid R, water for chromatography R* (0.4:100 *V/V*);
- *mobile phase B*: *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	75	25
2 - 27	75 → 65	25 → 35
27 - 42	65 → 32	35 → 68
42 - 43	32 → 10	68 → 90
43 - 52	10	90

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 210 nm.

Autosampler Set at 15 °C.

Injection 5 µL of the test solution and reference solutions (a) and (b).

Identification of impurities Use the chromatogram supplied with *riociguat for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B and C.

Relative retention With reference to riociguat (retention time = about 22 min): impurity B = about 0.97; impurity C = about 1.4.

System suitability Reference solution (b):
- *resolution*: minimum 1.5 between the peaks due to impurity B and riociguat.

Calculation of percentage contents:
- for each impurity, use the concentration of riociguat in reference solution (a).

Limits:
- *impurity C*: maximum 0.20 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.3 per cent;
- *reporting threshold*: 0.05 per cent.

Impurity E

Head-space gas chromatography (*2.2.28*).

Test solution Dissolve 50 mg of the substance to be examined in 1.0 mL of *dimethyl sulfoxide R* in a head-space vial.

Reference solution Dilute 50 µL of *benzene R* (impurity E) to 10.0 mL with *dimethyl sulfoxide R*. Dilute 11.5 µL of the solution to 10.0 mL with *dimethyl sulfoxide R*. Transfer 20 µL of this solution into a head-space vial and add 1.0 mL of *dimethyl sulfoxide R*.

Column:
- *material*: fused silica;
- *size*: *l* = 60 m, Ø = 0.32 mm;
- *stationary phase*: *macrogol 20 000 R* (film thickness 0.5 µm).

Carrier gas *helium for chromatography R*.

Flow rate 3.3 mL/min.

Split ratio 1:20.

Static head-space conditions that may be used:
- *equilibration temperature*: 100 °C;
- *equilibration time*: 30 min;
- *transfer-line temperature*: 120 °C;
- *pressurisation time*: 30 s;
- *injection volume*: 1.0 mL;
- *injection time*: 1 min.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 10	40 → 60
	10 - 15	60
	15 - 18.2	60 → 140
	18.2 - 35	140
Injection port		120
Detector		250

Detection Flame ionisation.

Identification of impurities Use the chromatogram obtained with the reference solution to identify the peak due to impurity E.

Retention time Impurity E = about 7.1 min.

System suitability Reference solution:
- *signal-to-noise ratio*: minimum 15 for the peak due to impurity E.

Limit:
- *impurity E*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (2 ppm).

Water (*2.5.32*)

Maximum 0.2 per cent, determined on 0.250 g using the evaporation technique at 150 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

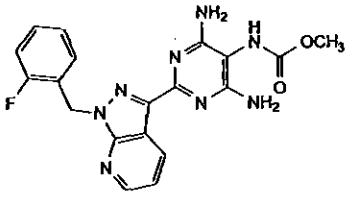
Injection Test solution and reference solution (c).

Calculate the percentage content of $C_{20}H_{19}FN_8O_2$ taking into account the assigned content of *riociguat CRS*.

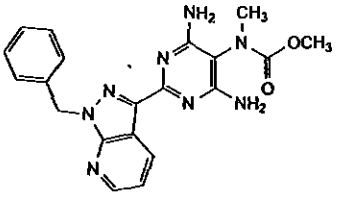
IMPURITIES

Specified impurities C, E.

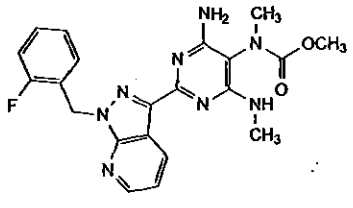
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) A, B, D.



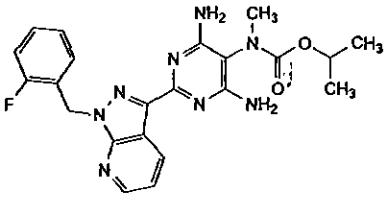
A. methyl [4,6-diamino-2-[1-[(2-fluorophenyl)methyl]-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]pyrimidin-5-yl]carbamate,



B. methyl [4,6-diamino-2-(1-benzyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)pyrimidin-5-yl](methyl)carbamate,



C. methyl [4-amino-2-[1-[(2-fluorophenyl)methyl]-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]-6-(methylamino)pyrimidin-5-yl](methyl)carbamate,



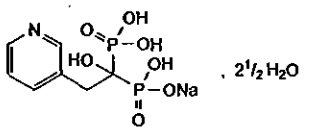
D. propan-2-yl [4,6-diamino-2-[1-[(2-fluorophenyl)methyl]-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]pyrimidin-5-yl](methyl)carbamate,

E. benzene.

Ph Eur

Risedronate Sodium 2.5-Hydrate

(Ph. Eur. monograph 2572)



$C_7H_{10}NNaO_7P_2,2½H_2O$ 350.1 *329003-65-8*

Action and use

Bisphosphonate; treatment of osteoporosis, Paget's disease.

Preparation

Risedronate Sodium Tablets

Ph Eur

DEFINITION

Sodium hydrogen [1-hydroxy-1-phosphono-2-(pyridin-3-yl) ethyl]phosphonate hemipentahydrate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Soluble in water, practically insoluble in methanol.
It dissolves in dilute solutions of alkali hydroxides and mineral acids.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *risedronate sodium 2.5-hydrate CRS*.

B. It gives reaction (a) of sodium (*2.3.1*). Dissolution of the substance to be examined is achieved after the addition of the 150 g/L solution of *potassium carbonate R*.

C. Water (see Tests).

TESTS

pH (*2.2.3*)
4.0 to 5.0.

Dissolve 0.10 g in *carbon dioxide-free water R* with the aid of an ultrasonic bath and dilute to 10 mL with the same solvent.

Related substances

A. Liquid chromatography (*2.2.29*).

Buffer solution Dissolve 0.410 g of *sodium edetate R*, 1.7 g of *dipotassium hydrogen phosphate R* and 1.7 g of *tetrabutylammonium dihydrogen phosphate R* in 900 mL of *water R*, adjust to pH 7.5 with *1 M sodium hydroxide* and dilute to 1000 mL with *water R*.

Solution A Dissolve 100 mg of *sodium chloride R* in the mobile phase and dilute to 10.0 mL with the mobile phase.

Test solution Dissolve 50 mg of the substance to be examined in the mobile phase by gentle swirling and heating for 5-10 min and dilute to 20.0 mL with the mobile phase.

Reference solution (a) To 2.0 mL of the test solution add 5 mg of *risedronate impurity E CRS* and dilute to 50.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:
- — *size*: *l* = 0.15 m, Ø = 4.6 mm;
- — *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm);
- — *temperature*: 40 °C.

Mobile phase *acetonitrile R*, buffer solution (10:90 *V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 263 nm.

Injection 20 µL.

Run time Twice the retention time of risedronate.

Relative retention With reference to risedronate (retention time = about 17 min): impurity E = about 0.95.

System suitability Reference solution (a):
- — *resolution*: minimum 3.0 between the peaks due to impurity E and risedronate.

Limits:
- — *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- — *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- — *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak due to solution A.

B. Liquid chromatography (*2.2.29*) as described in test A for related substances, with the following modifications.

Reference solution (a) Dissolve 5.0 mg of the substance to be examined in 50.0 mL of the mobile phase by gentle swirling and heating for 5-10 min, using an ultrasonic bath if necessary.

Reference solution (b) Dissolve 5.0 mg of *risedronate impurity A CRS* in the mobile phase by gentle swirling and heating for 5-10 min, using an ultrasonic bath if necessary, and dilute to 50.0 mL with the same solvent.

Reference solution (c) Dilute 0.5 mL of reference solution (b) to 20.0 mL with the mobile phase.

Reference solution (d) Mix 1.0 mL of reference solution (a) and 1.0 mL of reference solution (b) and dilute to 20.0 mL with the mobile phase.

Mobile phase *acetonitrile R*, buffer solution (25:75 *V/V*).

Injection 10 µL of the test solution, reference solutions (b), (c) and (d) and solution A.

Run time 8 times the retention time of risedronate.

Identification Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity A.

Relative retention With reference to risedronate (retention time = about 4 min): impurity A = about 2.2.

System suitability Reference solution (d):
- — *resolution*: minimum 10.0 between the peaks due to risedronate and impurity A.

Limits:
- — *impurity A*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.15 per cent);
- — *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);
- — *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);
- — *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent); disregard any peak due to solution A and any peak eluting before the peak due to risedronate.

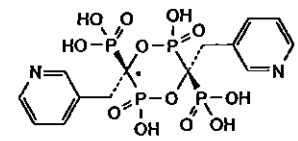
Water (*2.5.12*)
11.9 per cent to 13.9 per cent, determined on 0.100 g.

ASSAY
Dissolve 0.125 g in 50 mL of *carbon dioxide-free water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

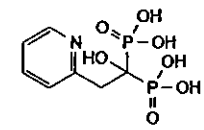
1 mL of *0.1 M sodium hydroxide* is equivalent to 15.26 mg of $C_7H_{10}NNaO_7P_2$.

IMPURITIES
Specified impurities *A*.

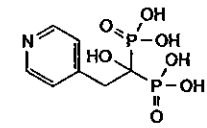
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D, E.*



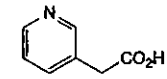
A. [(3*RS*,6*RS* and 3*R*,6*S*-*meso*)-2,5-dihydroxy-2,5-dioxo-3,6-bis[(pyridin-3-yl)methyl]-1,4,2λ^5,5λ^5-dioxadiphosphinane-3,6-diyl]bis(phosphonic acid),



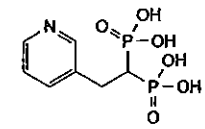
B. [1-hydroxy-2-(pyridin-2-yl)ethylidene]bis(phosphonic acid),



C. [1-hydroxy-2-(pyridin-4-yl)ethylidene]bis(phosphonic acid),



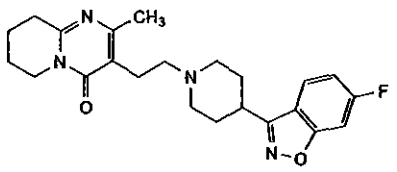
D. 2-(pyridin-3-yl)acetic acid,



E. [2-(pyridin-3-yl)ethylidene]bis(phosphonic acid).

Risperidone

(Ph. Eur. monograph 1559)



$C_{23}H_{27}FN_4O_2$ 410.5 *106266-06-2*

Action and use
Dopamine D_2 receptor antagonist; serotonin $5HT_2$ receptor antagonist; neuroleptic.

Preparations
Risperidone Oral Solution
Risperidone Tablets
Risperidone Dispersible Tablets

Ph Eur

DEFINITION

3-[2-[4-(6-Fluoro-1,2-benzisoxazol-3-yl)piperidin-1-yl]ethyl]-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in ethanol (96 per cent).
It dissolves in dilute acid solutions.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *risperidone CRS*.

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *acetone R*, evaporate to dryness and record new spectra using the residues.

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 0.1 g in a 7.5 g/L solution of *tartaric acid R* and dilute to 100 mL with the same acid solution.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dissolve 10 mg of *risperidone for system suitability CRS* (containing impurities A, B, C, D and E) in 1.0 mL of *methanol R*.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *methanol R*. Dilute 5.0 mL of this solution to 25.0 mL with *methanol R*.

Reference solution (c) Dissolve the contents of a vial of *risperidone impurity K CRS* in 1.0 mL of *methanol R*.

Column:
— *size*: *l* = 0.10 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
— *mobile phase A*: 5 g/L solution of *ammonium acetate R*;
— *mobile phase B*: *methanol R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	70	30
2 - 17	70 → 30	30 → 70
17 - 22	30	70

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 260 nm.

Injection 10 µL.

Identification of impurities Use the chromatogram supplied with *risperidone for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D and E; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity K.

Relative retention With reference to risperidone (retention time = about 12 min): impurity A = about 0.7; impurity B = about 0.75; impurity C = about 0.8; impurity K = about 0.9; impurity D = about 0.94; impurity E = about 1.1.

System suitability Reference solution (a):
— the chromatogram obtained is similar to the chromatogram supplied with *risperidone for system suitability CRS*;
— *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to risperidone.

Limits:
— *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
— *impurity K*: not more than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Dissolve 0.160 g in 70 mL of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*

and titrate with *0.1 M perchloric acid*. Determine the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 20.53 mg of $C_{23}H_{27}FN_4O_2$.

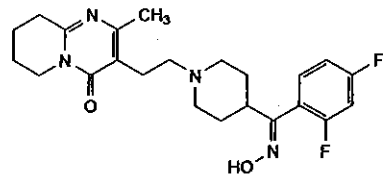
STORAGE

Protected from light.

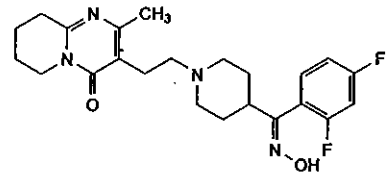
IMPURITIES

Specified impurities A, B, C, D, E, K.

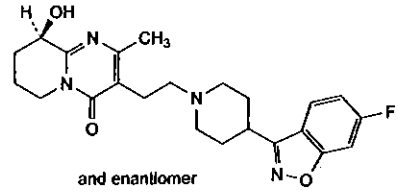
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) F, H, I, J, L, M.



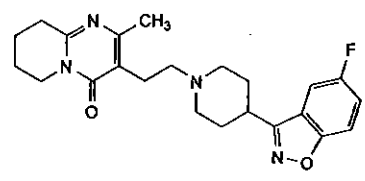
A. 3-[2-[4-[(*E*)-(2,4-difluorophenyl)(hydroxyimino)methyl] piperidin-1-yl]ethyl]-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one,



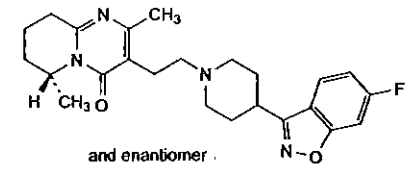
B. 3-[2-[4-[(*Z*)-(2,4-difluorophenyl)(hydroxyimino)methyl] piperidin-1-yl]ethyl]-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one,



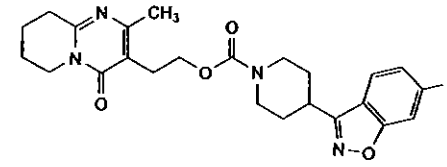
C. (9*RS*)-3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)piperidin-1-yl]ethyl]-9-hydroxy-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido [1,2-*a*]pyrimidin-4-one,



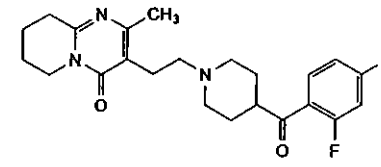
D. 3-[2-[4-(5-fluoro-1,2-benzisoxazol-3-yl)piperidin-1-yl] ethyl]-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*] pyrimidin-4-one,



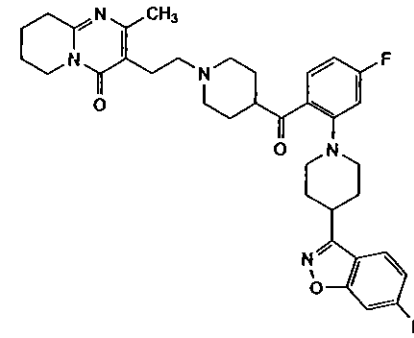
E. (6*RS*)-3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)piperidin-1-yl]ethyl]-2,6-dimethyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*] pyrimidin-4-one,



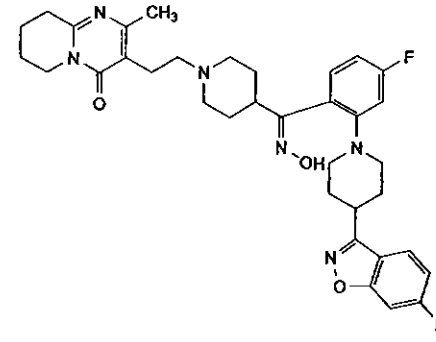
F. 2-[2-methyl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*] pyrimidin-3-yl]ethyl 4-(6-fluoro-1,2-benzisoxazol-3-yl) piperidin-1-carboxylate,



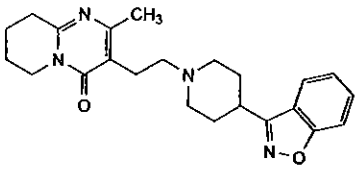
H. 3-[2-[4-(2,4-difluorobenzoyl)piperidin-1-yl]ethyl]-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one,



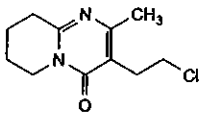
I. 3-[2-[4-[4-fluoro-2-[4-(6-fluoro-1,2-benzisoxazol-3-yl) piperidin-1-yl]benzoyl]piperidin-1-yl]ethyl]-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one,



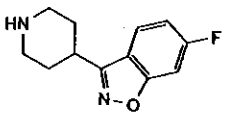
J. 3-[2-[4-[(*Z*)-[4-fluoro-2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)piperidin-1-yl]phenyl](hydroxyimino)methyl]piperidin-1-yl]ethyl]-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*] pyrimidin-4-one,



K. 3-[2-[4-(1,2-benzisoxazol-3-yl)piperidin-1-yl]ethyl]-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (desfluoro risperidone),



L. 3-(2-chloroethyl)-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (piperidopyrimidinone intermediate),

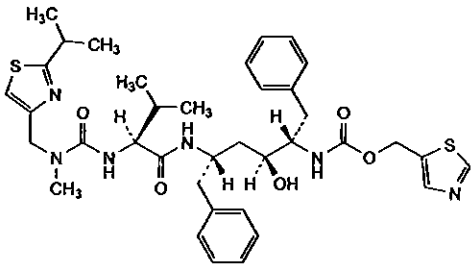


M. 6-fluoro-3-(piperidin-4-yl)-1,2-benzisoxazole.

Ph Eur

Ritonavir

(Ph. Eur. monograph 2136)



$C_{37}H_{48}N_6O_5S_2$ 721 *155213-67-5*

Action and use
Protease inhibitor; antiviral (HIV).

Preparations
Ritonavir Oral Solution
Ritonavir Tablets

Ph Eur

DEFINITION
Thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-1-benzyl-2-hydroxy-4-[[(2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanoyl]amino]-5-phenylpentyl]carbamate.

Content
97.0 per cent to 102.0 per cent (anhydrous substance).

PRODUCTION
The production method is validated to demonstrate suitable enantiomeric purity of the final product.

CHARACTERS
Appearance
White or almost white powder.

Solubility
Practically insoluble in water, freely soluble in methanol and in methylene chloride, very slightly soluble in acetonitrile.

It shows polymorphism (*5.9*).

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *ritonavir CRS.*

If the spectra obtained in the solid state show differences dissolve the substance to be examined and the reference substance separately in *methylene chloride R*, evaporate to dryness and record new spectra using the residues.

TESTS
Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture Mix equal volumes of *acetonitrile R* and a 4.1 g/L solution of *potassium dihydrogen phosphate R*.

Test solution (a) Dissolve 10.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture. Sonicate if necessary.

Test solution (b) Dilute 5.0 mL of test solution (a) to 10.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 20.0 mL with the solvent mixture.

Reference solution (a) Dissolve 5.0 mg of *ritonavir for peak identification CRS* (containing impurities E, F, L, O and T) in the solvent mixture and dilute to 5.0 mL with the solvent mixture. Sonicate if necessary.

Reference solution (b) Dilute 1.0 mL of test solution (a) to 10.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 100.0 mL with the solvent mixture.

Reference solution (c) Dissolve 10.0 mg of *ritonavir CRS* in the solvent mixture and dilute to 10.0 mL with the solvent mixture. Sonicate if necessary. Dilute 5.0 mL of this solution to 10.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 20.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped butylsilyl silica gel for chromatography R* (3 µm);
— *temperature*: 60 °C.

Mobile phase:
— *mobile phase A*: mix 5 volumes of *butanol R*, 8 volumes of *tetrahydrofuran R*, 18 volumes of *acetonitrile R* and 69 volumes of a 4.1 g/L solution of *potassium dihydrogen phosphate R* filtered through a 0.45 µm nylon membrane;
— *mobile phase B*: mix 5 volumes of *butanol R*, 8 volumes of *tetrahydrofuran R*, 40 volumes of a 4.1 g/L solution of *potassium dihydrogen phosphate R* filtered through a 0.45 µm nylon membrane and 47 volumes of *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 60	100	0
60 - 120	100 → 0	0 → 100
120 - 120.1	0 → 100	100 → 0
120.1 - 155	100	0

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 240 nm.

Injection 50 µL of test solution (a) and reference solutions (a) and (b).

Identification of impurities Use the chromatogram supplied with *ritonavir for peak identification CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities E, F, L, O and T.

Relative retention With reference to ritonavir (retention time = about 34 min): impurity E = about 0.39; impurity F = about 0.40; impurity L = about 0.8; impurity O = about 1.1; impurity T = about 2.6.

System suitability Reference solution (a):

— *peak-to-valley ratio*: minimum 1.2, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity F.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity F = 1.4; impurity L = 1.9; impurity T = 1.4;

— *impurities E, O*: for each impurity, not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *impurity T*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *impurities F, L*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.12*)
Maximum 0.5 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*)
Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (c).

Calculate the percentage content of $C_{37}H_{48}N_6O_5S_2$ from the declared content of *ritonavir CRS*.

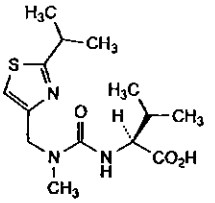
STORAGE

Protected from light.

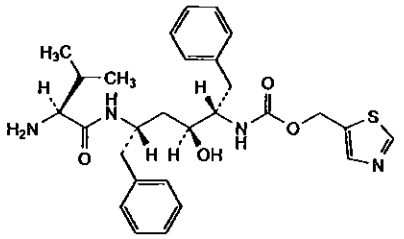
IMPURITIES

Specified impurities *E, F, L, O, T.*

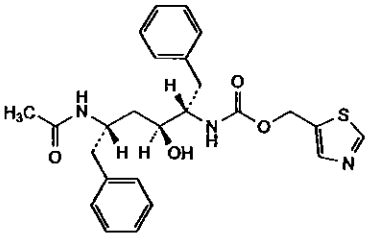
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, G, H, I, J, K, M, N, P, Q, R, S, U.*



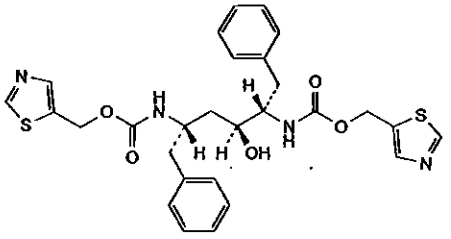
A. (2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanoic acid,



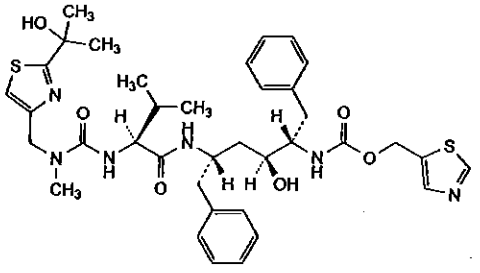
B. thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-4-[[(2*S*)-2-amino-3-methylbutanoyl]amino]-1-benzyl-2-hydroxy-5-phenylpentyl]carbamate,



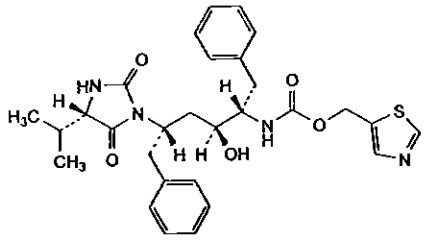
C. thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-4-(acetylamino)-1-benzyl-2-hydroxy-5-phenylpentyl]carbamate,



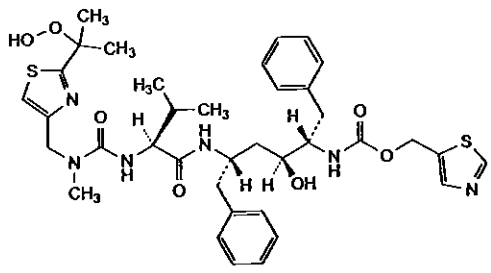
D. thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-1-benzyl-2-hydroxy-5-phenyl-4-[[(thiazol-5-ylmethoxy)carbonyl]amino]pentyl]carbamate,



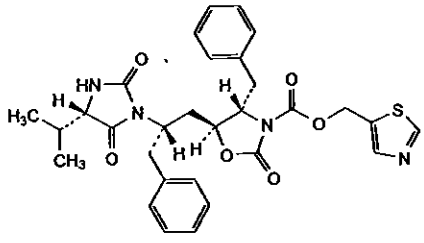
E. thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-1-benzyl-2-hydroxy-4-[[(2*S*)-2-[[[[2-(1-hydroxy-1-methylethyl)thiazol-4-yl]methyl]methylcarbamoyl]amino]-3-methylbutanoyl]amino]-5-phenylpentyl]carbamate,



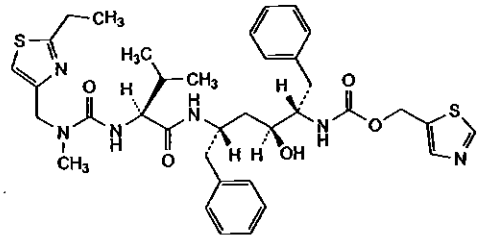
F. thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-1-benzyl-4-[[(2*S*)-1-benzyl-2-hydroxy-4-[(4*S*)-4-(1-methylethyl)-2,5-dioxoimidazolidin-1-yl]-5-phenylpentyl]carbamate,



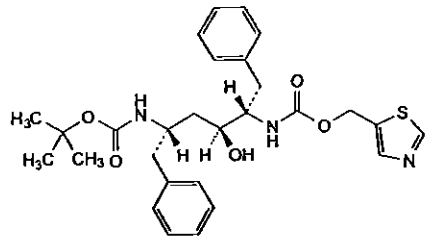
G. thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-1-benzyl-4-[[(2*S*)-2-[[[[2-(1-hydroperoxy-1-methylethyl)thiazol-4-yl]methyl]methylcarbamoyl]amino]-3-methylbutanoyl]amino]-2-hydroxy-5-phenylpentyl]carbamate,



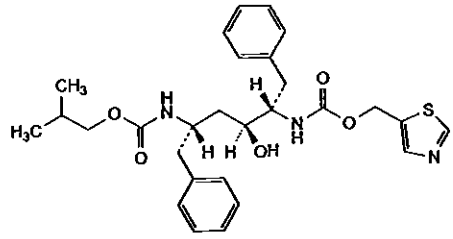
H. thiazol-5-ylmethyl (4*S*,5*S*)-4-benzyl-5-[(2*S*)-2-[(4*S*)-4-(1-methylethyl)-2,5-dioxoimidazolidin-1-yl]-3-phenylpropyl]-2-oxooxazolidine-3-carboxylate,



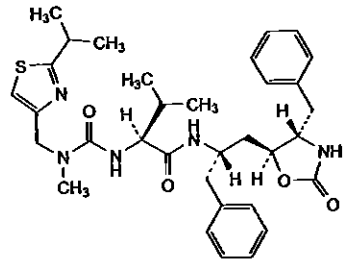
I. thiazol-5-ylmethyl [((1*S*,2*S*,4*S*)-1-benzyl-4-[[(2*S*)-2-[[[[2-ethylthiazol-4-yl]methyl]methylcarbamoyl]amino]-3-methylbutanoyl]amino]-2-hydroxy-5-phenylpentyl] carbamate,



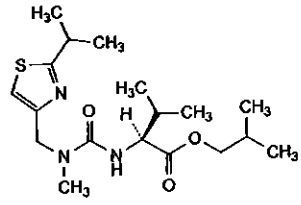
J. thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-1-benzyl-4-[[(1,1-dimethylethoxy)carbonyl]amino]-2-hydroxy-5-phenylpentyl]carbamate,



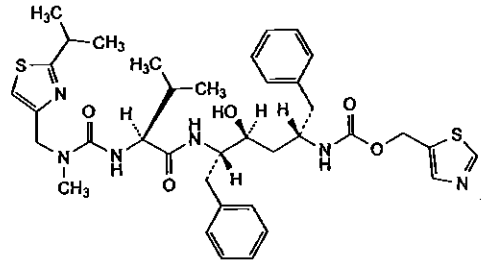
K. thiazol-5-ylmethyl (1*S*,2*S*,4*S*)-1-benzyl-2-hydroxy-4-[[(2-methylpropoxy)carbonyl]amino]-5-phenylpentyl] carbamate,



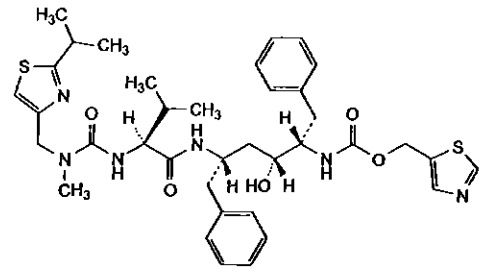
L. (4*S*,5*S*)-4-benzyl-5-[(2*S*)-2-[[(2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl] amino]butanoyl]amino]-3-phenylpropyl]oxazolidin-2-one,



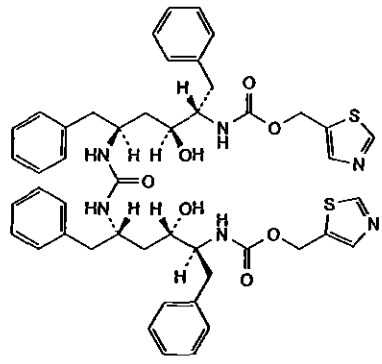
M. 2-methylpropyl (2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino] butanoate,



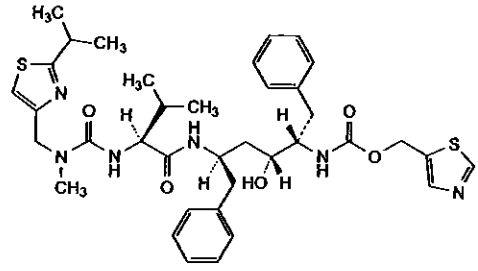
N. thiazol-5-ylmethyl [(1*S*,3*S*,4*S*)-1-benzyl-3-hydroxy-4-[[(2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl] methyl]carbamoyl]amino]butanoyl]amino]-5-phenylpentyl] carbamate,



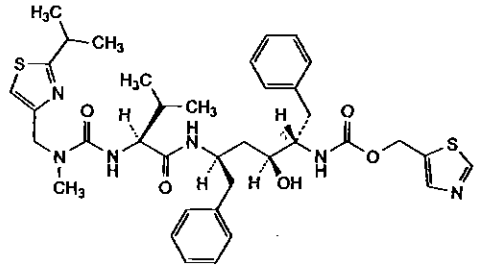
O. thiazol-5-ylmethyl [(1*S*,2*R*,4*S*)-1-benzyl-2-hydroxy-4-[[(2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanoyl]amino]-5-phenylpentyl] carbamate,



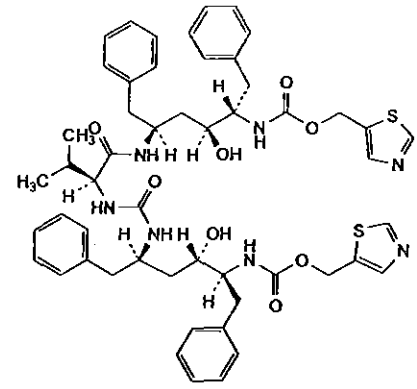
P. bis(thiazol-5-ylmethyl) [carbonylbis[imino[(2*S*,3*S*,5*S*)-3-hydroxy-1,6-diphenylhexane-5,2-diyl]]]dicarbamate,



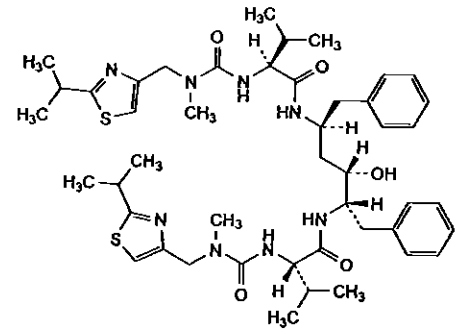
Q. thiazol-5-ylmethyl [(1*S*,2*R*,4*R*)-1-benzyl-2-hydroxy-4-[[(2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanoyl]amino]-5-phenylpentyl] carbamate,



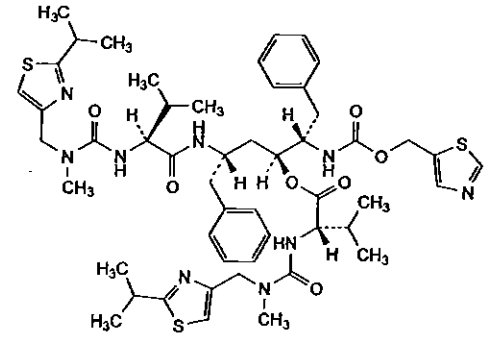
R. thiazol-5-ylmethyl [(1*S*,2*S*,4*R*)-1-benzyl-2-hydroxy-4-[[(2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanoyl]amino]-5-phenylpentyl] carbamate,



S. thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-1-benzyl-4-[[(2*S*)-2-[[[(1*S*,3*S*,4*S*)-1-benzyl-3-hydroxy-5-phenyl-4-[[(thiazol-5-ylmethoxy)carbonyl]amino]pentyl]carbamoyl]amino]-3-methylbutanoyl]amino]-2-hydroxy-5-phenylpentyl]carbamate,



T. (2*S*)-*N*-[(1*S*,2*S*,4*S*)-1-benzyl-2-hydroxy-4-[[(2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanoyl]amino]-5-phenylpentyl]-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanamide,

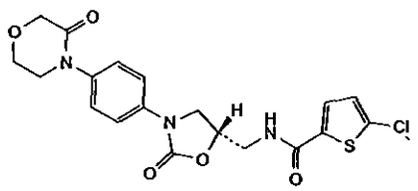


U. (1*S*,3*S*)-3-[[(2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanoyl]amino]-4-phenyl-1-[(1*S*)-2-phenyl-1-[[(thiazol-5-ylmethoxy)carbonyl]amino]ethyl]butyl (2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanoate.

Ph Eur

Rivaroxaban

(Ph. Eur. monograph 2932)



$C_{19}H_{18}ClN_3O_5S$ 435.9 *366789-02-8*

Action and use
Factor Xa inhibitor; anticoagulant

Preparation
Rivaroxaban Tablets

Ph Eur

DEFINITION

5-Chloro-*N*-[[(5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl]methyl]thiophene-2-carboxamide.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or yellowish powder.

Solubility
Practically insoluble in water, freely soluble in dimethyl sulfoxide, practically insoluble in anhydrous ethanol and in heptane.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *rivaroxaban CRS*.

B. Enantiomeric purity (see Tests).

TESTS

Enantiomeric purity
Liquid chromatography (*2.2.29*).

Test solution Dissolve 20 mg of the substance to be examined in 25 mL of *acetonitrile R* and dilute to 50 mL with *anhydrous ethanol R*.

Reference solution (a) Dissolve 1 mg of *rivaroxaban impurity A CRS* in 5 mL of *acetonitrile R* and dilute to 10 mL with *anhydrous ethanol R*.

Reference solution (b) Dissolve 20 mg of the substance to be examined in 25 mL of *acetonitrile R*, add 1 mL of reference solution (a) and dilute to 50 mL with *anhydrous ethanol R*.

Column:
— *size*: *l* = 0.25 m, Ø = 2.0 mm;
— *stationary phase*: *cellulose derivative of silica gel for chiral separation R* (10 µm);
— *temperature*: 50 °C.

Mobile phase *anhydrous ethanol R, heptane R* (30:70 *V/V*).

Flow rate 0.2 mL/min.

Detection Spectrophotometer at 250 nm.

Injection 3.0 µL of the test solution and reference solution (b).

Run time 1.5 times the retention time of rivaroxaban.

Relative retention With reference to rivaroxaban (retention time = about 17 min): impurity A = about 0.9.

System suitability Reference solution (b):
— *resolution*: minimum 1.5 between the peaks due to impurity A and rivaroxaban.

Limit:
— *impurity A*: maximum 0.5 per cent; calculate the ratio of the area of the peak due to impurity A to the sum of the areas of the peaks due to rivaroxaban and impurity A.

Related substances
Liquid chromatography (*2.2.29*).

Solution A Dissolve 1.36 g of *potassium dihydrogen phosphate R* in *water for chromatography R*, add 200 µL of *phosphoric acid R* and dilute to 1000 mL with *water for chromatography R*.

Solvent mixture *acetonitrile R*, solution A (40:60 *V/V*).

Test solution Dissolve 25.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dissolve 25.0 mg of *rivaroxaban CRS* in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 2.5 mg of *rivaroxaban for system suitability CRS* (containing impurity G) in the solvent mixture and dilute to 5 mL with the solvent mixture.

Column:
— *size*: *l* = 0.15 m, Ø = 3.0 mm;
— *stationary phase*: *end-capped extra-dense bonded octadecylsilyl silica gel for chromatography R* (3.5 µm);
— *temperature*: 60 °C.

Mobile phase:
— *mobile phase A*: mix 5 volumes of *methanol R* and 95 volumes of a 1.0 g/L solution of *sodium hexanesulfonate R* in solution A;
— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	98	2
2 - 8	98 → 84	2 → 16
8 - 25	84 → 64	16 → 36
25 - 37	64 → 20	36 → 80

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 250 nm.

Injection 3 µL of the test solution and reference solutions (b) and (c).

Identification of impurities Use the chromatogram supplied with *rivaroxaban for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peak due to impurity G.

Relative retention With reference to rivaroxaban (retention time = about 16 min): impurity G = about 0.9.

System suitability Reference solution (c):
— *resolution*: minimum 7.0 between the peaks due to impurity G and rivaroxaban.

Calculation of percentage contents:
— for each impurity, use the concentration of rivaroxaban in reference solution (b).

Limits:
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;

— *total*: maximum 0.3 per cent;
— *reporting threshold*: 0.05 per cent.

Water (*2.5.32*)
Maximum 0.5 per cent, determined on 0.150 g using the evaporation technique at 150 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 2.0 g.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

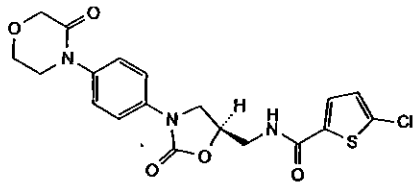
Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{19}H_{18}ClN_3O_5S$ taking into account the assigned content of *rivaroxaban CRS*.

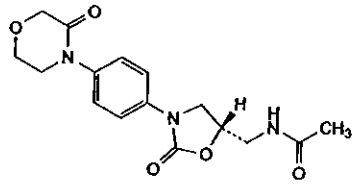
IMPURITIES

Specified impurities *A.*

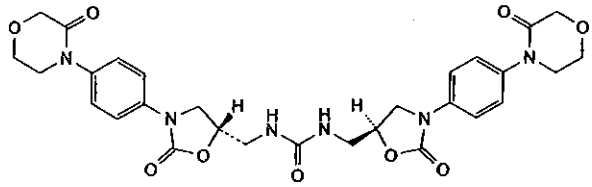
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, D, E, F, G, H, I, J.*



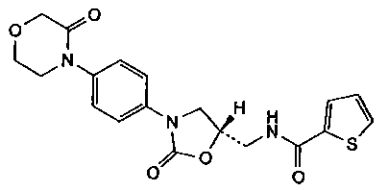
A. 5-chloro-*N*-[[(5*R*)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl]methyl]thiophene-2-carboxamide,



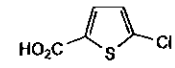
B. *N*-[[(5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl]methyl]acetamide,



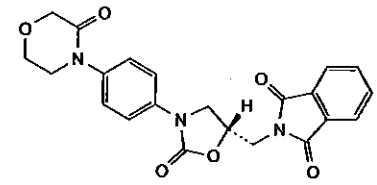
D. *N*,*N*′-bis[[(5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl]methyl]urea,



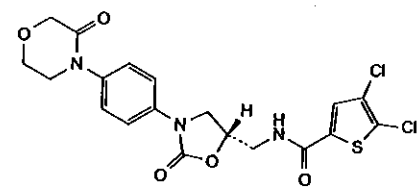
E. *N*-[[(5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl]methyl]thiophene-2-carboxamide,



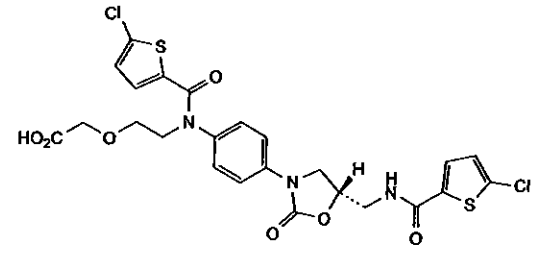
F. 5-chlorothiophene-2-carboxylic acid,



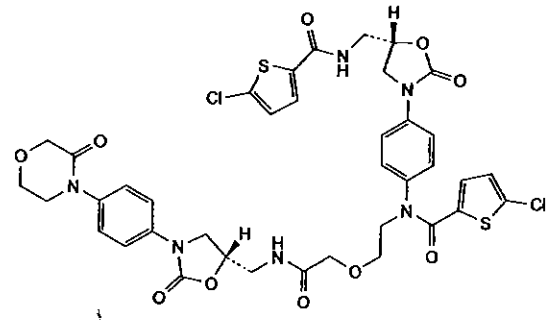
G. 2-[[(5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl]methyl]-2*H*-isoindole-1,3-dione,



H. 4,5-dichloro-*N*-[[(5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl]methyl]thiophene-2-carboxamide,



I. [2-[1^5,9^5-dichloro-2,5^2,8-trioxo-3,7-diaza-5(3,5)-1,3-oxazolidina-1,9(2)-dithiophena-4(1,4)-benzenanonaphan-3-yl]ethoxy]acetic acid,

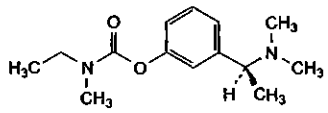


J. 5-chloro-*N*-[4-[(5*S*)-5-[(5-chlorothiophene-2-carboxamido)methyl]-2-oxo-1,3-oxazolidin-3-yl]phenyl]-*N*-[2-[2-oxo-2-[[[(5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl]methyl]amino]ethoxy]ethyl]thiophene-2-carboxamide.

Ph Eur

Rivastigmine

(Ph. Eur. monograph 2629)



$C_{14}H_{22}N_2O_2$ 250.3 *123441-03-2*

Action and use
Cholinesterase inhibitor; treatment of dementia in Alzheimer's disease and Parkinson's disease.

Preparation
Rivastigmine Transdermal Patches

Ph Eur

DEFINITION

3-[(1*S*)-1-(Dimethylamino)ethyl]phenyl ethyl (methyl)carbamate.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
Viscous, clear, colourless or yellow or very slightly brown, hygroscopic liquid.

Solubility
Sparingly soluble in water, very soluble in anhydrous ethanol and in heptane.

IDENTIFICATION

Carry out either tests A, B or tests B, C.

A. Specific optical rotation (*2.2.7*): −44.0 to −38.0 (anhydrous substance). *Prepare the solution immediately before use.*

Dissolve 0.300 g in *ethyl acetate R* and dilute to 50.0 mL with the same solvent.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Film.

Comparison *rivastigmine hydrogen tartrate CRS*, treated as follows: dissolve 0.100 g in 30 mL of *buffer solution pH 11 R*, then add 30 mL of *1,1-dimethylethyl methyl ether R* and shake vigorously for 2 min. Allow the layers to separate. Filter the upper organic layer through *anhydrous sodium sulfate R*. Evaporate the filtrate under reduced pressure at a temperature not exceeding 60 °C to obtain a residue. Record the reference spectrum using this residue.

C. Enantiomeric purity (see Tests).

TESTS

Enantiomeric purity
Liquid chromatography (*2.2.29*).

Solution A Solution containing 1.78 g/L of *disodium hydrogen phosphate dihydrate R* and 1.38 g/L of *sodium dihydrogen phosphate monohydrate R*. Adjust to pH 6.0 with *phosphoric acid R*.

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 5.0 mL of the solution to 20.0 mL with the mobile phase.

Reference solution (a) Dissolve 2.0 mg of *rivastigmine impurity D CRS* in the mobile phase and dilute to 200.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve 1 mg of *rivastigmine hydrogen tartrate CRS* in reference solution (a) and dilute to 10 mL with reference solution (a).

Column:
— *size*: *l* = 0.10 m, Ø = 4.0 mm;
— *stationary phase*: *α1-acid-glycoprotein silica gel for chiral separation R* (5 µm).

Mobile phase Mix 205 µL of *N,N-dimethyloctylamine R* and 20.0 mL of *acetonitrile R1* and dilute to 1000 mL with solution A.

Flow rate 0.5 mL/min.

Detection Spectrophotometer at 200 nm.

Injection 20 µL.

Run time Twice the retention time of rivastigmine.

Relative retention With reference to rivastigmine (retention time = about 9 min): impurity D = about 0.8.

System suitability Reference solution (b):
— *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to rivastigmine.

Calculation of percentage content:
— use the concentration of impurity D in reference solution (a).

Limit:
— *impurity D*: maximum 0.3 per cent.

Related substances
Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Test solution Dissolve 62.5 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve the contents of a vial of *rivastigmine for system suitability CRS* (containing impurities A, B and C) in 1 mL of the mobile phase.

Reference solution (c) Dissolve 50.0 mg of *rivastigmine hydrogen tartrate CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.0 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R1* (5 µm);
— *temperature*: 40 °C.

Mobile phase Mix 42 volumes of an 8.9 g/L solution of *disodium hydrogen phosphate dihydrate R* previously adjusted to pH 7.0 with *phosphoric acid R* and 58 volumes of *methanol R2*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 214 nm.

Injection 20 µL of the test solution and reference solutions (a) and (b).

Run time Twice the retention time of rivastigmine.

Identification of impurities Use the chromatogram supplied with *rivastigmine for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B and C.

Relative retention With reference to rivastigmine (retention time = about 10 min): impurity A = about 0.4; impurity C = about 0.6; impurity B = about 0.7.

System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the peaks due to impurities C and B.

Calculation of percentage contents:
— for each impurity, use the concentration of rivastigmine in reference solution (a).

Limits:
— *impurity A*: maximum 0.3 per cent;
— *impurity B*: maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.5 per cent;
— *reporting threshold*: 0.05 per cent.

Water (*2.5.12*)
Maximum 0.5 per cent, determined on 1.000 g.
Change the solvent after standardisation of the titrant and after every 3rd sample.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (c).

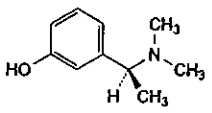
System suitability Reference solution (c):
— *symmetry factor*: maximum 2.5 for the peak due to rivastigmine.

Calculate the percentage content of $C_{14}H_{22}N_2O_2$ taking into account the assigned content of *rivastigmine hydrogen tartrate CRS* and a conversion factor of 0.625.

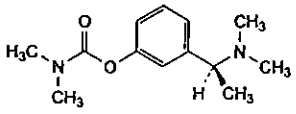
STORAGE
Under an inert gas, in an airtight container, protected from light, at a temperature of 2 °C to 8 °C.

IMPURITIES
Specified impurities *A, B, D.*

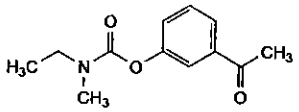
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C.*



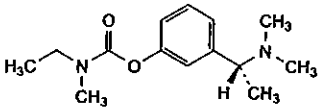
A. 3-[(1*S*)-1-(dimethylamino)ethyl]phenol (dimetol),



B. 3-[(1*S*)-1-(dimethylamino)ethyl]phenyl dimethylcarbamate,



C. 3-acetylphenyl ethyl(methyl)carbamate,

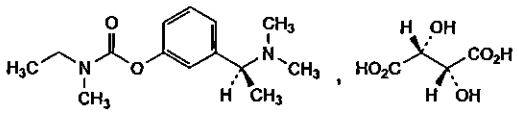


D. 3-[(1*R*)-1-(dimethylamino)ethyl]phenyl ethyl (methyl)carbamate ((*R*)-enantiomer).

Ph Eur

Rivastigmine Hydrogen Tartrate

(Ph. Eur. monograph 2630)



$C_{18}H_{28}N_2O_8$ 400.4 *129101-54-8*

Action and use
Cholinesterase inhibitor; treatment of dementia in Alzheimer's disease and Parkinson's disease.

Preparations
Rivastigmine Capsules
Rivastigmine Oral Solution

Ph Eur

DEFINITION
3-[(1*S*)-1-(Dimethylamino)ethyl]phenyl *N*-ethyl-*N*-methylcarbamate hydrogen (2*R*,3*R*)-2,3-dihydroxybutanedioate.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white, very hygroscopic, crystalline or fine crystalline powder.

Solubility
Very soluble in water, soluble in methanol, very slightly soluble in ethyl acetate.

It shows polymorphism (*5.9*).

IDENTIFICATION
Carry out either tests A, B or tests B, C.

A. Specific optical rotation (*2.2.7*): + 4.2 to + 5.1.

Dissolve 0.600 g in *methanol R* and dilute to 20.0 mL with the same solvent.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *rivastigmine hydrogen tartrate CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

C. Enantiomeric purity (see Tests).

TESTS

Enantiomeric purity

Liquid chromatography (*2.2.29*).

Solution A Solution containing 1.78 g/L of *disodium hydrogen phosphate dihydrate R* and 1.38 g/L of *sodium dihydrogen phosphate monohydrate R*, adjusted to pH 6.0 with *phosphoric acid R*.

Test solution Dissolve 10.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dissolve 2.0 mg of *rivastigmine impurity D CRS* in the mobile phase and dilute to 200.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve 1 mg of *rivastigmine hydrogen tartrate CRS* in reference solution (a) and dilute to 10.0 mL with the same solution.

Column:
- *size*: *l* = 0.10 m, Ø = 4.0 mm;
- *stationary phase*: *α1-acid-glycoprotein silica gel for chiral separation R* (5 µm).

Mobile phase Mix 205 µL of *N,N-dimethyloctylamine R* and 20.0 mL of *acetonitrile R1* and dilute to 1000 mL with solution A.

Flow rate 0.5 mL/min.

Detection Spectrophotometer at 200 nm.

Injection 20 µL.

Run time Twice the retention time of rivastigmine.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity D.

Relative retention With reference to rivastigmine (retention time = about 9 min): impurity D = about 0.8.

System suitability Reference solution (b):
- *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to rivastigmine.

Calculation of percentage content:
- for impurity D, use the concentration of impurity D in reference solution (a).

Limit:
- *impurity D*: maximum 0.3 per cent.

Related substances

Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve the contents of a vial of *rivastigmine for system suitability CRS* (containing impurities A, B and C) in 1.0 mL of the mobile phase.

Reference solution (c) Dissolve 50.0 mg of *rivastigmine hydrogen tartrate CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase.

Column:
- *size*: *l* = 0.25 m, Ø = 4.0 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase Mix 42 volumes of an 8.9 g/L solution of *disodium hydrogen phosphate dihydrate R* previously adjusted to pH 7.0 with *phosphoric acid R* and 58 volumes of *methanol R1*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 214 nm.

Injection 20 µL of the test solution and reference solutions (a) and (b).

Run time Twice the retention time of rivastigmine.

Identification of impurities Use the chromatogram supplied with *rivastigmine for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B and C.

Relative retention with reference to rivastigmine (retention time = about 10 min): impurity A = about 0.4; impurity C = about 0.6; impurity B = about 0.7.

System suitability Reference solution (b):
- *resolution*: minimum 2.0 between the peaks due to impurities C and B.

Calculation of percentage contents:
- for each impurity, use the concentration of rivastigmine hydrogen tartrate in reference solution (a).

Limits:
- *impurity A*: maximum 0.3 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.5 per cent;
- *reporting threshold*: 0.05 per cent; disregard the peak due to tartaric acid.

Water (*2.5.12*)

Maximum 0.5 per cent, determined on 1.00 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (c).

System suitability Reference solution (c):
- *symmetry factor*: maximum 2.5 for the peak due to rivastigmine.

Calculate the percentage content of $C_{18}H_{28}N_2O_8$ taking into account the assigned content of *rivastigmine hydrogen tartrate CRS*.

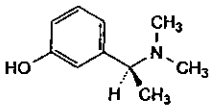
STORAGE

In an airtight container, protected from light.

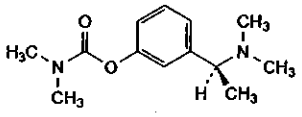
IMPURITIES

Specified impurities *A, D.*

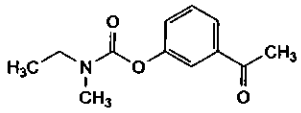
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, E, F, G, H.*



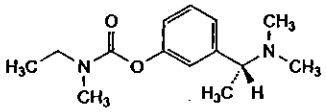
A. 3-[(1*S*)-1-(dimethylamino)ethyl]phenol,



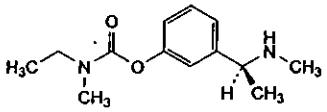
B. 3-[(1*S*)-1-(dimethylamino)ethyl]phenyl *N*,*N*-dimethylcarbamate,



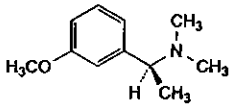
C. 3-acetylphenyl *N*-ethyl-*N*-methylcarbamate,



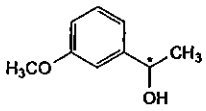
D. 3-[(1*R*)-1-(dimethylamino)ethyl]phenyl *N*-ethyl-*N*-methylcarbamate,



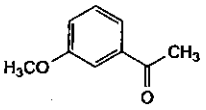
E. 3-[(1*S*)-1-(methylamino)ethyl]phenyl *N*-ethyl-*N*-methylcarbamate,



F. (1*S*)-1-(3-methoxyphenyl)-*N*,*N*-dimethylethanamine,



G. 1-(3-methoxyphenyl)ethan-1-ol,

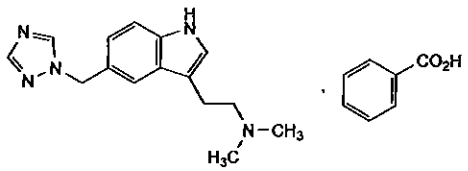


H. 1-(3-methoxyphenyl)ethan-1-one.

Ph Eur

Rizatriptan Benzoate

(Ph. Eur. monograph 2585)



$C_{22}H_{25}N_5O_2$ 391.5 *145202-66-0*

Action and use
Serotonin $5HT_1$ receptor agonist; treatment of migrane.

Preparations
Rizatriptan Orodispersible Tablets
Rizatriptan Tablets

Ph Eur

DEFINITION

N,*N*-Dimethyl-2-[5-(1*H*-1,2,4-triazol-1-ylmethyl)-1*H*-indol-3-yl]ethanamine benzoate.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white powder or crystals.

Solubility
Soluble in water, sparingly soluble in ethanol (96 per cent), slightly soluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *rizatriptan benzoate CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

B. Examine the chromatograms obtained in the assay.

Results The 2 principal peaks in the chromatogram obtained with the test solution are similar in retention time and size to the 2 principal peaks in the chromatogram obtained with reference solution (a).

TESTS

Related substances
Liquid chromatography (*2.2.29*): use the normalisation procedure. *Use silanised glass autosampler vials and freshly prepared solutions.*

Test solution Dissolve 50.0 mg of the substance to be examined in mobile phase A and dilute to 50.0 mL with mobile phase A.

Reference solution (a) Dissolve 50.0 mg of *rizatriptan benzoate CRS* in mobile phase A and dilute to 50.0 mL with mobile phase A.

Reference solution (b) Dissolve 5 mg of *rizatriptan for system suitability CRS* (containing impurity C) in mobile phase A and dilute to 5.0 mL with mobile phase A.

Reference solution (c) Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 20.0 mL with mobile phase A.

Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: phenylsilyl silica gel for chromatography R* (5 µm);
— *temperature:* 40 °C.

Mobile phase:
— *mobile phase A:* mix 160 mL of *acetonitrile R* and 840 mL of *water R*, add 1.0 mL of *trifluoroacetic acid R* and mix;
— *mobile phase B:* to 1000 mL of *acetonitrile R* add 1.0 mL of *trifluoroacetic acid R* and mix;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 8	100	0
8 - 17	100 → 70	0 → 30
17 - 20	70	30
20 - 20.1	70 → 100	30 → 0
20.1 - 23	100	0

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µL of the test solution and reference solutions (b) and (c).

Identification of impurities Use the chromatogram supplied with *rizatriptan for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity C.

Relative retention With reference to rizatriptan (retention time = about 5 min): impurity C = about 1.3; benzoic acid = about 2.1.

System suitability Reference solution (b):
— *resolution:* minimum 2.0 between the peaks due to rizatriptan and impurity C.

Limits:
— *unspecified impurities:* for each impurity, maximum 0.10 per cent;
— *total:* maximum 0.3 per cent;
— *disregard limit:* the area of the peak due to rizatriptan in the chromatogram obtained with reference solution (c) (0.05 per cent); disregard any peak due to benzoic acid.

Water (*2.5.12*)
Maximum 0.5 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

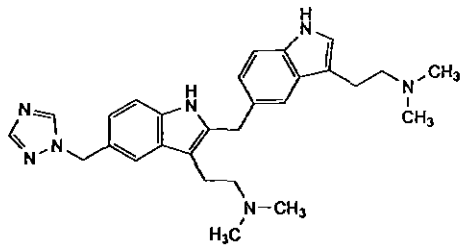
Injection Test solution and reference solution (a).

System suitability Reference solution (a):
— *symmetry factor:* maximum 3.5 for the peak due to rizatriptan.

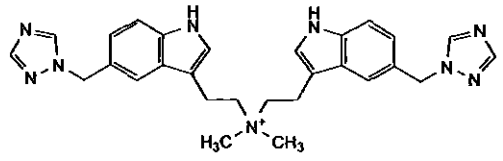
Calculate the percentage content of $C_{22}H_{25}N_5O_2$ from the declared content of *rizatriptan benzoate CRS*.

IMPURITIES

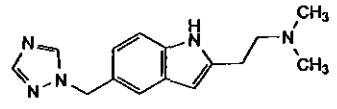
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, E, F, G, H, I.*



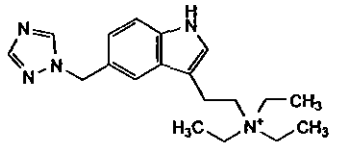
A. 2-[2-[[3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl]methyl]-5-(1*H*-1,2,4-triazol-1-ylmethyl)-1*H*-indol-3-yl]-*N*,*N*-dimethylethanamine,



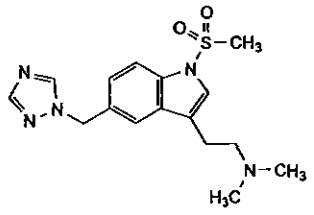
B. *N*,*N*-dimethyl-2-[5-(1*H*-1,2,4-triazol-1-ylmethyl)-1*H*-indol-3-yl]-*N*-[2-[5-(1*H*-1,2,4-triazol-1-ylmethyl)-1*H*-indol-3-yl]ethyl]ethanaminium,



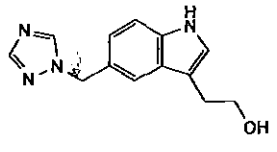
C. *N*,*N*-dimethyl-2-[5-(1*H*-1,2,4-triazol-1-ylmethyl)-1*H*-indol-2-yl]ethanamine,



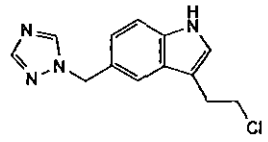
D. *N*,*N*,*N*-triethyl-2-[5-(1*H*-1,2,4-triazol-1-ylmethyl)-1*H*-indol-3-yl]ethanaminium,



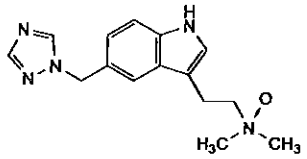
E. *N*,*N*-dimethyl-2-[1-(methylsulfonyl)-5-(1*H*-1,2,4-triazol-1-ylmethyl)-1*H*-indol-3-yl]ethanamine,



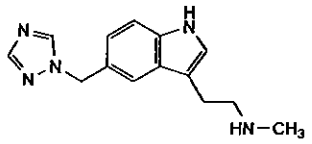
F. 2-[5-(1*H*-1,2,4-triazol-1-ylmethyl)-1*H*-indol-3-yl]ethanol,



G. 3-(2-chloroethyl)-5-(1*H*-1,2,4-triazol-1-ylmethyl)-1*H*-indole,



H. *N,N*-dimethyl-2-[5-(1*H*-1,2,4-triazol-1-ylmethyl)-1*H*-indol-3-yl]ethanamine *N*-oxide,

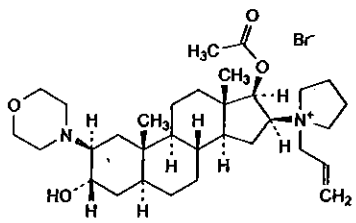


I. *N*-methyl-2-[5-(1*H*-1,2,4-triazol-1-ylmethyl)-1*H*-indol-3-yl]ethanamine.

Ph Eur

Rocuronium Bromide

(Ph. Eur. monograph 1764)



$C_{32}H_{53}BrN_2O_4$ 610 *119302-91-9*

Action and use
Non-depolarizing neuromuscular blocker.

Ph Eur

DEFINITION
1-[17β-Acetoxy-3α-hydroxy-2β-(morpholin-4-yl)-5α-androstan-16β-yl]-1-(prop-2-enyl)pyrrolidinium bromide.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance
Almost white or pale yellow, slightly hygroscopic powder.

Solubility
Freely soluble in water, very soluble in methylene chloride, freely soluble in anhydrous ethanol.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *rocuronium bromide CRS*.
B. Solution S (see Tests) gives reaction (a) of bromides (*2.3.1*).

TESTS

Solution S
Dissolve 0.10 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*)
+ 28.5 to + 32.0 (anhydrous substance).
Dissolve 0.250 g in a 5.15 g/L solution of *hydrochloric acid R* and dilute to 25.0 mL with the same solution.

pH (*2.2.3*)
8.9 to 9.5 for solution S.

Related substances
Liquid chromatography (*2.2.29*).
Solvent mixture *water R, acetonitrile R1* (10:90 *V/V*).
Test solution Dissolve 0.100 g of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.
Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.
Reference solution (b) Dissolve 5 mg of *rocuronium for peak identification CRS* (containing impurities A, B, C, F, G and H) in the solvent mixture and dilute to 5.0 mL with the solvent mixture.
Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *silica gel for chromatography R* (5 µm);
— *temperature*: 30 °C.
Mobile phase Mix 10 volumes of a 4.53 g/L solution of *tetramethylammonium hydroxide R* adjusted to pH 7.4 with *phosphoric acid R* and 90 volumes of *acetonitrile R1*.
Flow rate 2.0 mL/min.
Detection Spectrophotometer at 210 nm.
Injection 5 µL.
Run time 2.5 times the retention time of rocuronium.
Identification of impurities Use the chromatogram supplied with *rocuronium for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C, F, G and H.
Relative retention With reference to rocuronium (retention time = about 9 min): impurity A = about 0.2; impurity G = about 0.4; impurity F = about 0.75; impurity B = about 0.80; impurity H = about 0.95; impurity C = about 1.2.
System suitability Reference solution (b):
— *peak-to-valley ratio*: minimum 3.0, where H_p = height above the baseline of the peak due to impurity H and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to rocuronium.
Limits:
— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.5; impurity F = 1.3; impurity G = 0.4; impurity H = 0.4;
— *impurities A, B, C*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *impurities F, G, H*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent); disregard any peak eluting before impurity A.

Chlorides

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in *water R* and dilute to 20.0 mL with the same solvent.

Reference solution (a) Dissolve 0.644 g of *sodium bromide R* and 0.824 g of *sodium chloride R* in *water R* and dilute to 1000.0 mL with the same solvent. Dilute 1.0 mL of the solution to 50.0 mL with *water R*.

Reference solution (b) Dissolve 0.824 g of *sodium chloride R* in *water R* and dilute to 1000.0 mL with the same solvent. Dilute 5.0 mL of the solution to 50.0 mL with *water R*. Dilute 2.0 mL of this solution to 50.0 mL with *water R*.

Blank solution *water R*.

Precolumn:
— *size*: *l* = 0.05 m, Ø = 4.0 mm;
— *stationary phase*: *anion-exchange resin R* (13 µm).

Column:
— *size*: *l* = 0.25 m, Ø = 4.0 mm;
— *stationary phase*: *anion-exchange resin R* (13 µm).

Mobile phase A solution containing 0.063 g/L of *sodium hydrogen carbonate R* and 0.212 g/L of *anhydrous sodium carbonate R*.

Flow rate 2.0 mL/min.

Detection Conductivity detector set at 100 µS/V and maintained at 30 °C.

Use a self-regenerating anion suppressor.

Injection 25 µL.

Retention times Chloride = about 1.7 min; bromide = about 2.8 min.

System suitability Reference solution (a):
— *resolution*: minimum 2.5 between the peaks due to chloride and bromide.

Limit:
— *chlorides*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

2-Propanol (*2.4.24, System A*)

Maximum 1.0 per cent.

Water (*2.5.12*)

Maximum 4.5 per cent, determined on 0.400 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.400 g in 40 mL of *glacial acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 60.97 mg of $C_{32}H_{53}BrN_2O_4$.

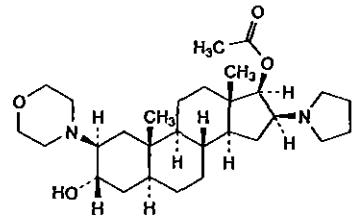
STORAGE

In an airtight container, protected from light, at a temperature below −15 °C.

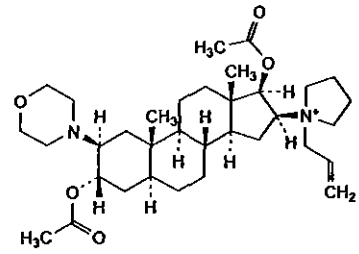
IMPURITIES

Specified impurities *A, B, C, F, G, H.*

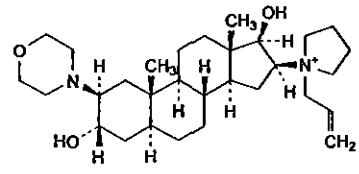
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *D, E.*



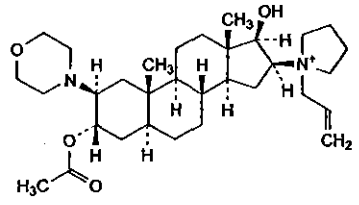
A. 3α-hydroxy-2β-(morpholin-4-yl)-16β-(pyrrolidin-1-yl)-5α-androstan-17β-yl acetate,



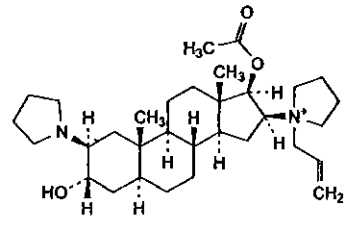
B. 1-[3α,17β-diacetoxy-2β-(morpholin-4-yl)-5α-androstan-16β-yl]-1-(prop-2-enyl)pyrrolidinium,



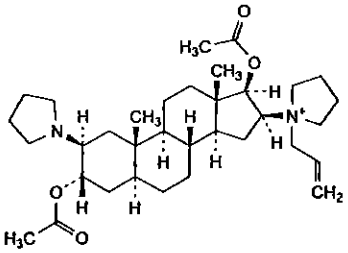
C. 1-[3α,17β-dihydroxy-2β-(morpholin-4-yl)-5α-androstan-16β-yl]-1-(prop-2-enyl)pyrrolidinium,



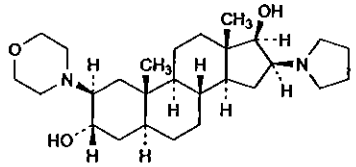
D. 1-[3α-acetoxy-17β-hydroxy-2β-(morpholin-4-yl)-5α-androstan-16β-yl]-1-(prop-2-enyl)pyrrolidinium,



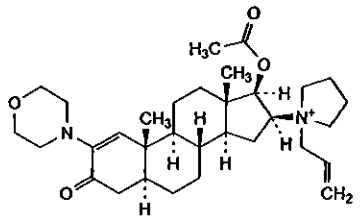
E. 1-[17β-acetoxy-3α-hydroxy-2β-(pyrrolidin-1-yl)-5α-androstan-16β-yl]-1-(prop-2-enyl)pyrrolidinium,



F. 1-[3α,17β-acetoxy-2β-(pyrrolidin-1-yl)-5α-androstan-16β-yl]-1-(prop-2-enyl)pyrrolidinium,



G. 2β-(morpholin-4-yl)-16β-(pyrrolidin-1-yl)-5α-androstane-3α,17β-diol,

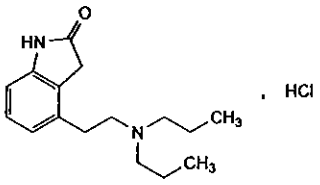


H. 1-[17β-acetoxy-2-(morpholin-4-yl)-3-oxo-5α-androst-1-en-16β-yl]-1-(prop-2-enyl)pyrrolidinium.

Ph Eur

Ropinirole Hydrochloride

(Ph. Eur. monograph 2604)



$C_{16}H_{25}ClN_2O$ 296.8 *91374-20-8*

Action and use
Dopamine receptor agonist.

Ph Eur

DEFINITION
4-[2-(Dipropylamino)ethyl]-1,3-dihydro-2*H*-indol-2-one hydrochloride.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or yellowish, crystalline powder.

Solubility
Freely soluble in water and in methanol, slightly soluble in ethanol (96 per cent), practically insoluble in heptane.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *ropinirole hydrochloride CRS.*
B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS
Related substances
Liquid chromatography (*2.2.29*).
Test solution (a) Dissolve, using sonication, 50.0 mg of the substance to be examined in 4 mL of mobile phase B and dilute to 25.0 mL with mobile phase A.
Test solution (b) Dilute 1.0 mL of test solution (a) to 20.0 mL with mobile phase A.
Reference solution (a) Dissolve, using sonication, 7.5 mg of *ropinirole impurity A CRS* and 5.0 mg of the substance to be examined in 16 mL of mobile phase B and dilute to 100.0 mL with mobile phase A.
Reference solution (b) To 1.0 mL of reference solution (a) add 4 mL of mobile phase B and dilute to 25.0 mL with mobile phase A.
Reference solution (c) Dissolve, using sonication, 50.0 mg of *ropinirole hydrochloride CRS* in 4 mL of mobile phase B and dilute to 25.0 mL with mobile phase A. Dilute 1.0 mL of the solution to 20.0 mL with mobile phase A.
Reference solution (d) Dissolve 5.0 mg of *ropinirole for peak identification 1 CRS* (containing impurity D) in mobile phase A and dilute to 5.0 mL with mobile phase A.
Reference solution (e) Dissolve 5.0 mg of *ropinirole for peak identification 2 CRS* (containing impurities G and H) in mobile phase A and dilute to 5.0 mL with mobile phase A.
Reference solution (f) Dissolve 5.0 mg of *ropinirole for peak identification 3 CRS* (containing impurity E) in mobile phase A and dilute to 5.0 mL with mobile phase A.
Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 40 °C.
Mobile phase:
— *mobile phase A*: dissolve 3.85 g of *ammonium acetate R* in 950 mL of *water for chromatography R*, adjust to pH 2.5 with *dilute phosphoric acid R* and dilute to 1000 mL with *water for chromatography R*;
— *mobile phase B*: *methanol R*, *acetonitrile R1* (30:70 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	92	8
15 - 30	92 → 80	8 → 20
30 - 50	80 → 60	20 → 40
50 - 75	60	40

Flow rate 1.0 mL/min.
Detection Spectrophotometer at 250 nm.
Injection 10 µL of test solution (a) and reference solutions (a), (b), (d), (e) and (f).
Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity A; use the chromatogram supplied with *ropinirole for peak identification 1 CRS* and the chromatogram obtained with reference solution (d) to identify the peak due to impurity D; use the chromatogram supplied with *ropinirole for peak identification 2 CRS* and the chromatogram obtained with reference solution (e) to identify the peaks due to

impurities G and H; use the chromatogram supplied with *ropinirole for peak identification 3 CRS* and the chromatogram obtained with reference solution (f) to identify the peak due to impurity E.

Relative retention With reference to ropinirole (retention time = about 30 min): impurity D = about 0.5; impurity A = about 0.9; impurity E = about 1.1; impurity H = about 1.2; impurity G = about 1.4.

System suitability:
- *resolution*: minimum 3.0 between the peaks due to impurity A and ropinirole in the chromatogram obtained with reference solution (a);
- *signal-to-noise ratio*: minimum 60 for the peak due to ropinirole in the chromatogram obtained with reference solution (b).

Calculation of percentage contents:
- *correction factors*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity G = 2.7; impurity H = 0.5;
- for impurity A, use the concentration of impurity A in reference solution (b);
- for impurities other than A, use the concentration of ropinirole hydrochloride in reference solution (b).

Limits:
- *impurities A, D, E, G, H*: for each impurity, maximum 0.15 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.5 per cent;
- *reporting threshold*: 0.05 per cent.

Water (*2.5.12*)
Maximum 0.5 per cent, determined on 1.000 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Mobile phase Mobile phase B, mobile phase A (19:81 *V/V*).

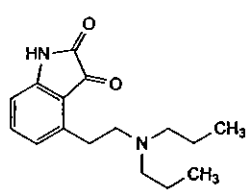
Injection Test solution (b) and reference solution (c).

Calculate the percentage content of $C_{16}H_{25}ClN_2O$ taking into account the assigned content of *ropinirole hydrochloride CRS*.

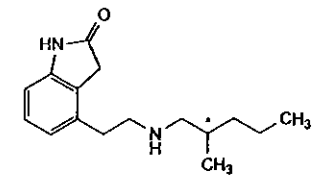
IMPURITIES

Specified impurities *A, D, E, G, H.*

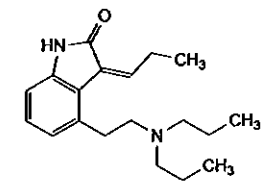
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, F.*



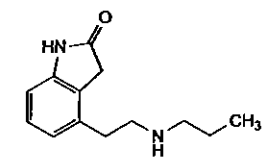
A. 4-[2-(dipropylamino)ethyl]-1*H*-indole-2,3-dione,



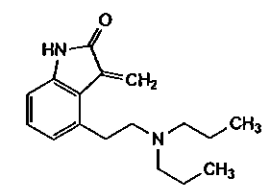
B. 4-[2-[(2-methylpentyl)amino]ethyl]-1,3-dihydro-2*H*-indol-2-one,



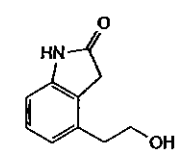
C. (3*Z*)-4-[2-(dipropylamino)ethyl]-3-propylidene-1,3-dihydro-2*H*-indol-2-one,



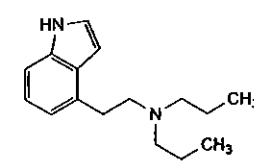
D. 4-[2-(propylamino)ethyl]-1,3-dihydro-2*H*-indol-2-one,



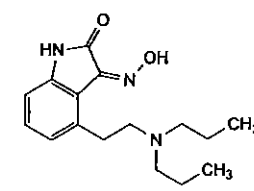
E. 4-[2-(dipropylamino)ethyl]-3-methylidene-1,3-dihydro-2*H*-indol-2-one,



F. 4-(2-hydroxyethyl)-1,3-dihydro-2*H*-indol-2-one,



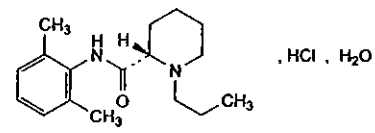
G. 4-[2-(dipropylamino)ethyl]-1*H*-indole,



H. (3*Z*)-4-[2-(dipropylamino)ethyl]-3-(hydroxyimino)-1,3-dihydro-2*H*-indol-2-one.

Ropivacaine Hydrochloride Monohydrate

(Ph. Eur. monograph 2335)



$C_{17}H_{27}ClN_2O,H_2O$ 328.9 *132112-35-7*

Action and use
Local anaesthetic.

Ph Eur

DEFINITION
(−)-(2*S*)-*N*-(2,6-Dimethylphenyl)-1-propylpiperidine-2-carboxamide hydrochloride monohydrate.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Soluble in water and in ethanol (96 per cent), slightly soluble in methylene chloride.

IDENTIFICATION
Carry out either tests A, C, D or tests A, B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *ropivacaine hydrochloride monohydrate CRS*.

B. Specific optical rotation (*2.2.7*): −74.0 to −64.0 (anhydrous substance).

Mix 2 mL of a 200 g/L solution of *sodium hydroxide R* and 30 mL of *water R* and dilute to 100.0 mL with *ethanol (96 per cent) R* (solution A). Dissolve 0.500 g of the substance to be examined in solution A and dilute to 50.0 mL with solution A.

C. It gives reaction (a) of chlorides (*2.3.1*).

D. Enantiomeric purity (see Tests).

TESTS

Solution S
Dissolve 0.50 g in *carbon dioxide-free water R* and dilute to 25.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*).

pH (*2.2.3*)
4.5 to 6.0 for solution S.

Absorbance (*2.2.25*)
Maximum 0.030 at 405 nm and maximum 0.025 at 436 nm, determined on solution S prepared immediately before use, with a path length of 5 cm and using *water R* as the compensation liquid.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 55 mg of the substance to be examined in the mobile phase and dilute to 20 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 5 mg of the substance to be examined and 5 mg of *bupivacaine hydrochloride CRS* (impurity A) in the mobile phase and dilute to 5 mL with the mobile phase. Dilute 1 mL of this solution to 100 mL with the mobile phase.

Column:
— *size*: *l* = 0.15 m, Ø = 3.9 mm;
— *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (4 µm).

Mobile phase Mix 1.3 mL of a 138 g/L solution of *sodium dihydrogen phosphate R* and 32.5 mL of an 89 g/L solution of *disodium hydrogen phosphate dodecahydrate R* and dilute to 1000 mL with *water R*; mix equal volumes of this solution (pH 8.0) and *acetonitrile R*.

Flow rate 1.0 mL/min.

Injection 20 µL.

Detection Spectrophotometer at 240 nm.

Run time 2.5 times the retention time of ropivacaine.

Relative retention With reference to ropivacaine (retention time = about 6 min): impurity A = about 1.6.

System suitability Reference solution (b):
— *resolution*: minimum 6.0 between the peaks due to ropivacaine and impurity A.

Limits:
— *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Impurity H
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution Dissolve 13.0 mg of *2,6-dimethylaniline hydrochloride R* in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Retention time Impurity H = about 2-3 min.

Limit:
— *impurity H*: not more than the area of the principal peak in the chromatogram obtained with the reference solution (10 ppm).

Enantiomeric purity
Capillary electrophoresis (*2.2.47*): use the normalisation procedure.

Test solution Dissolve 50 mg of the substance to be examined in *water R* and dilute to 25 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 200.0 mL with *water R*.

Reference solution (b) Dissolve 1.5 mg of the substance to be examined and 1.5 mg of *ropivacaine impurity G CRS* in *water R* and dilute to 100 mL with the same solvent.

Capillary:
— *material*: fused silica;
— *size*: effective length = about 72 cm, total length = 80 cm, Ø = 50 µm.

Temperature 30 °C.

CZE buffer Prepare a 13.3 g/L solution of *dimethyl-β-cyclodextrin R* in an 11.5 g/L solution of *phosphoric acid R* previously adjusted to pH 3.0 with *triethanolamine R*. The CZE buffer is prepared and filtered through a membrane filter (nominal pore size 0.45 µm) immediately before use.

Detection Spectrophotometer at 206 nm.

Preconditioning of the capillary Rinse the capillary at 100 kPa with *water R* for 1 min, with *0.1 M sodium hydroxide* for 10 min and with *water R* for 3 min. If the capillary is new or dry, increase the sodium hydroxide rinse to 30 min.

Between-run rinsing Rinse the capillary at 100 kPa with *water R* for 1 min, with *0.1 M sodium hydroxide* for 4 min, with *water R* for 1 min and with the CZE buffer for 4 min.

Injection Under pressure (5 kPa) for 5 s.

Migration Apply a field strength of 375 V/cm with an initial ramp of 500 V/s and a positive polarity corresponding to a current of 40-45 µA.

Run time 30 min.

System suitability:
— *resolution*: minimum 3.7 between the peaks due to impurity G (1st peak) and (*S*)-ropivacaine in the electropherogram obtained with reference solution (b); if necessary, increase the dimethyl-β-cyclodextrin concentration in the CZE buffer or vary the pH between 2.9 and 3.1 or lower the temperature;
— *signal-to-noise ratio*: minimum 10 for the principal peak in the electropherogram obtained with reference solution (a).

Limit:
— *impurity G*: maximum 0.5 per cent.

Water (*2.5.12*)
5.0 per cent to 6.0 per cent, determined on 0.100 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

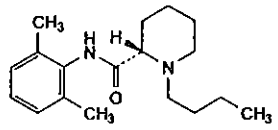
Dissolve 0.250 g in a mixture of 10 mL of *water R* and 40 mL of *ethanol (96 per cent) R*. Add 5.0 mL of *0.01 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 31.09 mg of $C_{17}H_{27}ClN_2O$.

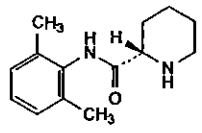
IMPURITIES

Specified impurities *A, G, H.*

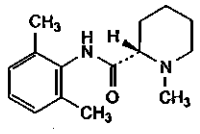
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D, E, F.*



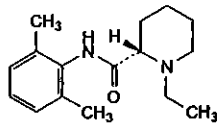
A. (*S*)-bupivacaine,



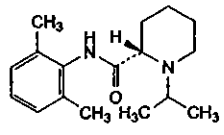
B. (−)-(2*S*)-*N*-(2,6-dimethylphenyl)piperidine-2-carboxamide,



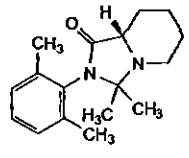
C. (−)-(2*S*)-*N*-(2,6-dimethylphenyl)-1-methylpiperidine-2-carboxamide ((*S*)-mepivacaine),



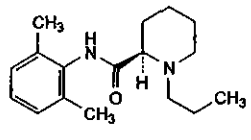
D. (−)-(2*S*)-*N*-(2,6-dimethylphenyl)-1-ethylpiperidine-2-carboxamide,



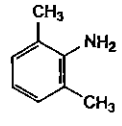
E. (−)-(2*S*)-*N*-(2,6-dimethylphenyl)-1(1-methylethyl)piperidine-2-carboxamide,



F. (8a*S*)-2-(2,6-dimethylphenyl)-3,3-dimethylhexahydroimidazo[1,5-*a*]pyridin-1(5*H*)-one (acetone adduct),



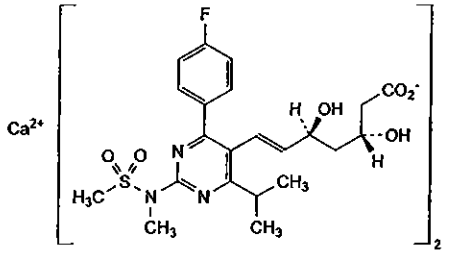
G. (+)-(2*R*)-*N*-(2,6-dimethylphenyl)-1-propylpiperidine-2-carboxamide ((*R*)-ropivacaine),



H. 2,6-dimethylaniline.

Rosuvastatin Calcium

(Ph. Eur. monograph 2631)



$C_{44}H_{54}CaF_2N_6O_{12}S_2$ 1001 *147098-20-2*

Action and use
HMG Co-A reductase inhibitor; lipid-regulating drug.

Preparation
Rosuvastatin Tablets

Ph Eur

DEFINITION

Calcium bis[(3*R*,5*S*,6*E*)-7-[4-(4-fluorophenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoate].

Content
97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, hygroscopic powder.

Solubility
Slightly soluble in water, freely soluble in methylene chloride, practically insoluble in anhydrous ethanol.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *rosuvastatin calcium CRS.*

B. Enantiomeric purity (see Tests).

C. It gives reaction (b) of calcium (*2.3.1*).

TESTS

Enantiomeric purity
Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Solvent mixture *acetonitrile R, water R* (25:75 *V/V*).

Test solution Dissolve 25.0 mg of the substance to be examined in 6 mL of *acetonitrile R* and dilute to 25.0 mL with *water R.*

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve the contents of a vial of *rosuvastatin impurity G CRS* in 1 mL of the test solution.

Column:
— *size:* *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase: cellulose derivative of silica gel for chiral separation R* (5 µm);
— *temperature:* 35 °C.

Mobile phase *acetonitrile for chromatography R,* 0.1 per cent *V/V* solution of *trifluoroacetic acid R* (25:75 *V/V*).

Flow rate 0.5 mL/min.

Detection Spectrophotometer at 242 nm.

Injection 10 µL.

Run time 2.6 times the retention time of rosuvastatin.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity G.

Relative retention With reference to rosuvastatin (retention time = about 29 min): impurity G = about 0.9.

System suitability Reference solution (b):
— *resolution:* minimum 1.5 between the peaks due to impurity G and rosuvastatin.

Calculation of percentage content:
— for impurity G, use the concentration of rosuvastatin calcium in reference solution (a).

Limit:
— *impurity G:* maximum 0.15 per cent.

Impurity L
Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Solvent mixture *acetonitrile R, water R* (50:50 *V/V*).

Test solution Dissolve 20.0 mg of the substance to be examined in 50 mL of *acetonitrile R* and dilute to 100.0 mL with *water R.*

Reference solution (a) Dissolve 5 mg of *rosuvastatin for impurity L identification CRS* in 10 mL of *acetonitrile R* and dilute to 20 mL with *water R.*

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Column:
— *size:* *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase: end-capped solid core octylsilyl silica gel for chromatography R* (2.7 µm).

Mobile phase To 650 mL of a 0.02 per cent *V/V* solution of *trifluoroacetic acid R,* add 350 mL of a mixture of 1 volume of *ethanol (96 per cent) R* and 2 volumes of *acetonitrile for chromatography R.*

Flow rate 0.7 mL/min.

Detection Spectrophotometer at 243 nm.

Injection 10 µL.

Run time 3 times the retention time of rosuvastatin.

Identification of impurities Use the chromatogram supplied with *rosuvastatin for impurity L identification CRS* and the chromatogram obtained with reference solution (a) to identify the peak due to impurity L.

Relative retention With reference to rosuvastatin (retention time = about 22 min): impurity L = about 1.1.

System suitability Reference solution (a):
— *peak-to-valley ratio:* minimum 2.5, where H_p = height above the baseline of the peak due to impurity L and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to rosuvastatin.

Calculation of percentage content:
— *correction factor:* multiply the peak area of impurity L by 1.8;
— for impurity L, use the concentration of rosuvastatin calcium in reference solution (b).

Limit:
— *impurity L:* maximum 0.15 per cent.

Related substances
Liquid chromatography (*2.2.29*). *Carry out the test protected from light and prepare the solutions immediately before use.*

Solvent mixture *acetonitrile R, water R* (25:75 *V/V*).

Test solution Dissolve 35.0 mg of the substance to be examined in 12 mL of *acetonitrile R* and dilute to 50.0 mL with *water R*.

Reference solution (a) Dissolve 35.0 mg of *rosuvastatin calcium CRS* in 12 mL of *acetonitrile R* and dilute to 50.0 mL with *water R*.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 7 mg of *rosuvastatin for system suitability CRS* (containing impurities A, B and C) in 2.5 mL of *acetonitrile R* and dilute to 10 mL with *water R*.

Reference solution (d) Dissolve the contents of a vial of *rosuvastatin impurity mixture CRS* (impurities D and K) in 1 mL of the solvent mixture.

Reference solution (e) Dissolve 7 mg of *rosuvastatin for peak identification CRS* (containing impurity M) in 2.5 mL of *acetonitrile R* and dilute to 10 mL with *water R*.

Column:
- *size*: l = 0.15 m, Ø = 3.0 mm;
- *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (3 µm);
- *temperature*: 40 °C.

Mobile phase:
- *mobile phase A*: 1 per cent *V/V* solution of *trifluoroacetic acid R, acetonitrile for chromatography R, water for chromatography R* (1:29:70 *V/V/V*);
- *mobile phase B*: 1 per cent *V/V* solution of *trifluoroacetic acid R, water for chromatography R, acetonitrile for chromatography R* (1:24:75 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	100	0
30 - 50	100 → 60	0 → 40
50 - 60	60 → 0	40 → 100
60 - 70	0	100

Flow rate 0.75 mL/min.

Detection Spectrophotometer at 242 nm.

Injection 10 µL of the test solution and reference solutions (b), (c), (d) and (e).

Identification of impurities Use the chromatogram supplied with *rosuvastatin for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B and C; use the chromatogram supplied with *rosuvastatin impurity mixture CRS* and the chromatogram obtained with reference solution (d) to identify the peaks due to impurities D and K; use the chromatogram supplied with *rosuvastatin for peak identification CRS* and the chromatogram obtained with reference solution (e) to identify the peak due to impurity M.

Relative retention With reference to rosuvastatin (retention time = about 25 min): impurity M = about 0.8; impurity A = about 0.9; impurity B = about 1.1; impurity C = about 1.5; impurity D = about 1.9; impurity K = about 2.0.

System suitability Reference solution (c):
- *resolution*: minimum 2.0 between the peaks due to rosuvastatin and impurity B.

Calculation of percentage contents:
- *correction factor*: multiply the peak area of impurity C by 1.4;
- for each impurity, use the concentration of rosuvastatin calcium in reference solution (b).

Limits:
- *impurity C*: maximum 0.8 per cent;
- *impurity B*: maximum 0.5 per cent;
- *impurity A*: maximum 0.2 per cent;
- *impurities D, K, M*: for each impurity, maximum 0.15 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 1.2 per cent;
- *reporting threshold*: 0.05 per cent.

Water (*2.5.12*)
Maximum 6.1 per cent, determined on 0.100 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{44}H_{54}CaF_2N_6O_{12}S_2$ taking into account the assigned content of *rosuvastatin calcium CRS*.

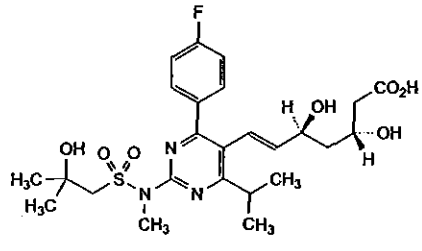
STORAGE

In an airtight container, protected from light, at a temperature of 2 °C to 8 °C.

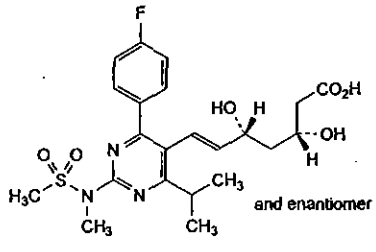
IMPURITIES

Specified impurities *A, B, C, D, G, K, L, M.*

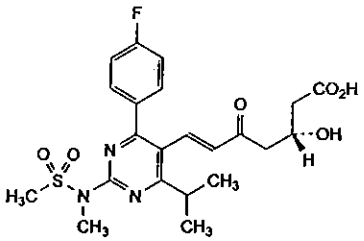
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *E, F, J.*



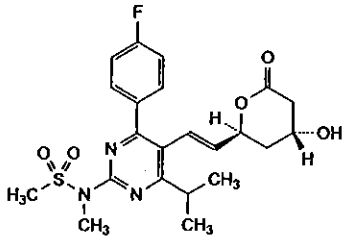
A. (3*R*,5*S*,6*E*)-7-[2-(2,*N*-dimethyl-2-hydroxypropane-1-sulfonamido)-4-(4-fluorophenyl)-6-(propan-2-yl) pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid,



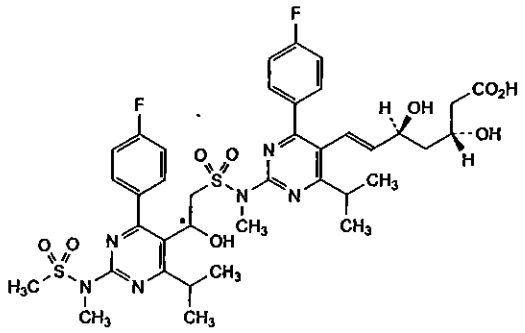
B. (3*RS*,5*RS*,6*E*)-7-[4-(4-fluorophenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid,



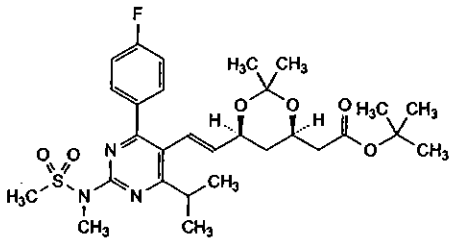
C. (3*R*,6*E*)-7-[4-(4-fluorophenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3-hydroxy-5-oxohept-6-enoic acid,



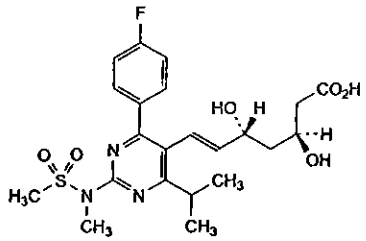
D. *N*-[4-(4-fluorophenyl)-5-[(1*E*)-2-[(2*S*,4*R*)-4-hydroxy-6-oxooxan-2-yl]ethen-1-yl]-6-(propan-2-yl)pyrimidin-2-yl]-*N*-methylmethanesulfonamide,



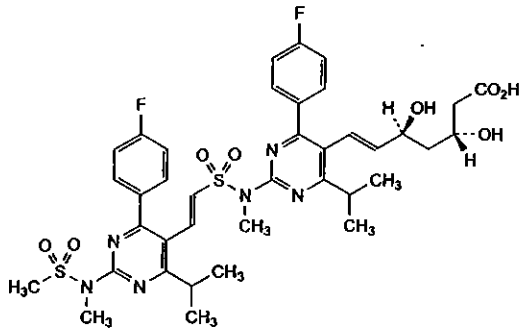
E. (3*R*,5*S*,6*E*)-7-[4-(4-fluorophenyl)-2-[(2*E*)-2-[4-(4-fluorophenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-2-hydroxy-*N*-methylethane-1-sulfonamido]-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid,



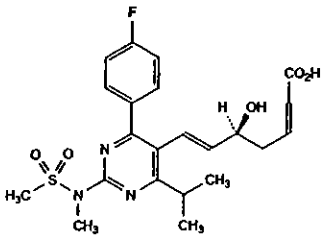
F. *tert*-butyl[(4*R*,6*S*)-6-[(1*E*)-2-[4-(4-fluorophenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]ethen-1-yl]-2,2-dimethyl-1,3-dioxan-4-yl]acetate,



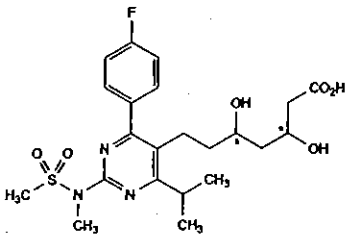
G. (3*S*,5*R*,6*E*)-7-[4-(4-fluorophenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid,



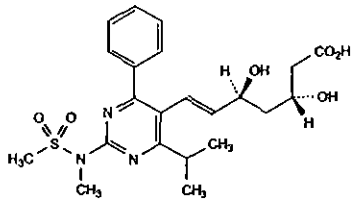
J. (3*R*,5*S*,6*E*)-7-[4-(4-fluorophenyl)-2-[(1*E*)-2-[4-(4-fluorophenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-*N*-methylethene-1-sulfonamido]-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid,



K. (2*E*,5*S*,6*E*)-7-[4-(4-fluorophenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-5-hydroxyhepta-2,6-dienoic acid,



L. (3*E*,5*E*)-7-[4-(4-fluorophenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyheptanoic acid,

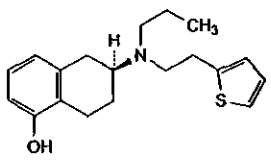


M.(3*R*,5*S*,6*E*)-7-[2-(*N*-methylmethanesulfonamido)-4-phenyl-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid,

Ph Eur

Rotigotine

(Ph. Eur. monograph 3014)



$C_{19}H_{25}NOS$ 315.5 *99755-59-6*

Action and use
Dopamine D2 receptor agonist; treatment of Parkinson's disease and restless legs syndrome.

Preparation
Rotigotine Transdermal Patches

Ph Eur

DEFINITION
(6*S*)-6-[Propyl[2-(thiophen-2-yl)ethyl]amino]-5,6,7,8-tetrahydronaphthalen-1-ol.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or light brown powder.

Solubility
Practically insoluble in water, soluble in anhydrous ethanol, very slightly soluble in heptane.

It shows polymorphism (*5.9*).

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *rotigotine CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *anhydrous ethanol R*, evaporate to dryness and record new spectra using the residues.

B. Enantiomeric purity (see Tests).

TESTS

Enantiomeric purity
Liquid chromatography (*2.2.29*).

Solution A *methanol R1, 2-propanol R, anhydrous ethanol R* (5:5:90 *V/V/V*).

Solution B 0.1 per cent *V/V* solution of *diethylamine R* in solution A.

Test solution Dissolve 10 mg of the substance to be examined in solution B and dilute to 10 mL with solution B.

Reference solution Dissolve 2 mg of *racemic rotigotine CRS* in 1 mL of solution B.

Column:
— *size:* l = 0.25 m, Ø = 4.6 mm;
— *stationary phase: cellulose derivative of silica gel for chiral separation R* (10 µm).

Mobile phase *diethylamine R*, solution A, *heptane R* (0.1:2:98 *V/V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 225 nm.

Injection 10 µL.

Run time 1.5 times the retention time of rotigotine.

Relative retention With reference to rotigotine (retention time = about 22 min): impurity A = about 0.9.

System suitability Reference solution:
— *resolution:* minimum 1.5 between the peaks due to impurity A and rotigotine.

Limit:
— *impurity A:* maximum 0.15 per cent, calculate the ratio of the area of the peak due to impurity A to the sum of the areas of the peaks due to rotigotine and impurity A.

Related substances
Liquid chromatography (*2.2.29*).

Solution A 0.1 per cent *V/V* solution of *trifluoroacetic acid R.*

Solution B 0.1 per cent *V/V* solution of *trifluoroacetic acid R* in *acetonitrile R.*

Test solution (a) Suspend 30.0 mg of the substance to be examined in 2.5 mL of solution B and add 20 mL of solution A. Sonicate for at least 20 min, shaking vigorously at 5 min intervals, until dissolution is complete, then dilute to 50.0 mL with solution A.

Test solution (b) Dilute 1.0 mL of test solution (a) to 10.0 mL with solution A.

Reference solution (a) Suspend 33.0 mg of *rotigotine hydrochloride CRS* in 2.5 mL of solution B and add 20 mL of solution A. Sonicate for at least 20 min, shaking vigorously at 5 min intervals, until dissolution is complete, then dilute to 50.0 mL with solution A. Dilute 1.0 mL of this solution to 10.0 mL with solution A.

Reference solution (b) Suspend 3 mg of *rotigotine impurity B CRS*, 5 mg of *rotigotine impurity C CRS*, 5 mg of *rotigotine impurity G CRS* and 3 mg of *rotigotine impurity H CRS* in 5 mL of solution B and add 40 mL of solution A. Sonicate for at least 20 min, shaking vigorously at 5 min intervals, until dissolution is complete, then dilute to 100.0 mL with solution A. Dilute 1.0 mL of this solution to 50.0 mL with solution A.

Reference solution (c) To 1.0 mL of test solution (a) add 5 mL of solution B and dilute to 100.0 mL with solution A. Dilute 1.0 mL of this solution to 10.0 mL with solution A.

Column:
— *size:* l = 0.15 m, Ø = 4.6 mm;
— *stationary phase: end-capped extra-dense bonded octylsilyl silica gel for chromatography R* (5 µm);
— *temperature:* 40 °C.

Mobile phase:
— *mobile phase A: trifluoroacetic acid R, water for chromatography R* (0.3:1000 *V/V*);
— *mobile phase B: trifluoroacetic acid R, acetonitrile for chromatography R* (0.2:1000 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	82	18
2 - 24	82 → 50	18 → 50

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µL of test solution (a) and reference solutions (b) and (c).

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B, C, G and H.

Relative retention With reference to rotigotine (retention time = about 10 min): impurity B = about 0.2; impurity C = about 0.6; impurity G = about 1.6; impurity H = about 1.7.

System suitability Reference solution (b):
— *resolution*: minimum 1.5 between the peaks due to impurities G and H.

Calculation of percentage contents:
— for each impurity, use the concentration of rotigotine in reference solution (c).

Limits:
— *impurities C, G*: for each impurity, maximum 0.2 per cent;
— *impurity B*: maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.6 per cent;
— *reporting threshold*: 0.05 per cent.

Water (*2.5.12*)
Maximum 0.20 per cent, determined on 1.00 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution (b) and reference solution (a).

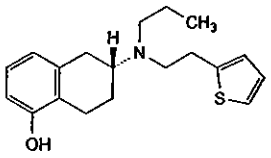
System suitability Reference solution (a):
— *symmetry factor*: maximum 2.2 for the principal peak.

Calculate the percentage content of $C_{19}H_{25}NOS$ taking into account the assigned content of *rotigotine hydrochloride CRS* and a conversion factor of 0.8964.

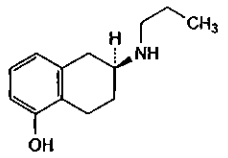
IMPURITIES

Specified impurities *A, B, C, G.*

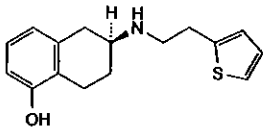
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *D, E, F, H, I, J, K.*



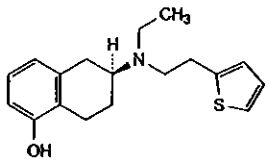
A. (6*R*)-6-[propyl[2-(thiophen-2-yl)ethyl]amino]-5,6,7,8-tetrahydronaphthalen-1-ol ((*R*)-rotigotine),



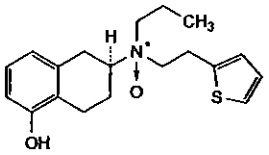
B. (6*S*)-6-(propylamino)-5,6,7,8-tetrahydronaphthalen-1-ol (desthienylethyl rotigotine),



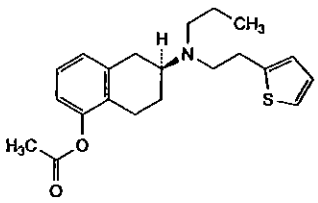
C. (6*S*)-6-[[2-(thiophen-2-yl)ethyl]amino]-5,6,7,8-tetrahydronaphthalen-1-ol (despropyl rotigotine),



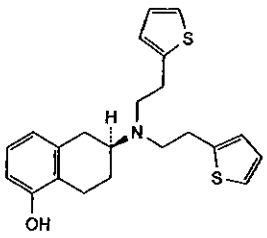
D. (6*S*)-6-[ethyl[2-(thiophen-2-yl)ethyl]amino]-5,6,7,8-tetrahydronaphthalen-1-ol (ethyl rotigotine),



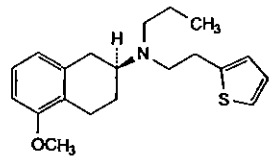
E. (2*S*)-5-hydroxy-*N*-propyl-*N*-[2-(thiophen-2-yl)ethyl]-1,2,3,4-tetrahydronaphthalen-2-amine *N*-oxide (rotigotine *N*-oxide),



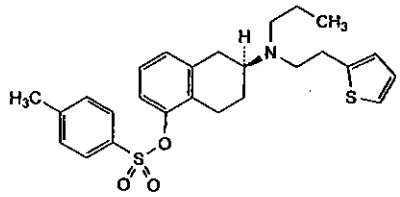
F. (6*S*)-6-[propyl[2-(thiophen-2-yl)ethyl]amino]-5,6,7,8-tetrahydronaphthalen-1-yl acetate (acetyl rotigotine),



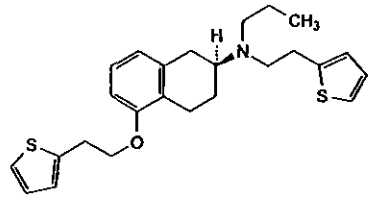
G. (6*S*)-6-[*N*,*N*-bis[2-(thiophen-2-yl)ethyl]amino]-5,6,7,8-tetrahydronaphthalen-1-ol,



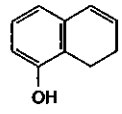
H. (2*S*)-5-methoxy-*N*-propyl-*N*-[2-(thiophen-2-yl)ethyl]-1,2,3,4-tetrahydronaphthalen-2-amine (methoxy rotigotine),



I. (6*S*)-6-[propyl[2-(thiophen-2-yl)ethyl]amino]-5,6,7,8-tetrahydronaphthalen-1-yl 4-methylbenzenesulfonate (rotigotine toluene sulfonic acid ester),



J. (2*S*)-*N*-propyl-5-[2-(thiophen-2-yl)ethoxy]-*N*-[2-(thiophen-2-yl)ethyl]-1,2,3,4-tetrahydronaphthalen-2-amine (rotigotine thienylethyl ether),

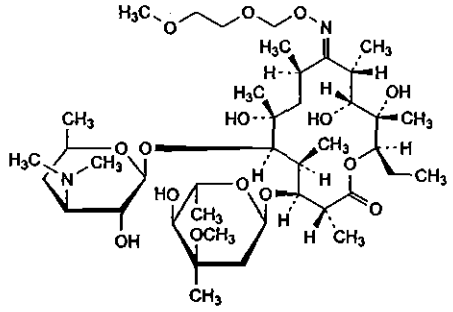


K. 7,8-dihydronaphthalen-1-ol.

Ph Eur

Roxithromycin

(Ph. Eur. monograph 1146)



$C_{41}H_{76}N_2O_{15}$ 837 *80214-83-1*

Action and use
Antibacterial.

Ph Eur

DEFINITION
(3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*S*,12*R*,13*S*,14*R*)-4-[(2,6Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-10-[(*E*)[(2-methoxyethoxy)methoxy]imino]-3,5,7,9,11,13hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*hexopyranosyl]oxy]oxacyclotetradecan-2-one(erythromycin 9-(*E*)-[*O*-[(2-methoxyethoxy)methyl]oxime]).

Semi-synthetic product derived from a fermentation product.

Content
96.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Very slightly soluble in water, freely soluble in acetone, in alcohol and in methylene chloride. It is slightly soluble in dilute hydrochloric acid.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *roxithromycin CRS.*

If the spectra obtained shows differences, prepare further spectra using 90 g/L solutions in *methylene chloride R.*

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 0.2 g in *methanol R* and dilute to 20 mL with the same solvent.

Specific optical rotation (*2.2.7*)
−93 to −96 (anhydrous substance).

Dissolve 0.500 g in *acetone R* and dilute to 50.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Solution A Mix 30 volumes of *acetonitrile R* and 70 volumes of a 48.6 g/L solution of *ammonium dihydrogen phosphate R*, adjusted to pH 5.3 with *dilute sodium hydroxide solution R.*

Test solution Dissolve 50.0 mg of the substance to be examined in solution A and dilute to 25.0 mL with solution A.

Reference solution (a) Dissolve 50.0 mg of *roxithromycin CRS* in solution A and dilute to 25.0 mL with solution A.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 100.0 mL with solution A.

Reference solution (c) Dissolve 2.0 mg of *roxithromycin for system suitability CRS* in solution A and dilute to 1.0 mL with solution A.

Reference solution (d) Dilute 1.0 mL of *toluene R* to 100.0 mL with *acetonitrile R.* Dilute 0.2 mL of this solution to 200.0 mL with solution A.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm,
— *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm) with a 10 nm pore size and a carbon loading of about 19 per cent,
— *temperature*: 15 °C.

Mobile phase:
— *mobile phase A*: mix 26 volumes of *acetonitrile R* and 74 volumes of a 59.7 g/L solution of *ammonium*

dihydrogen phosphate R, adjusted to pH 4.3 with *dilute sodium hydroxide solution R*,
— *mobile phase B*: *water R*, *acetonitrile R* (30:70 *V/V*),

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 50	100	0
50 - 51	100 → 90	0 → 10
51 - 80	90	10
80 - 81	90 → 100	10 → 0
81 - 100	100	0

Flow rate 1.1 mL/min.

Detection Spectrophotometer at 205 nm.

Injection 20 µL, using an injector maintained at 8 °C, of the test solution and reference solutions (b), (c) and (d).

Relative retention With reference to roxithromycin (retention time = about 22 min): impurity A = about 0.28; impurity B = about 0.31; impurity C = about 0.33; impurity D = about 0.62; impurity E = about 0.67; impurity F = about 0.83; impurity G = about 1.15; impurity K = about 1.7; impurity H = about 1.85; impurity J = about 2.65; impurity I = about 3.1.

System suitability Reference solution (c):
— *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to roxithromycin.

Limits:
— *impurity G*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent),
— *impurities A, B, C, D, E, F, H, I, J*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),
— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent),
— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent). Disregard any peak due to toluene (use reference solution (d) to identify it).

Water (*2.5.12*)
Maximum 3.0 per cent, determined on 0.200 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances, with the following modifications.

Column:
— *size*: *l* = 0.25 m.

Mobile phase Mix 307 volumes of *acetonitrile R* and 693 volumes of a 49.1 g/L solution of *ammonium dihydrogen phosphate R* adjusted to pH 5.3 with *dilute sodium hydroxide solution R*.

Flow rate 1.5 mL/min.

Injection Test solution and reference solutions (a) and (c).

Retention time Roxithromycin = about 12 min.

System suitability Reference solution (c):
— *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to roxithromycin.

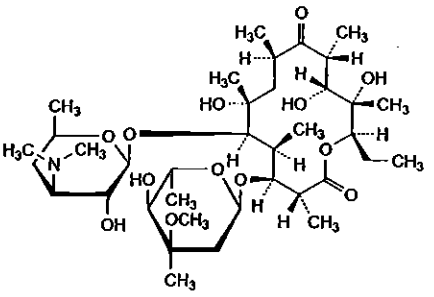
STORAGE

In an airtight container.

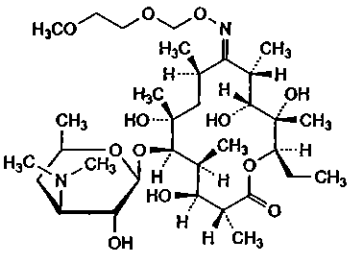
IMPURITIES

Specified impurities *A, B, C, D, E, F, G, H, I, J.*

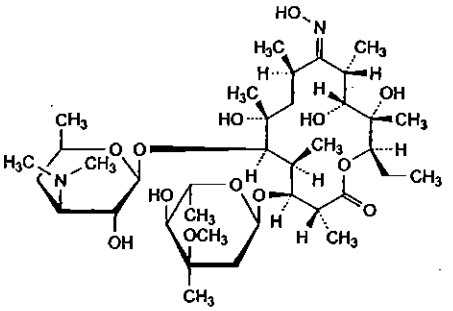
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *K.*



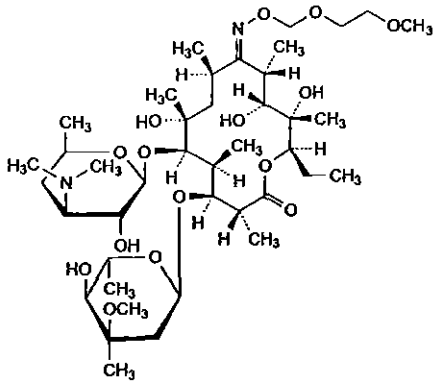
A. (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-4-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]oxacyclotetradecane-2,10-dione (erythromycin A),



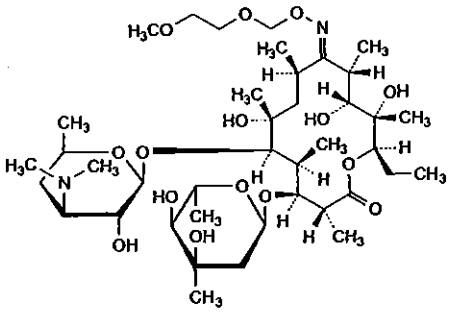
B. 3-*O*-de(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)erythromycin 9-(*E*)-[*O*-[(2-methoxyethoxy)methyl]oxime],



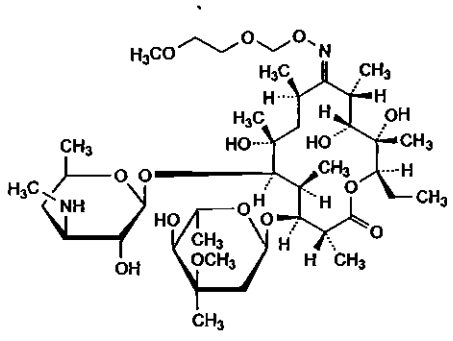
C. erythromycin 9-(*E*)-oxime,



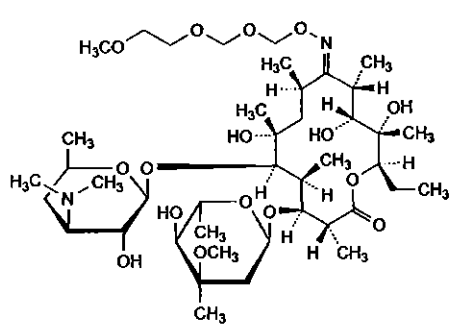
D. erythromycin 9-(*Z*)-[*O*-[(2-methoxyethoxy)methyl]oxime],



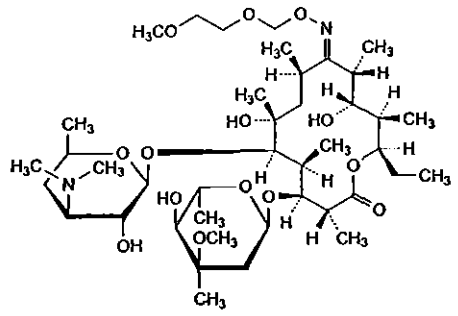
E. 3″-*O*-demethylerythromycin 9-(*E*)-[*O*-[(2-methoxyethoxy) methyl]oxime],



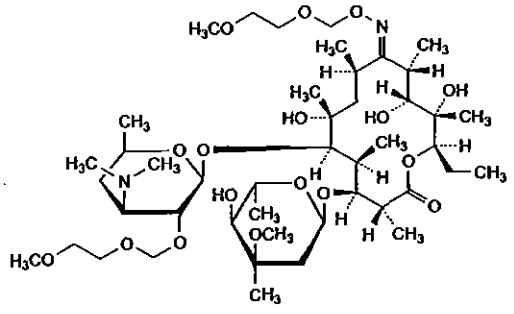
F. 3′-*N*-demethylerythromycin 9-(*E*)-[*O*-[(2-methoxyethoxy) methyl]oxime],



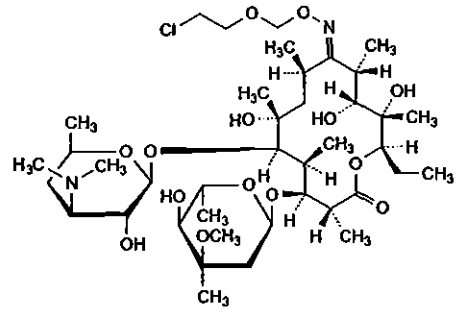
G. erythromycin 9-(*E*)-[*O*-[[(2-methoxyethoxy) methoxy]methyl]oxime],



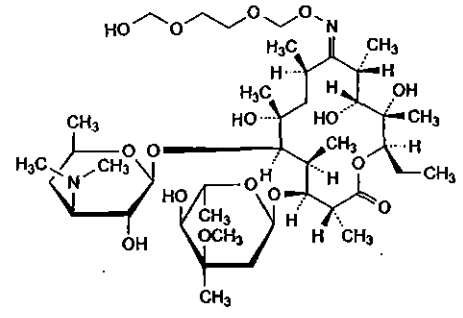
H. 12-deoxyerythromycin 9-(*E*)-[*O*-[(2-methoxyethoxy)methyl]oxime],



I. 2′-*O*-[(2-methoxyethoxy)methyl]erythromycin 9-(*E*)-[*O*-[(2-methoxyethoxy)methyl]oxime],



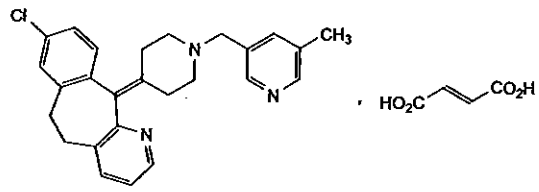
J. erythromycin 9-(*E*)-[*O*-[(2-chloroethoxy)methyl]oxime],



K. erythromycin 9-(*E*)-[*O*-[[2-(hydroxymethoxy) ethoxy]methyl]oxime].

Rupatadine Fumarate

(Ph. Eur. monograph 2888)



$C_{30}H_{30}ClN_3O_4$ 532.0 *182349-12-8*

Ph Eur

DEFINITION

8-Chloro-11-[1-[(5-methylpyridin-3-yl)methyl]piperidin-4-ylidene]-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine (2*E*)-but-2-enedioate.

Content

98.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or slightly pinkish powder.

Solubility

Very slightly soluble in water, slightly soluble in anhydrous ethanol, very slightly soluble in heptane.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *rupatadine fumarate CRS.*

TESTS

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Solvent mixture *acetonitrile R*, mobile phase A (20:80 *V/V*).

Test solution Dissolve 32.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 3 mg of *rupatadine for system suitability CRS* (containing impurities A and B) in the solvent mixture and dilute to 5 mL with the solvent mixture.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 30 °C.

Mobile phase:

— *mobile phase A*: 7.0 g/L solution of *sodium dihydrogen phosphate monohydrate R* in *water for chromatography R*;

— *mobile phase B*: *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	80	20
2 - 27	80 → 50	20 → 50

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 50 µL.

Identification of impurities Use the chromatogram supplied with *rupatadine for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and B.

Relative retention With reference to rupatadine (retention time = about 17 min): fumaric acid = about 0.1; impurity A = about 0.6; impurity B = about 0.7.

System suitability Reference solution (b):

— *resolution*: minimum 5.0 between the peaks due to impurities A and B.

Calculation of percentage contents:

— *correction factor*: multiply the peak area of impurity A by 1.3;

— for each impurity, use the concentration of rupatadine fumarate in reference solution (a).

Limits:

— *impurity B*: maximum 0.5 per cent;

— *impurity A*: maximum 0.15 per cent;

— *unspecified impurities*: for each impurity, maximum 0.10 per cent;

— *total*: maximum 0.7 per cent;

— *reporting threshold*: 0.05 per cent; disregard the peak due to fumaric acid.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* in an oven at 80 °C for 3 h.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 50 mL of *glacial acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 17.73 mg of $C_{30}H_{30}ClN_3O_4$.

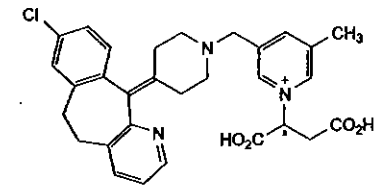
STORAGE

Protected from light.

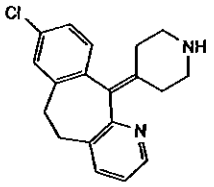
IMPURITIES

Specified impurities *A, B.*

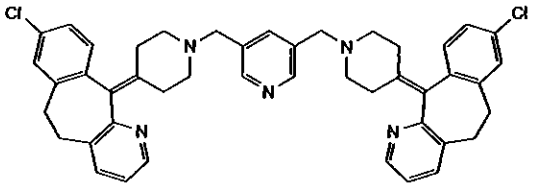
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C.*



A. 3-[[4-(8-chloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-ylidene)piperidin-1-yl]methyl]-1-(1,2-dicarboxyethyl)-5-methylpyridin-1-ium,



B. 8-chloro-11-(piperidin-4-ylidene)-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine,

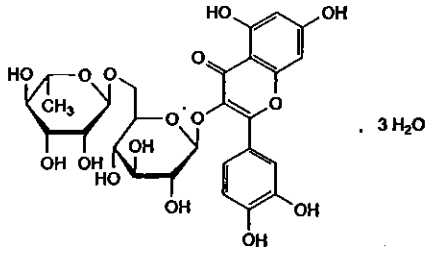


C. 11,11′-[pyridine-3,5-diylbis(methylenepiperidin-1-yl-4-ylidene)]bis(8-chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine).

Ph Eur

Rutoside Trihydrate

(Ph. Eur. monograph 1795)



$C_{27}H_{30}O_{16},3H_2O$ 665 *250249-75-3*

Action and use
Bioflavinoid.

Ph Eur

DEFINITION

3-[[6-*O*-(6-Deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4*H*-1-benzopyran-4-one trihydrate.

Content
95.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance
Yellow or greenish-yellow, crystalline powder.

Solubility
Practically insoluble in water, soluble in methanol, sparingly soluble in ethanol (96 per cent), practically insoluble in methylene chloride. It dissolves in solutions of alkali hydroxides.

IDENTIFICATION

First identification: *B.*

Second identification: *A, C, D.*

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 50.0 mg in *methanol R*, dilute to 250.0 mL with the same solvent and filter if necessary. Dilute 5.0 mL of the solution to 50.0 mL with *methanol R*.

Spectral range 210-450 nm.

Absorption maxima At 257 nm and 358 nm.

Specific absorbance at the absorption maximum at 358 nm 305 to 330 (anhydrous substance).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *rutoside trihydrate CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution Dissolve 25 mg of *rutoside trihydrate CRS* in *methanol R* and dilute to 10.0 mL with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *butanol R, anhydrous acetic acid R, water R, methyl ethyl ketone R, ethyl acetate R* (5:10:10:30:50 *V/V/V/V/V*).

Application 10 µL.

Development Over a path of 10 cm.

Drying In air.

Detection Spray with a mixture of 2.5 mL of *ferric chloride solution R1* and 7.5 mL of a 10 g/L solution of *potassium ferricyanide R* and examine for 10 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve 10 mg in 5 mL of *ethanol (96 per cent) R*, add 1 g of *zinc R* and 2 mL of *hydrochloric acid R1*. A red colour develops.

TESTS

Light-absorbing impurities
The absorbance (*2.2.25*) is not greater than 0.10 at wavelengths between 450 nm and 800 nm.

Dissolve 0.200 g in 40 mL of *2-propanol R*. Stir for 15 min, dilute to 50.0 mL with *2-propanol R* and filter.

Substances insoluble in methanol
Maximum 3 per cent.

Shake 2.5 g of the substance to be examined for 15 min in 50 mL of *methanol R* at 20-25 °C. Filter under reduced pressure through a sintered-glass filter (1.6) (*2.1.2*) previously dried for 15 min at 100-105 °C, allowed to cool in a desiccator and tared. Wash the filter 3 times with 20 mL of *methanol R*. Dry the filter for 30 min at 100-105 °C. Allow to cool and weigh. The residue weighs a maximum of 75 mg.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.10 g of the substance to be examined in 20 mL of *methanol R* and dilute to 100.0 mL with mobile phase B.

Reference solution (a) Dissolve 10.0 mg of *rutoside trihydrate CRS* in 2.0 mL of *methanol R* and dilute to 10.0 mL with mobile phase B.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 50.0 mL with mobile phase B.

Column:
— *size*: *l* = 0.25 m, Ø = 4.0 mm;
— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 30 °C.

Mobile phase:
— *mobile phase A*: mix 5 volumes of *tetrahydrofuran R* and 95 volumes of a 15.6 g/L solution of *sodium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*;
— *mobile phase B*: mix 40 volumes of *tetrahydrofuran R* and 60 volumes of a 15.6 g/L solution of *sodium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	50 → 0	50 → 100
10 - 15	0	100
15 - 16	0 → 50	100 → 50
16 - 20	50	50

Flow rate 1 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µL.

Relative retention With reference to rutoside (retention time = about 7 min): impurity B = about 1.1; impurity A = about 1.2; impurity C = about 2.5.

System suitability Reference solution (a):
— *resolution*: minimum 2.5 between the peaks due to rutoside and impurity B.

Limits Locate the impurities by comparison with the chromatogram provided with *rutoside trihydrate CRS*:
— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.8; impurity C = 0.5;
— *impurities A, B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (4.0 per cent);
— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Water (*2.5.12*)
7.5 per cent to 9.5 per cent, determined on 0.100 g.

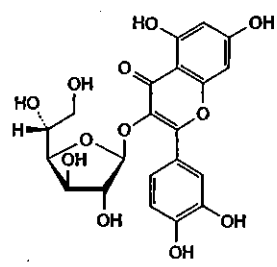
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.200 g in 20 mL of *dimethylformamide R*. Titrate with 0.1 M tetrabutylammonium hydroxide, determining the end-point potentiometrically (*2.2.20*).

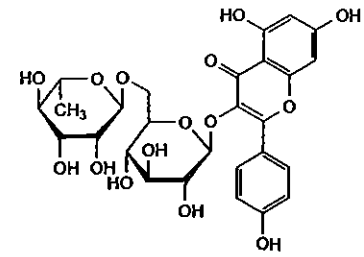
1 mL of 0.1 M tetrabutylammonium hydroxide is equivalent to 30.53 mg of $C_{27}H_{30}O_{16}$.

STORAGE
Protected from light.

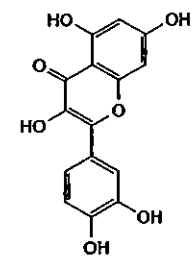
IMPURITIES



A. 2-(3,4-dihydroxyphenyl)-3-(β-D-glucofuranosyloxy)-5,7-dihydroxy-4*H*-1-benzopyran-4-one (isoquercitroside),



B. 3-[[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5,7-dihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (kaempferol 3-rutinoside),

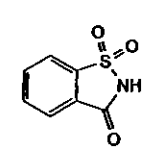


C. 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one (quercetin).

Ph Eur

Saccharin

(Ph. Eur. monograph 0947)



$C_7H_5NO_3S$ 183.2 *81-07-2*

Action and use
Sweetening agent.

Ph Eur

DEFINITION
1,2-Benzisothiazol-3(2*H*)-one 1,1-dioxide.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder or colourless crystals.

Solubility
Sparingly soluble in boiling water and in ethanol (96 per cent), slightly soluble in cold water. It dissolves in dilute solutions of alkali hydroxides and carbonates.

IDENTIFICATION
First identification: C.

Second identification: A, B, D, E.

A. A saturated solution, prepared without heating, turns *blue litmus paper R* red.

B. Melting point (*2.2.14*): 226 °C to 230 °C.

C. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *saccharin CRS.*

D. Mix about 10 mg with about 10 mg of *resorcinol R*, add 0.25 mL of *sulfuric acid R* and carefully heat the mixture over a naked flame until a dark green colour is produced. Allow to cool, add 10 mL of *water R* and *dilute sodium hydroxide solution R* until an alkaline reaction is produced. An intense green fluorescence develops.

E. To 0.2 g add 1.5 mL of *dilute sodium hydroxide solution R*, evaporate to dryness and heat the residue carefully until it melts, avoiding carbonisation. Allow to cool, dissolve the mass in about 5 mL of *water R*, add *dilute hydrochloric acid R* until a weak acid reaction is produced and filter, if necessary. To the filtrate add 0.2 mL of *ferric chloride solution R2*. A violet colour develops.

TESTS

Solution S

Dissolve 5.0 g in 20 mL of a 200 g/L solution of *sodium acetate R* and dilute to 25 mL with the same solution.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

o- and *p*-Toluenesulfonamide

Gas chromatography (*2.2.28*).

Internal standard solution Dissolve 25 mg of *caffeine R* in *methylene chloride R* and dilute to 100 mL with the same solvent.

Test solution Suspend 10.0 g of the substance to be examined in 20 mL of *water R* and dissolve using 5-6 mL of *strong sodium hydroxide solution R*. If necessary adjust the solution to pH 7-8 with *1 M sodium hydroxide* or *1 M hydrochloric acid* and dilute to 50 mL with *water R*. Shake the solution with 4 quantities, each of 50 mL, of *methylene chloride R*. Combine the lower layers, dry over *anhydrous sodium sulfate R* and filter. Wash the filter and the sodium sulfate with 10 mL of *methylene chloride R*. Combine the solution and the washings and evaporate almost to dryness in a water-bath at a temperature not exceeding 40 °C. Using a small quantity of *methylene chloride R*, quantitatively transfer the residue into a suitable 10 mL tube, evaporate to dryness in a current of nitrogen and dissolve the residue in 1.0 mL of the internal standard solution.

Blank solution Evaporate 200 mL of *methylene chloride R* to dryness in a water-bath at a temperature not exceeding 40 °C. Dissolve the residue in 1 mL of *methylene chloride R*.

Reference solution Dissolve 20.0 mg of *o-toluenesulfonamide R* and 20.0 mg of *toluenesulfonamide R* in *methylene chloride R* and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of the solution to 50.0 mL with *methylene chloride R*. Evaporate 5.0 mL of the final solution to dryness in a current of nitrogen. Dissolve the residue in 1.0 mL of the internal standard solution.

Column:
- *material*: fused silica;
- *size*: *l* = 10 m, Ø = 0.53 mm;
- *stationary phase*: *phenyl(50)methyl(50)polysiloxane R* (film thickness 2 µm).

Carrier gas *nitrogen for chromatography R.*

Flow rate 10 mL/min.

Split ratio 1:2.

Temperature:
- *column*: 180 °C;
- *injection port and detector*: 250 °C.

Detection Flame ionisation.

Injection 1 µL.

Order of elution *o*-toluenesulfonamide, *p*-toluenesulfonamide, caffeine.

System suitability:
- *resolution*: minimum 1.5 between the peaks due to *o*-toluenesulfonamide and *p*-toluenesulfonamide in the chromatogram obtained with the reference solution;
- the chromatogram obtained with the blank solution does not show any peak with the same retention times as the internal standard, *o*-toluenesulfonamide and *p*-toluenesulfonamide.

Limits:
- *o-toluenesulfonamide*: the ratio of its area to that of the internal standard is not greater than the corresponding ratio in the chromatogram obtained with the reference solution (10 ppm);
- *p-toluenesulfonamide*: the ratio of its area to that of the internal standard is not greater than the corresponding ratio in the chromatogram obtained with the reference solution (10 ppm).

Readily carbonisable substances

Dissolve 0.20 g in 5 mL of *sulfuric acid R* and keep at 48-50 °C for 10 min. When viewed against a white background, the solution is not more intensely coloured than a solution prepared by mixing 0.1 mL of red primary solution, 0.1 mL of blue primary solution and 0.4 mL of yellow primary solution (*2.2.2*) with 4.4 mL of *water R*.

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulfated ash (*2.4.14*)

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.500 g in 40 mL of *ethanol (96 per cent) R*. Add 40 mL of *water R*. Titrate with *0.1 M sodium hydroxide*, using a 10 g/L solution of *phenolphthalein R* in *ethanol (96 per cent) R* as indicator. Carry out a blank titration.

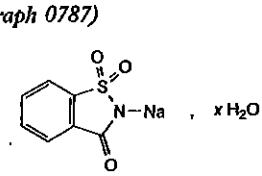
1 mL of *0.1 M sodium hydroxide* is equivalent to 18.32 mg of $C_7H_5NO_3S$.

Ph Eur

Saccharin Sodium

Soluble Saccharin

(Ph. Eur. monograph 0787)



$C_7H_4NNaO_3S,xH_2O$ 205.2 (anhydrous substance)

Anhydrous saccharin sodium *128-44-9*

Action and use

Sweetening agent.

Ph Eur

DEFINITION

2-Sodio-1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

It may be anhydrous or contain a variable quantity of water.

CHARACTERS

Appearance
White or almost white, crystalline powder or colourless crystals, efflorescent in dry air.

Solubility
Freely soluble in water, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: B, E.

Second identification: A, C, D, E.

A. Melting point (*2.2.14*): 226 °C to 230 °C.

To 5 mL of solution S (see Tests) add 3 mL of *dilute hydrochloric acid R*. A white precipitate is formed. Filter and wash with *water R*. Dry the precipitate at 100-105 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs; dry the substances to constant mass at 105 °C before use.

Comparison *saccharin sodium CRS*.

C. Mix about 10 mg with about 10 mg of *resorcinol R*, add 0.25 mL of *sulfuric acid R* and carefully heat the mixture over a naked flame until a dark green colour is produced. Allow to cool, add 10 mL of *water R* and *dilute sodium hydroxide solution R* until an alkaline reaction is produced. An intense green fluorescence develops.

D. To 0.2 g add 1.5 mL of *dilute sodium hydroxide solution R*, evaporate to dryness and heat the residue carefully until it melts, avoiding carbonisation. Allow to cool, dissolve the mass in about 5 mL of *water R*, add *dilute hydrochloric acid R* until a weak acid reaction is produced and filter, if necessary. To the filtrate add 0.2 mL of *ferric chloride solution R2*. A violet colour develops.

E. Solution S gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S
Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50.0 mL with the same solvent.

Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 2.0 g in 10 mL of *water R*.

Acidity or alkalinity
To 10 mL of solution S add about 0.05 mL of a 10 g/L solution of *phenolphthalein R* in *ethanol (96 per cent) R*. The solution is neither pink nor red. Add 0.05 mL of *0.1 M sodium hydroxide*. The solution becomes pink or red.

o- and p-Toluenesulfonamide
Gas chromatography (*2.2.28*).

Internal standard solution Dissolve 25 mg of *caffeine R* in *methylene chloride R* and dilute to 100 mL with the same solvent.

Test solution Dissolve 10.0 g of the substance to be examined in 50 mL of *water R*. If necessary adjust the solution to pH 7-8 by addition of *1 M sodium hydroxide* or *1 M hydrochloric acid*. Shake the solution with 4 quantities, each of 50 mL, of *methylene chloride R*. Combine the lower layers, dry over *anhydrous sodium sulfate R* and filter. Wash the filter and the sodium sulfate with 10 mL of *methylene chloride R*. Combine the solution and the washings and evaporate almost to dryness in a water-bath at a temperature not exceeding 40 °C. Using a small quantity of *methylene chloride R*, quantitatively transfer the residue into a suitable 10 mL tube, evaporate to dryness in a current of *nitrogen R* and add 1.0 mL of the internal standard solution.

Blank solution Evaporate 200 mL of *methylene chloride R* to dryness in a water-bath at a temperature not exceeding 40 °C. Dissolve the residue in 1 mL of *methylene chloride R*.

Reference solution Dissolve 20.0 mg of *o-toluenesulfonamide R* and 20.0 mg of *toluenesulfonamide R* in *methylene chloride R* and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of the solution to 50.0 mL with *methylene chloride R*. Evaporate 5.0 mL of the final solution to dryness in a current of *nitrogen R*. Take up the residue using 1.0 mL of the internal standard solution.

Column:
- *material*: fused silica;
- *size*: *l* = 10 m, Ø = 0.53 mm;
- *stationary phase*: *phenyl(50)methyl(50)polysiloxane R* (film thickness 2 µm).

Carrier gas *nitrogen for chromatography R*.

Flow rate 10 mL/min.

Split ratio 1:2.

Temperature:
- *column*: 180 °C;
- *injection port and detector*: 250 °C.

Detection Flame ionisation.

Injection 1 µL.

Elution order *o*-toluenesulfonamide, *p*-toluenesulfonamide, caffeine.

System suitability:
- *resolution*: minimum 1.5 between the peaks due to *o*-toluenesulfonamide and *p*-toluenesulfonamide in the chromatogram obtained with the reference solution;
- the chromatogram obtained with the blank solution does not show any peak with the same retention times as the internal standard, *o*-toluenesulfonamide and *p*-toluenesulfonamide.

Limits:
- *o-toluenesulfonamide*: the ratio of its area to that of the internal standard is not greater than the corresponding ratio in the chromatogram obtained with the reference solution (10 ppm);
- *p-toluenesulfonamide*: the ratio of its area to that of the internal standard is not greater than the corresponding ratio in the chromatogram obtained with the reference solution (10 ppm).

Readily carbonisable substances
Dissolve 0.200 g in 5 mL of *sulfuric acid R* and keep at 48-50 °C for 10 min. When viewed against a white background, the solution is not more intensely coloured than a solution prepared by mixing 0.1 mL of red primary solution, 0.1 mL of blue primary solution and 0.4 mL of yellow primary solution (*2.2.2*) with 4.4 mL of *water R*.

Water (*2.5.12*)
Maximum 15.0 per cent, determined on 0.200 g.

ASSAY

Dissolve 0.150 g in 50 mL of *anhydrous acetic acid R*, with slight heating if necessary. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 mL of *0.1 M perchloric acid* is equivalent to 20.52 mg of $C_7H_4NNaO_3S$.

STORAGE

In an airtight container.

Ph Eur

Refined Safflower Oil

(Ph. Eur. monograph 2088)

Ph Eur

DEFINITION

Fatty oil obtained from seeds of *Carthamus tinctorius* L. (type I) or from seeds of hybrids of *Carthamus tinctorius* L. (type II), by expression and/or extraction followed by refining. Type II refined safflower oil is rich in oleic ((9*Z*)-octadec-9-enoic) acid. A suitable antioxidant may be added.

PRODUCTION

The oil is prepared using materials and methods designed to ensure that the content of brassicasterol (*2.4.23*) in the sterol fraction of the oil is not greater than 0.3 per cent.

CHARACTERS

Appearance

Clear, viscous, yellow or pale yellow liquid.

Solubility

Practically insoluble in ethanol (96 per cent), miscible with light petroleum (bp: 40-60 °C).

	Type I refined safflower oil	Type II refined safflower oil
Relative density	about 0.922	about 0.914
Refractive index	about 1.476	about 1.472

IDENTIFICATION

First identification: B.

Second identification: A.

A. Identification of fatty oils by thin-layer chromatography (*2.3.2*).

Results The chromatogram obtained is similar to the corresponding chromatogram for type I or type II shown in Figure 2.3.2.-1.

B. Composition of fatty acids (see Tests).

TESTS

Acid value (*2.5.1*)

Maximum 0.5.

Peroxide value (*2.5.5, Method A*)

Maximum 10.0, or maximum 5.0 if intended for use in the manufacture of parenteral preparations.

Unsaponifiable matter (*2.5.7*)

Maximum 1.5 per cent, determined on 5.0 g.

Alkaline impurities (*2.4.19*)

It complies with the test.

Composition of fatty acids (*2.4.22, Method A*)

Use the mixture of calibrating substances in Table 2.4.22.-3.

Composition of the fatty-acid fraction of type I refined safflower oil:

- *saturated fatty acids of chain length less than C14*: maximum 0.2 per cent;
- *myristic acid*: maximum 0.2 per cent;
- *palmitic acid*: 4.0 per cent to 10.0 per cent;
- *stearic acid*: 1.0 per cent to 5.0 per cent;
- *oleic acid*: 8.0 per cent to 21.0 per cent;
- *linoleic acid*: 68.0 per cent to 83.0 per cent;
- *linolenic acid*: maximum 0.5 per cent;
- *arachidic acid*: maximum 0.5 per cent;
- *eicosenoic acid*: maximum 0.5 per cent;
- *behenic acid*: maximum 1.0 per cent.

Composition of the fatty-acid fraction of type II refined safflower oil:

- *saturated fatty acids of chain length less than C14*: maximum 0.2 per cent;
- *myristic acid*: maximum 0.2 per cent;
- *palmitic acid*: 3.6 per cent to 6.0 per cent;
- *stearic acid*: 1.0 per cent to 5.0 per cent;
- *oleic acid*: 70.0 per cent to 84.0 per cent;
- *linoleic acid*: 7.0 per cent to 23.0 per cent;
- *linolenic acid*: maximum 0.5 per cent;
- *arachidic acid*: maximum 1.0 per cent;
- *eicosenoic acid*: maximum 1.0 per cent;
- *behenic acid*: maximum 1.2 per cent.

Water (*2.5.32*)

Maximum 0.1 per cent, determined on 1.00 g.

STORAGE

In a well-filled, airtight container, protected from light.

LABELLING

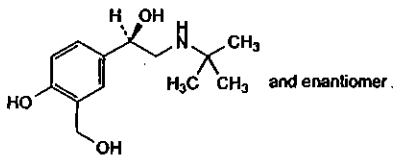
The label states:

- where applicable, that the substance is suitable for use in the manufacture of parenteral preparations;
- the type of oil (type I or type II).

Ph Eur

Salbutamol

(Ph. Eur. monograph 0529)



$C_{13}H_{21}NO_3$ 239.3 *18559-94-9*

Action and use

Beta2-adrenoceptor agonist; bronchodilator.

Preparations

Salbutamol Pressurised Inhalation

Salbutamol Prolonged-release Capsules

Ph Eur

DEFINITION

(1*RS*)-2-[(1,1-Dimethylethyl)amino]-1-[4-hydroxy-3-(hydroxymethyl)phenyl]ethanol.

Content

98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Sparingly soluble in water, soluble in ethanol (96 per cent).

mp

About 155 °C, with decomposition.

IDENTIFICATION

First identification: B.

Second identification: A, C, D.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 80.0 mg in a 10 g/L solution of *hydrochloric acid R* and dilute to 100.0 mL with the same acid. Dilute 10.0 mL of the solution to 100.0 mL with a 10 g/L solution of *hydrochloric acid R*.

Spectral range 230-350 nm.

Absorption maximum At 276 nm.

Specific absorbance at the absorption maximum 66 to 75.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *salbutamol CRS*.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 50 mL with the same solvent.

Reference solution Dissolve 10 mg of *salbutamol CRS* in *methanol R* and dilute to 50 mL with the same solvent.

Plate *TLC silica gel plate R*.

Mobile phase *concentrated ammonia R, water R, ethyl acetate R, 2-propanol R, methyl isobutyl ketone R* (3:18:35:45:50 *V/V/V/V/V*).

Application 5 µL.

Development Over 3/4 of the plate.

Drying In air.

Detection Spray with a 1 g/L solution of *methylbenzothiazolone hydrazone hydrochloride R* in a 90 per cent *V/V* solution of *methanol R*, followed by a 20 g/L solution of *potassium ferricyanide R* in a mixture of 1 volume of *concentrated ammonia R1* and 3 volumes of *water R*, followed by a further spraying with a 1 g/L solution of *methylbenzothiazolone hydrazone hydrochloride R* in a 90 per cent *V/V* solution of *methanol R*.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve about 10 mg in 50 mL of a 20 g/L solution of *disodium tetraborate R*. Add 1 mL of a 30 g/L solution of *aminopyrazolone R*, 10 mL of *methylene chloride R* and 10 mL of a 20 g/L solution of *potassium ferricyanide R*. Shake and allow to separate. An orange-red colour develops in the methylene chloride layer.

TESTS

Solution S

Dissolve 0.50 g in *methanol R* and dilute to 25.0 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

Optical rotation (*2.2.7*)

−0.10° to + 0.10°, determined on solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 2.0 mg of *salbutamol CRS*, 2 mg of *salbutamol impurity B CRS*, 3.0 mg of *salbutamol impurity D CRS*, 3.0 mg of *salbutamol impurity F CRS* and 3.0 mg of *salbutamol impurity G CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase. Dilute 2.0 mL of this solution to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve the contents of a vial of *salbutamol impurity I CRS* in 1.0 mL of the mobile phase.

Reference solution (c) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 20.0 mL with the mobile phase.

Column:

— *size*: *l* = 0.15 m, Ø = 3.9 mm;

— *stationary phase*: spherical *end-capped octylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 335 m^2/g, a pore size of 10 nm and a carbon loading of 11.7 per cent.

Mobile phase Mix 22 volumes of *acetonitrile R1* and 78 volumes of a solution containing 2.87 g/L of *sodium heptanesulfonate R* and 2.5 g/L of *potassium dihydrogen phosphate R* previously ajusted to pH 3.65 with *dilute phosphoric acid R*.

Flow rate 1 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µL.

Run time 25 times the retention time of salbutamol.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peaks due to impurities B, D, F and G; use the chromatogram obtained with reference solution (b) to identify the peak due to impurity I.

Relative retention With reference to salbutamol (retention time = about 2 min): impurity B = about 1.3; impurity A = about 1.7; impurity C = about 2.0; impurity D = about 2.7; impurity H = about 3.0; impurity E = about 3.1; impurity G = about 4.1; impurity F = about 6.2; impurity I = about 23.2.

System suitability Reference solution (a):

— *resolution*: minimum 3.0 between the peaks due to salbutamol and impurity B.

Limits:

— *impurity D*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *impurity F*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *impurity G*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *impurities A, B, C, E, H, I*: for each impurity, not more than 1.5 times the area of the peak due to salbutamol in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the peak due to salbutamol in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: maximum 1.0 per cent;

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Impurity J

Maximum 0.2 per cent.

Dissolve 50.0 mg in a 1 g/L solution of *hydrochloric acid R* and dilute to 25.0 mL with the same solvent.

The absorbance (*2.2.25*) of the solution measured at 310 nm is not greater than 0.10.

Boron

Maximum 50 ppm.

Test solution To 50 mg of the substance to be examined add 5 mL of a solution containing 13 g/L of *anhydrous sodium*

carbonate R and 17 g/L of *potassium carbonate R*. Evaporate to dryness on a water-bath and dry at 120 °C. Ignite the residue rapidly until the organic matter has been destroyed, allow to cool and add 0.5 mL of *water R* and 3.0 mL of a freshly prepared 1.25 g/L solution of *curcumin R* in *glacial acetic acid R*. Warm gently to effect solution, allow to cool and add 3.0 mL of a mixture prepared by adding 5 mL of *sulfuric acid R*, slowly and with stirring, to 5 mL of *glacial acetic acid R*. Mix and allow to stand for 30 min. Dilute to 100.0 mL with *ethanol (96 per cent) R*, filter and use the filtrate.

Reference solution Dissolve 0.572 g of *boric acid R* in 1000.0 mL of *water R*. Dilute 1.0 mL of the solution to 100.0 mL with *water R*. To 2.5 mL of this solution add 5 mL of a solution containing 13 g/L of *anhydrous sodium carbonate R* and 17 g/L of *potassium carbonate R*, and treat this mixture in the same manner as the test solution.

Measure the absorbance (*2.2.25*) of the test solution and of the reference solution at the absorption maximum at about 555 nm. The absorbance of the test solution is not greater than that of the reference solution.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 30 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

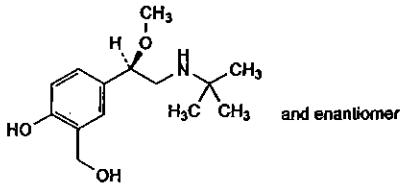
1 mL of *0.1 M perchloric acid* is equivalent to 23.93 mg of $C_{13}H_{21}NO_3$.

STORAGE

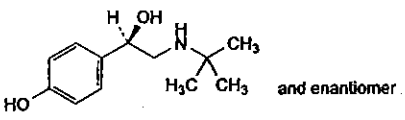
Protected from light.

IMPURITIES

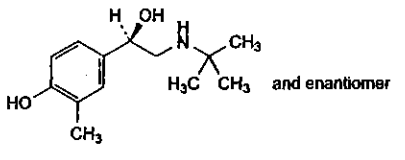
Specified impurities *A, B, C, D, E, F, G, H, I, J.*



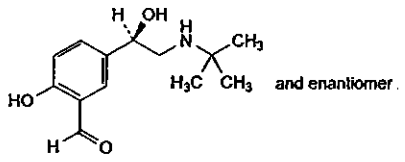
A. 5-[(1*RS*)-2-[(1,1-dimethylethyl)amino]-1-methoxyethyl]-2-hydroxyphenyl]methanol,



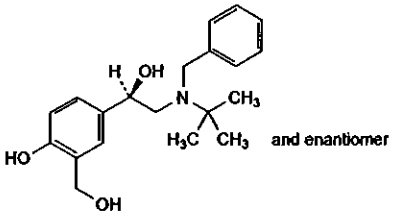
B. (1*RS*)-2-[(1,1-dimethylethyl)amino]-1-(4-hydroxyphenyl) ethanol,



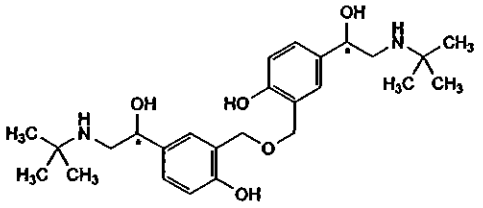
C. (1*RS*)-2-[(1,1-dimethylethyl)amino]-1-(4-hydroxy-3-methylphenyl)ethanol,



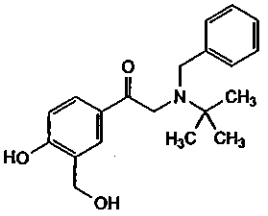
D. 5-[(1*RS*)-2-[(1,1-dimethylethyl)amino]-1-hydroxyethyl]-2-hydroxybenzaldehyde,



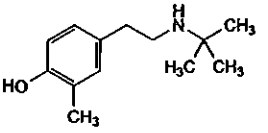
E. (1*RS*)-2-[benzyl(1,1-dimethylethyl)amino]-1-[4-hydroxy-3-(hydroxymethyl)phenyl]ethanol,



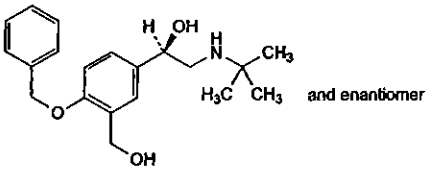
F. 1,1′-[oxybis[methylene(4-hydroxy-1,3-phenylene)]]bis[2-[(1,1-dimethylethyl)amino]ethanol],



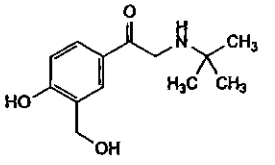
G. 2-[benzyl(1,1-dimethylethyl)amino]-1-[4-hydroxy-3-(hydroxymethyl)phenyl]ethanone,



H. 4-[2-[(1,1-dimethylethyl)amino]ethyl]-2-methylphenol,



I. (1*RS*)-2-[(1,1-dimethylethyl)amino]-1-[4-(benzyloxy)-3-(hydroxymethyl)phenyl]ethanol,

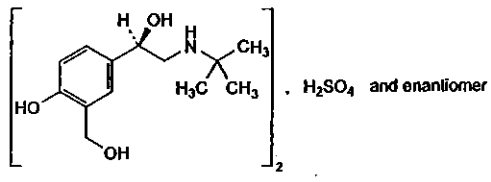


J. 2-[(1,1-dimethylethyl)amino]-1-[4-hydroxy-3-(hydroxymethyl)phenyl]ethanone (salbutamone).

Salbutamol Sulfate

Salbutamol Sulphate

(Ph. Eur. monograph 0687)



$C_{26}H_{44}N_2O_{10}S$ 576.7 *51022-70-9*

Action and use
$Beta_2$-adrenoceptor agonist; bronchodilator.

Preparations
Salbutamol Inhalation Powder
Salbutamol Inhalation Powder, pre-metered
Salbutamol Injection
Salbutamol Nebuliser Solution
Salbutamol Oral Solution
Salbutamol Pressurised Inhalation
Salbutamol Tablets
Salbutamol Prolonged-release Capsules

Ph Eur

DEFINITION

Bis[(1*RS*)-2-[(1,1-dimethylethyl)amino]-1-[4-hydroxy-3-(hydroxymethyl)phenyl]ethanol] sulfate.

Content
98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water, practically insoluble or very slightly soluble in ethanol (96 per cent) and in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification: B, E.

Second identification: A, C, D, E.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 80.0 mg in a 10 g/L solution of *hydrochloric acid R* and dilute to 100.0 mL with the same acid. Dilute 10.0 mL of the solution to 100.0 mL with a 10 g/L solution of *hydrochloric acid R*.

Spectral range 230-350 nm.

Absorption maximum At 276 nm.

Specific absorbance at the absorption maximum 55 to 64.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *salbutamol sulfate CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *anhydrous ethanol R*. Dry the residues and record new spectra using the residues.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 12 mg of the substance to be examined in *water R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 12 mg of *salbutamol sulfate CRS* in *water R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *concentrated ammonia R, water R, ethyl acetate R, 2-propanol R, methyl isobutyl ketone R* (3:18:35:45:50 *V/V/V/V/V*).

Application 1 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with a 1 g/L solution of *methylbenzothiazolone hydrazone hydrochloride R* in a 90 per cent *V/V* solution of *methanol R*, followed by a 20 g/L solution of *potassium ferricyanide R* in a mixture of 1 volume of *concentrated ammonia R1* and 3 volumes of *water R*, followed by a further spraying with a 1 g/L solution of *methylbenzothiazolone hydrazone hydrochloride R* in a 90 per cent *V/V* solution of *methanol R*.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve about 10 mg in 50 mL of a 20 g/L solution of *disodium tetraborate R*. Add 1 mL of a 30 g/L solution of *aminopyrazolone R*, 10 mL of *methylene chloride R* and 10 mL of a 20 g/L solution of *potassium ferricyanide R*. Shake and allow to separate. An orange-red colour develops in the methylene chloride layer.

E. It gives reaction (a) of sulfates (*2.3.1*).

TESTS

Solution S
Dissolve 0.250 g in *carbon dioxide-free water R* and dilute to 25.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Optical rotation (*2.2.7*)
−0.10° to + 0.10°, determined on solution S.

Acidity or alkalinity
To 10 mL of solution S add 0.15 mL of *methyl red solution R* and 0.2 mL of *0.01 M sodium hydroxide*. The solution is yellow. Not more than 0.4 mL of *0.01 M hydrochloric acid* is required to change the colour of the indicator to red.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in mobile phase A and dilute to 50.0 mL with mobile phase A.

Reference solution (a) Dissolve 3.0 mg of *salbutamol impurity D CRS* and 3.0 mg of *salbutamol impurity F CRS* in mobile phase A and dilute to 50.0 mL with mobile phase A. Dilute 2.0 mL of the solution to 100.0 mL with mobile phase A.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

Reference solution (c) With the aid of ultrasound, dissolve the contents of a vial of *salbutamol impurity J CRS* in 1.0 mL of the test solution.

Reference solution (d) Dissolve 1 mg of *salbutamol impurity D CRS* in mobile phase A and dilute to 100.0 mL with mobile phase A.

Reference solution (e) Dissolve 4 mg of *salbutamol sulfate for system suitability CRS* (containing impurities C, F, N and O)

in mobile phase A, add 0.4 mL of reference solution (d) and dilute to 10.0 mL with mobile phase A.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: spherical *end-capped octylsilyl silica gel for chromatography R* (3 µm);
— *temperature*: 30 °C.

Mobile phase:
— *mobile phase A*: dissolve 3.45 g of *sodium dihydrogen phosphate monohydrate R* in 900 mL of a 0.05 per cent *V/V* solution of *triethylamine R*, adjust to pH 3.0 with *dilute phosphoric acid R* and dilute to 1000 mL with a 0.05 per cent *V/V* solution of *triethylamine R*;
— *mobile phase B*: *methanol R*, *acetonitrile R* (35:65 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	95	5
5 - 30	95 → 10	5 → 90

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 273 nm.

Injection 20 µl of the test solution and reference solutions (a), (b), (c) and (e).

Relative retention With reference to salbutamol (retention time = about 7 min): impurity J = about 0.9; impurity C = about 1.6; impurity N = about 1.67; impurity D = about 1.68; impurity F = about 1.77; impurity O = about 1.82.

Identification of impurities Use the chromatogram supplied with *salbutamol sulfate for system suitability CRS* and the chromatogram obtained with reference solution (e) to identify the peaks due to impurities C, D, F, N and O; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity J.

System suitability:
— *peak-to-valley ratio*: minimum 1.2, where H_p = height above the baseline of the peak due to impurity N and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity D in the chromatogram obtained with reference solution (e); minimum 2.0, where H_p = height above the baseline of the peak due to impurity J and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to salbutamol in the chromatogram obtained with reference solution (c).

Limits:
— *impurities D, F*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
— *impurities C, N, O*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: maximum 0.9 per cent;
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Boron
Maximum 50 ppm.

Test solution To 50 mg of the substance to be examined add 5 mL of a solution containing 13 g/L of *anhydrous sodium carbonate R* and 17 g/L of *potassium carbonate R*. Evaporate to dryness on a water-bath and dry at 120 °C. Ignite the residue rapidly until the organic matter has been destroyed, allow to cool and add 0.5 mL of *water R* and 3.0 mL of a freshly prepared 1.25 g/L solution of *curcumin R* in *glacial acetic acid R*. Warm gently to effect solution, allow to cool and add 3.0 mL of a mixture prepared by adding 5 mL of *sulfuric acid R*, slowly and with stirring, to 5 mL of *glacial acetic acid R*. Mix and allow to stand for 30 min. Dilute to 100.0 mL with *ethanol (96 per cent) R*, filter and use the filtrate.

Reference solution Dissolve 0.572 g of *boric acid R* in 1000.0 mL of *water R*. Dilute 1.0 mL of the solution to 100.0 mL with *water R*. To 2.5 mL of this solution add 5 mL of a solution containing 13 g/L of *anhydrous sodium carbonate R* and 17 g/L of *potassium carbonate R*, and treat this mixture in the same manner as the test solution.

Measure the absorbance (*2.2.25*) of the test solution and of the reference solution at the absorption maximum at about 555 nm. The absorbance of the test solution is not greater than that of the reference solution.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

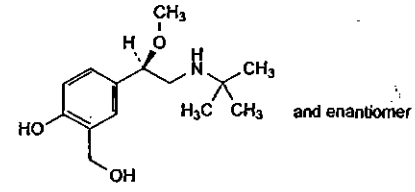
ASSAY
Dissolve 0.400 g in 5 mL of *anhydrous formic acid R* and add 35 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 57.67 mg of $C_{26}H_{44}N_2O_{10}S$.

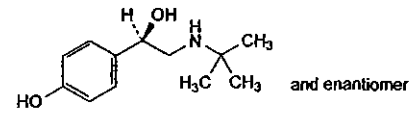
STORAGE
Protected from light.

IMPURITIES
Specified impurities *C, D, F, N, O.*

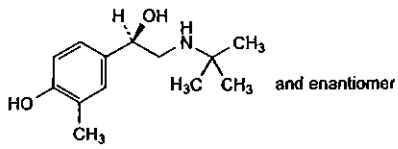
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, E, G, I, J, K, L, M.*



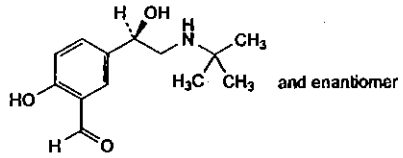
A. [5-[(1*RS*)-2-[(1,1-dimethylethyl)amino]-1-methoxyethyl]-2-hydroxyphenyl]methanol,



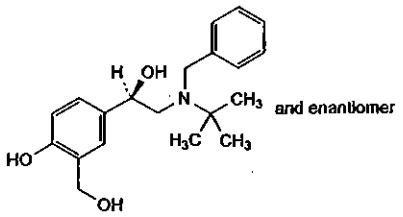
B. (1*RS*)-2-[(1,1-dimethylethyl)amino]-1-(4-hydroxyphenyl)ethanol,



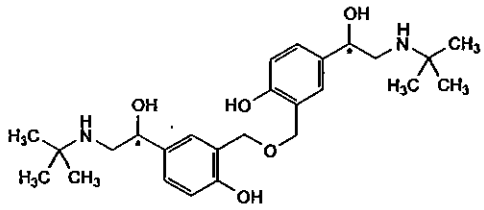
C. (1*RS*)-2-[(1,1-dimethylethyl)amino]-1-(4-hydroxy-3-methylphenyl)ethanol,



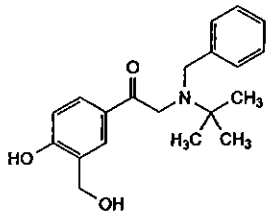
D. 5-[(1*RS*)-2-[(1,1-dimethylethyl)amino]-1-hydroxyethyl]-2-hydroxybenzaldehyde,



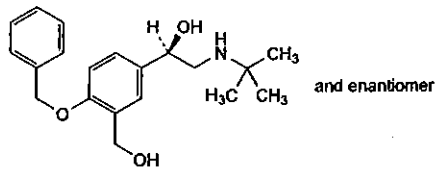
E. (1*RS*)-2-[benzyl(1,1-dimethylethyl)amino]-1-[4-hydroxy-3-(hydroxymethyl)phenyl]ethanol,



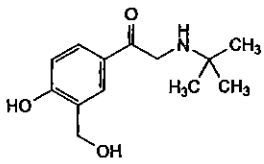
F. 1,1′-[oxybis[methylene(4-hydroxy-1,3-phenylene)]]bis[2-[(1,1-dimethylethyl)amino]ethanol],



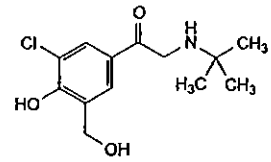
G. 2-[benzyl(1,1-dimethylethyl)amino]-1-[4-hydroxy-3-(hydroxymethyl)phenyl]ethanone,



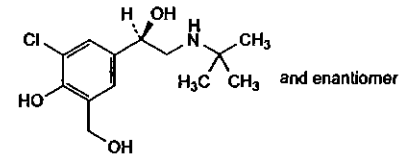
I. (1*RS*)-2-[(1,1-dimethylethyl)amino]-1-[4-(benzyloxy)-3-(hydroxymethyl)phenyl]ethanol,



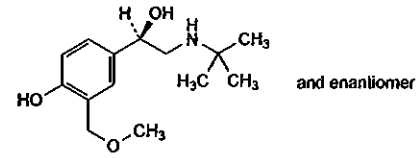
J. 2-[(1,1-dimethylethyl)amino]-1-[4-hydroxy-3-(hydroxymethyl)phenyl]ethanone (salbutamone),



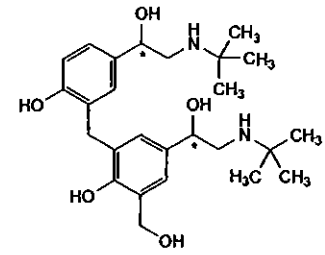
K. 2-[(1,1-dimethylethyl)amino]-1-[3-chloro-4-hydroxy-5-(hydroxymethyl)phenyl]ethanone,



L. (1*RS*)-2-[(1,1-dimethylethyl)amino]-1-[3-chloro-4-hydroxy-5-(hydroxymethyl)phenyl]ethanol,



M.(1*RS*)-2-[(1,1-dimethylethyl)amino]-1-[4-hydroxy-3-(methoxymethyl)phenyl]ethanol,



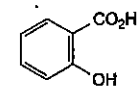
N. 2-[(1,1-dimethylethyl)amino]-1-[3-[[5-[2-[(1,1-dimethylethyl)amino]-1-hydroxyethyl]-2-hydroxyphenyl]methyl]-4-hydroxy-5-(hydroxymethyl)phenyl]ethanol,

O. unknown structure.

Ph Eur

Salicylic Acid

(Ph. Eur. monograph 0366)



$C_7H_6O_3$ 138.1 *69-72-7*

Action and use
Keratolytic.

Preparations
Coal Tar and Salicylic Acid Ointment
Dithranol and Salicylic Acid Ointment
Salicylic Acid Collodion
Salicylic Acid Cream
Salicylic Acid Ointment
Zinc and Salicylic Acid Paste

Ph Eur

DEFINITION

2-Hydroxybenzenecarboxylic acid.

Content

99.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or white or colourless, acicular crystals.

Solubility

Slightly soluble in water, freely soluble in ethanol (96 per cent), sparingly soluble in methylene chloride.

IDENTIFICATION

First identification: A, B.

Second identification: A, C.

A. Melting point (*2.2.14*): 158 °C to 161 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *salicylic acid CRS.*

C. Dissolve about 30 mg in 5 mL of *0.05 M sodium hydroxide*, neutralise if necessary and dilute to 20 mL with *water R*. 1 mL of the solution gives reaction (a) of salicylates (*2.3.1*).

TESTS

Solution S

Dissolve 2.5 g in 50 mL of boiling *distilled water R*, cool and filter.

Appearance of solution

The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 1 g in 10 mL of *ethanol (96 per cent) R*.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.50 g of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dissolve 10 mg of *phenol R* (impurity C) in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve 5 mg of *salicylic acid impurity B CRS* in the mobile phase and dilute to 20.0 mL with the mobile phase.

Reference solution (c) Dissolve 50 mg of *4-hydroxybenzoic acid R* (impurity A) in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (d) Dilute 1.0 mL of reference solution (a) to 10.0 mL with the mobile phase.

Reference solution (e) Dilute a mixture of 1.0 mL of each of reference solutions (a), (b) and (c) to 10.0 mL with the mobile phase.

Reference solution (f) Dilute a mixture of 0.1 mL of each of reference solutions (a), (b) and (c) to 10.0 mL with the mobile phase.

Column:

— *size: l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *glacial acetic acid R, methanol R, water R* (1:40:60 *V/V/V*).

Flow rate 0.5 mL/min.

Detection Spectrophotometer at 270 nm.

Injection 10 µL of the test solution and reference solutions (d), (e) and (f).

Identification of impurities Use the chromatogram obtained with reference solution (e) to identify the peaks due to impurities A, B and C.

Relative retention With reference to impurity C (retention time = about 9.5 min): impurity A = about 0.6; impurity B = about 0.8.

System suitability Reference solution (e):

— the 3rd peak in the chromatogram corresponds to the peak due to impurity C in the chromatogram obtained with reference solution (d);

— *resolution*: minimum 1.0 between the peaks due to impurities B and C; if necessary, adjust the quantity of acetic acid in the mobile phase.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (0.1 per cent);

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (0.05 per cent);

— *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (0.02 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the peak due to impurity B in the chromatogram obtained with reference solution (f) (0.05 per cent);

— *total*: not more than twice the area of the peak due to impurity A in the chromatogram obtained with reference solution (f) (0.2 per cent);

— *disregard limit*: 0.3 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (f) (0.03 per cent). Do not disregard the peak due to impurity C.

Chlorides (*2.4.4*)

Maximum 100 ppm.

Dilute 10 mL of solution S to 15 mL with *water R*.

Sulfates

Maximum 200 ppm.

Dissolve 1.0 g in 5 mL of *dimethylformamide R* and add 4 mL of *water R*. Mix thoroughly. Add 0.2 mL of *dilute hydrochloric acid R* and 0.5 mL of a 25 per cent *m/m* solution of *barium chloride R*. After 15 min any opalescence in the solution is not more intense than that in a standard prepared as follows: to 2 mL of *sulfate standard solution (100 ppm SO_4) R* add 0.2 mL of *dilute hydrochloric acid R*, 0.5 mL of a 25 per cent *m/m* solution of *barium chloride R*, 3 mL of *water R* and 5 mL of *dimethylformamide R*.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in a desiccator.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 2.0 g.

ASSAY

Dissolve 0.120 g in 30 mL of *ethanol (96 per cent) R* and add 20 mL of *water R*. Titrate with *0.1 M sodium hydroxide*, using 0.1 mL of *phenol red solution R* as indicator.

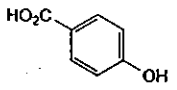
1 mL of *0.1 M sodium hydroxide* is equivalent to 13.81 mg of $C_7H_6O_3$.

STORAGE

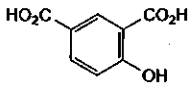
Protected from light.

IMPURITIES

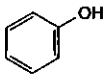
Specified impurities *A, B, C.*



A. 4-hydroxybenzoic acid,



B. 4-hydroxyisophthalic acid,

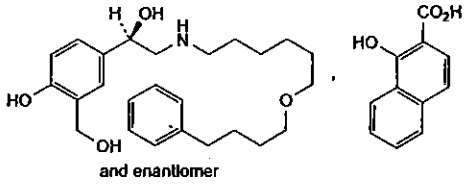


C. phenol.

Ph Eur

Salmeterol Xinafoate

(Ph. Eur. monograph 1765)



and enantiomer

$C_{36}H_{45}NO_7$ 604 *94749-08-3*

Action and use

$Beta_2$-adrenoceptor agonist; bronchodilator.

Preparations

Fluticasone and Salmeterol Inhalation Powder, pre-metered

Fluticasone and Salmeterol Pressurised Inhalation, Suspension

Fluticasone and Salmeterol Inhalation Powder, Metered-Dose

Salmeterol Inhalation Powder, pre-metered

Salmeterol Pressurised Inhalation, Suspension

Ph Eur

DEFINITION

(1*RS*)-1-[4-Hydroxy-3-(hydroxymethyl)phenyl]-2-[[6-(4-phenylbutoxy)hexyl]amino]ethanol 1-hydroxynaphthalene-2-carboxylate.

Content

97.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, soluble in methanol, slightly soluble in anhydrous ethanol, practically insoluble in methylene chloride.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *salmeterol xinafoate CRS.*

TESTS

Related substances

Liquid chromatography (*2.2.29*). *Protect the solutions from light.*

Solvent mixture *acetonitrile R, water R* (50:50 *V/V*).

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Test solution (b) Dissolve 25.0 mg of the substance to be examined in mobile phase A and dilute to 100.0 mL with mobile phase A.

Reference solution (a) Dissolve 11 mg of *salmeterol xinafoate for system suitability CRS* (containing impurities E and G) in the solvent mixture and dilute to 2.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 5 mg of *salmeterol xinafoate for peak identification CRS* (containing impurity D) in the solvent mixture and dilute to 1.0 mL with the solvent mixture.

Reference solution (d) Dissolve 25.0 mg of *salmeterol xinafoate CRS* in mobile phase A and dilute to 100.0 mL with mobile phase A.

Reference solution (e) Dilute 1.0 mL of reference solution (a) to 20.0 mL with mobile phase A.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: mix 24 volumes of a 7.71 g/L solution of *ammonium acetate R* and 24 volumes of a 28.84 g/L solution of *sodium dodecyl sulfate R* and adjust to pH 2.7 with *glacial acetic acid R*; mix with 52 volumes of *acetonitrile R*;

— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 16	100	0
16 - 36	100 → 30	0 → 70
36 - 45	30	70

Flow rate 2 mL/min.

Detection Spectrophotometer at 278 nm.

Injection 20 µL of test solution (a) and reference solutions (a), (b) and (c).

Identification of impurities Use the chromatogram supplied with *salmeterol xinafoate for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities E and G; use the chromatogram supplied with *salmeterol xinafoate for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peak due to impurity D.

Relative retention With reference to salmeterol (retention time = about 13 min): xinafoic acid = about 0.2; impurity D = about 0.8; impurity E = about 0.9; impurity G = about 2.7.

System suitability Reference solution (a):

— *peak-to-valley ratio*: minimum 10, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the

curve separating this peak from the peak due to salmeterol.

Calculation of percentage contents:

— for each impurity, use the concentration of salmeterol xinafoate in reference solution (b).

Limits:

— *impurities D, G*: for each impurity, maximum 0.2 per cent;

— *unspecified impurities*: for each impurity, maximum 0.10 per cent;

— *total*: maximum 0.5 per cent;

— *reporting threshold*: 0.05 per cent; disregard the peak due to xinafoic acid.

Water (*2.5.12*)

Maximum 0.5 per cent, determined on 1.00 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Mobile phase Mobile phase A.

Injection 20 µL of test solution (b) and reference solutions (d) and (e).

Run time Until complete elution of the peak due to salmeterol (about 16 min).

System suitability Reference solution (e):

— *peak-to-valley ratio*: minimum 10, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to salmeterol.

Calculate the percentage content of $C_{36}H_{45}NO_7$ using the chromatogram obtained with reference solution (d) and taking into account the assigned content of *salmeterol xinafoate CRS*.

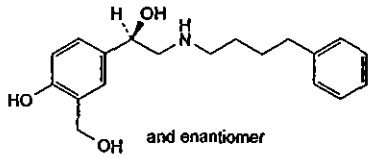
STORAGE

Protected from light.

IMPURITIES

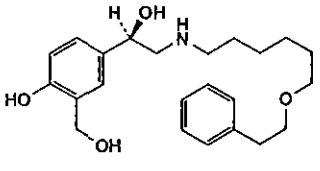
Specified impurities *D, G.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, E, F.*



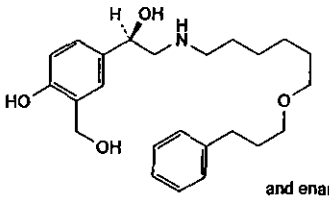
and enantiomer

A. (1*RS*)-1-[4-hydroxy-3-(hydroxymethyl)phenyl]-2-[(4-phenylbutyl)amino]ethanol,



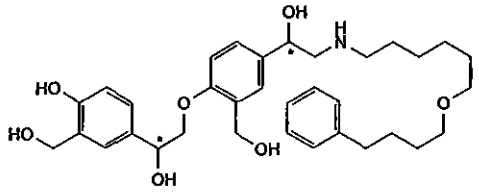
and enantiomer

B. (1*RS*)-1-[4-hydroxy-3-(hydroxymethyl)phenyl]-2-[[6-(2-phenylethoxy)hexyl]amino]ethanol,

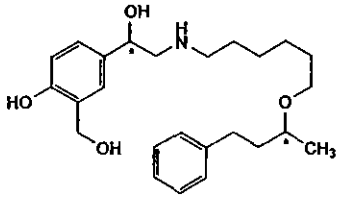


and enantiomer

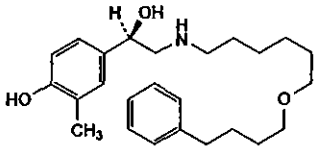
C. (1*RS*)-1-[4-hydroxy-3-(hydroxymethyl)phenyl]-2-[[6-(3-phenylpropoxy)hexyl]amino]ethanol,



D. 1-[4-[2-hydroxy-2-[4-hydroxy-3-(hydroxymethyl)phenyl]ethoxy]-3-(hydroxymethyl)phenyl]-2-[[6-(4-phenylbutoxy)hexyl]amino]ethanol,

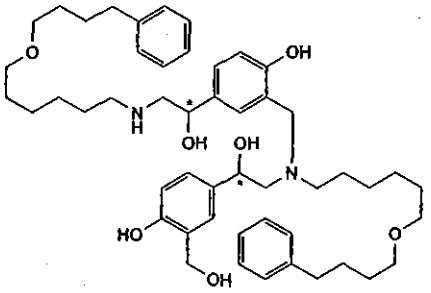


E. 1-[4-hydroxy-3-(hydroxymethyl)phenyl]-2-[[6-(1-methyl-3-phenylpropoxy)hexyl]amino]ethanol,



and enantiomer

F. (1*RS*)-1-(4-hydroxy-3-methylphenyl)-2-[[6-(4-phenylbutoxy)hexyl]amino]ethanol,



G. 1-[4-hydroxy-3-[[[2-hydroxy-2-[4-hydroxy-3-(hydroxymethyl)phenyl]ethyl][6-(4-phenylbutoxy)hexyl]amino]methyl]phenyl]-2-[[6-(4-phenylbutoxy)hexyl]amino]ethanol.

Ph Eur

Farmed Salmon Oil

(Ph. Eur. monograph 1910)

Ph Eur

DEFINITION

Purified fatty oil obtained from fresh farmed *Salmo salar*. The positional distribution (β(2)-acyl) is 60-70 per cent for cervonic (docosahexaenoic) acid (C22:6 n-3; DHA), 25-35 per cent for timnodonic (eicosapentaenoic) acid (C20:5 n-3; EPA) and 40-55 per cent for moroctic acid (C18:4 n-3).

Content

— *sum of the contents of EPA and DHA (expressed as triglycerides)*: 10.0 per cent to 28.0 per cent.

A suitable antioxidant may be added.

PRODUCTION

The fish shall only be given feed with a composition that is in accordance with the relevant European Union or other applicable regulations.

The content of dioxins and dioxin-like PCBs (polychlorinated biphenyls) is controlled using methods and limits in accordance with the requirements set in the European Union or other applicable regulations.

The oil is produced by mechanical expression of fresh raw materials, either from the whole fish, or fish where the fillets have been removed, at a temperature not exceeding 100 °C, and without using solvents. After centrifugation, solid substances may be removed from the oil by cooling and filtering (winterisation).

CHARACTERS

Appearance

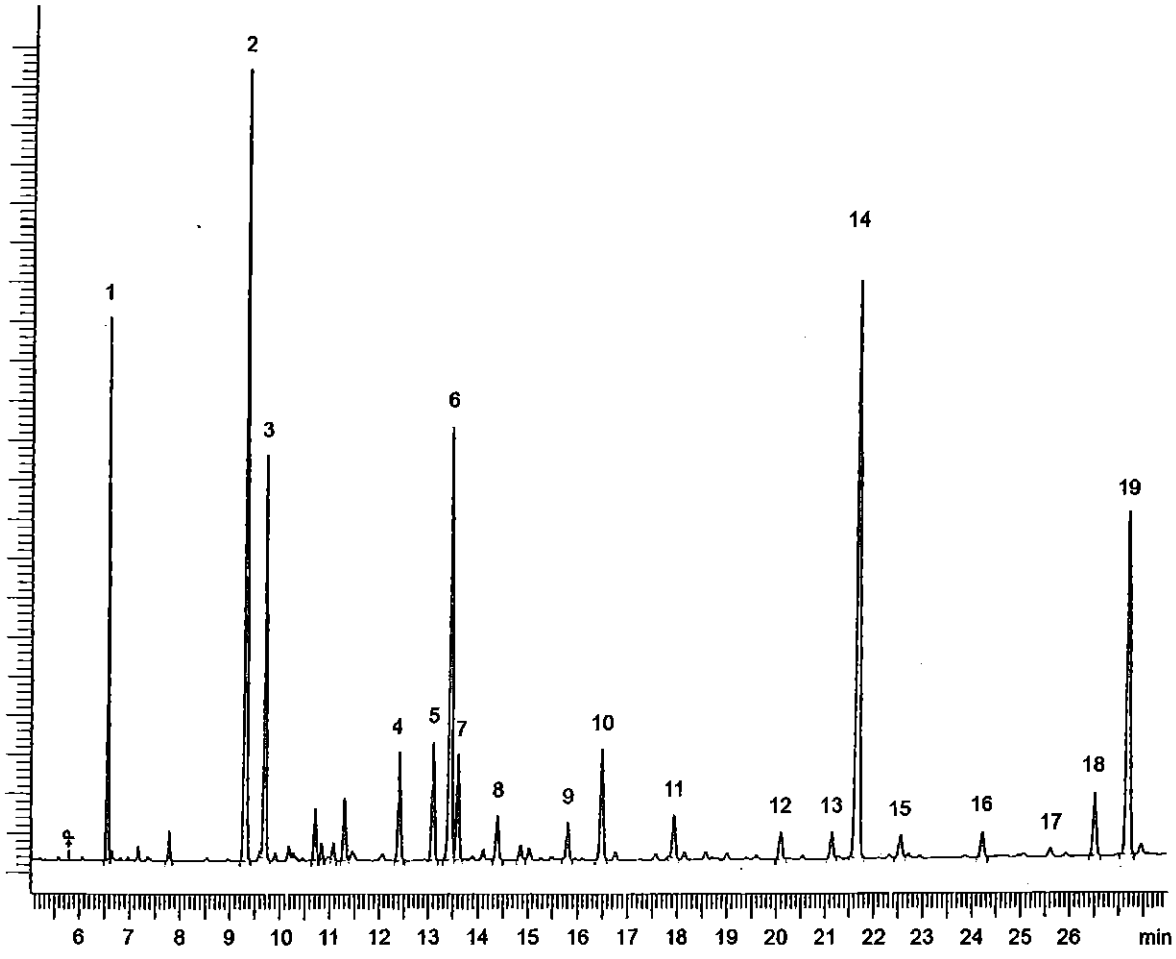
Pale pink liquid.

Solubility

Practically insoluble in water, very soluble in acetone and in heptane, slightly soluble in anhydrous ethanol.

IDENTIFICATION

Examine the ^{13}C NMR spectra obtained in the assay for positional distribution (β(2)-acyl) of fatty acids. The spectra contain peaks between 172 ppm and 173 ppm with shifts



1. C14:0
2. C16:0
3. C16:1 n-7
4. C16:4 n-1
5. C18:0
6. C18:1 n-9
7. C18:1 n-7
8. C18:2 n-6
9. C18:3 n-3
10. C18:4 n-3
11. C20:1 n-9
12. C20:4 n-6
13. C20:4 n-3
14. EPA
15. C22:1 n-11
16. C21:5 n-3
17. C22:5 n-6
18. C22:5 n-3
19. DHA

Figure 1910.-1. – *Chromatogram for the composition of fatty acids in farmed salmon oil*

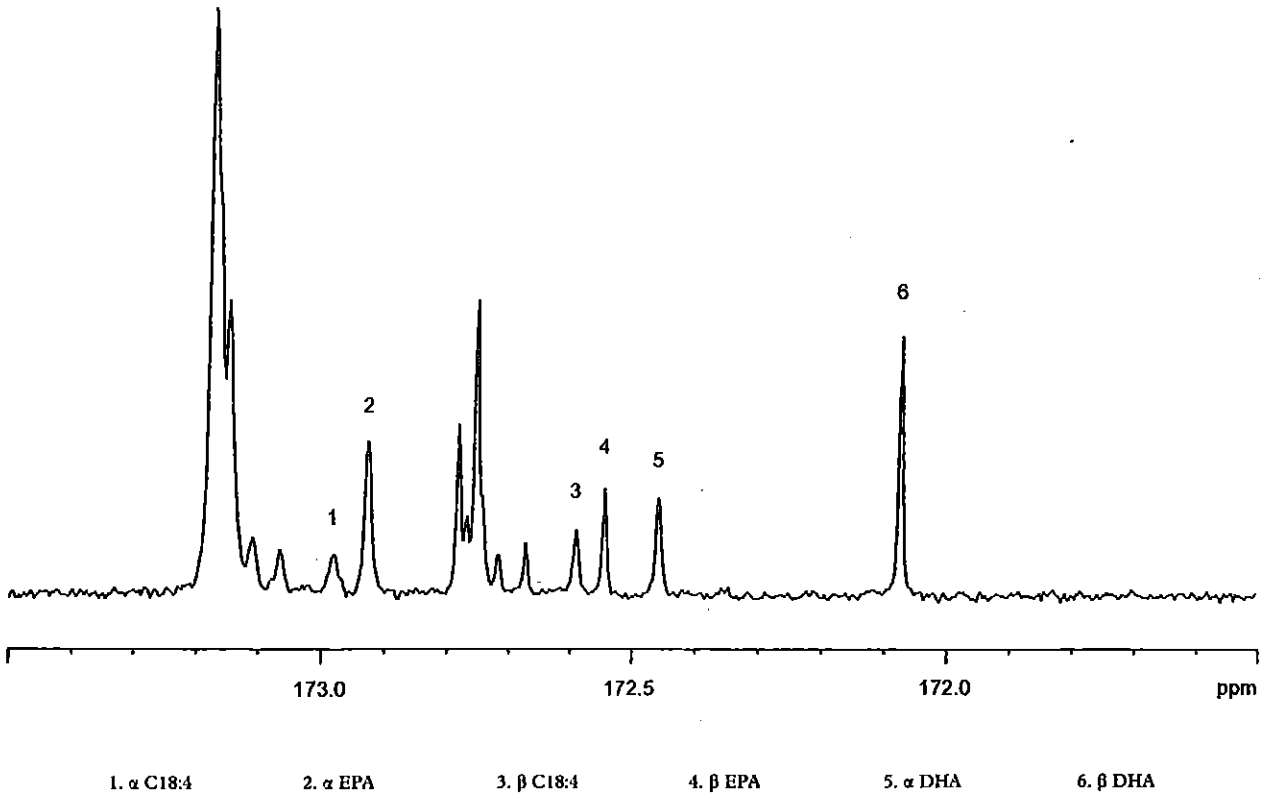


Figure 1910.-2. – *^{13}C NMR spectrum: carbonyl region of farmed salmon oil*

similar to those in the type spectrum (Figure 1910.-2).
The oil to be examined complies with the limits of this assay.

TESTS

Absorbance (*2.2.25*)
Minimum 0.10, measured at the absorption maximum between 470 nm and 480 nm.

Dissolve 5.0 mL in 5.0 mL of *trimethylpentane R*.

Acid value (*2.5.1*)
Maximum 2.0.

Anisidine value (*2.5.36*)
Maximum 10.0.

Peroxide value (*2.5.5, Method A*)
Maximum 5.0.

Unsaponifiable matter (*2.5.7*)
Maximum 1.5 per cent, determined on 5.0 g.

Linoleic acid (*2.4.29*)
Maximum 11.0 per cent.

Identify the peak due to linoleic acid using the chromatogram in Figure 1910.-1. Determine the percentage content by normalisation.

ASSAY

Positional distribution (β(2)-acyl) of fatty acids
Nuclear magnetic resonance spectrometry (*2.2.33*).

Apparatus High resolution FT-NMR spectrometer operating at minimum 300 MHz.

Test solution Dissolve 190-210 mg of fresh salmon oil in 500 µL of *deuterated chloroform R*. Prepare at least 3 samples and examine within 3 days.

Acquisition of ^{13}C NMR spectra The following parameters may be used:
— *sweep width*: 200 ppm (−5 to 195 ppm);
— *irradiation frequency offset*: 95 ppm;
— *time domain*: 64 K;
— *pulse delay*: 2 s;
— *pulse program*: zgig 30 (inverse gated, 30° excitation pulse);
— *dummy scans*: 4;
— *number of scans*: 4096.

Processing and plotting The following parameters may be used:
— *size*: 64 K (zero-filling);
— *window multiplication*: exponential;
— *Lorentzian broadening factor*: 0.2 Hz.

Use the $CDCl_3$ signal for shift referencing. The shift of the central peak of the 1:1:1 triplet is set to 77.16 ppm.

Plot the spectral region δ 171.5-173.5 ppm. Compare the spectrum with the reference spectrum in Figure 1910.-2. The shift values lie within the ranges given in Table 1910.-1.

Table 1910.-1. – *Shift values*

Signal	Shift range (ppm)
β DHA	172.05 - 172.09
α DHA	172.43 - 172.47
β EPA	172.52 - 172.56
α EPA	172.90 - 172.94
β C18:4	172.56 - 172.60
α C18:4	172.95 - 172.99

System suitability:
— *signal-to-noise ratio*: minimum 5 for the smallest relevant peak corresponding to α C18:4 signal (in the range δ 172.95-172.99 ppm);
— *peak width at half-height* maximum 0.02 ppm for the central $CDCl_3$ signal (at δ 77.16 ppm).

Calculation of positional distribution (β(2)-acyl) Use the following expression:

$$\frac{\beta}{\alpha+\beta} \times 100$$

α = peak area of the corresponding α-carbonyl peak;
β = peak area of β-carbonyl peak from C22:6 n-3, C20:5 n-3 or C18:4 n-3, respectively.

Limits:
— *cervonic (docosahexaenoic) acid (C22:6 n-3; DHA)*: 60 per cent to 70 per cent;
— *timnodonic (eicosapentaenoic) acid (C20:5 n-3; EPA)*: 25 per cent to 35 per cent;
— *moroctic acid (C18:4 n-3)*: 40 per cent to 55 per cent.

EPA and DHA *(2.4.29)*
See Figure 1910.-1.

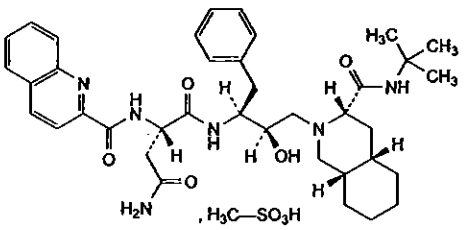
STORAGE
In an airtight, well-filled container, protected from light, under inert gas.

LABELLING
The label states:
— the concentration of EPA and DHA, expressed as triglycerides.

Ph Eur

Saquinavir Mesilate

(Ph. Eur. monograph 2267)



$C_{39}H_{54}N_6O_8S$ 767 *149845-06-7*

Action and use
Protease inhibitor; antiviral (HIV).

Ph Eur

DEFINITION
(2*S*)-*N*^{1}-[(1*S*,2*R*)-1-Benzyl-3-[(3*S*,4a*S*,8a*S*)-3-[(1,1-dimethylethyl)carbamoyl]octahydroisoquinolin-2(1*H*)-yl]-2-hydroxypropyl]-2-[(quinolin-2-ylcarbonyl)amino]butanediamide methanesulfonate.

Content
97.5 per cent to 102.0 per cent (anhydrous substance).

PRODUCTION
It is considered that alkyl methanesulfonate esters are genotoxic and are potential impurities in saquinavir mesilate. The manufacturing process should be developed taking into consideration the principles of quality risk management, together with considerations of the quality of starting materials, process capability and validation. The general methods *2.5.37. Methyl, ethyl and isopropyl methanesulfonate in methanesulfonic acid*, *2.5.38. Methyl, ethyl and isopropyl methanesulfonate in active substances* and *2.5.39. Methanesulfonyl chloride in methanesulfonic acid* are available to assist manufacturers.

CHARACTERS
Appearance
White or almost white, slightly hygroscopic powder.

Solubility
Practically insoluble in water, sparingly soluble in methanol, slightly soluble in ethanol (96 per cent).

IDENTIFICATION
A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry *(2.2.24)*.
Comparison *saquinavir mesilate CRS*.

TESTS
Specific optical rotation *(2.2.7)*
−42.0 to −35.0 (anhydrous substance).
Dissolve 0.25 g in *anhydrous methanol R* and dilute to 50.0 mL with the same solvent.

Related substances
Liquid chromatography *(2.2.29)*.
Solvent mixture *water for chromatography R, acetonitrile R1* (47:53 *V/V*).
Test solution Dissolve 30.0 mg of the substance to be examined in the solvent mixture, using sonication, and dilute to 100.0 mL with the same solvent.
Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.
Reference solution (b) Dissolve the contents of a vial of *saquinavir for system suitability CRS* (containing impurities A, B, C and D) in 1.0 mL of the solvent mixture and sonicate for 2 min.
Reference solution (c) Dissolve 30.0 mg of *saquinavir mesilate CRS* in the solvent mixture, using sonication, and dilute to 100.0 mL with the same solvent.
Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (3.5 µm).
Mobile phase:
— *mobile phase A*: to 2.5 mL of *strong sodium hydroxide solution R* add 900 mL of *water for chromatography R*, adjust to pH 1.8 with *perchloric acid R* and dilute to 1000 mL with *water for chromatography R*;
— *mobile phase B*: mobile phase A, *acetonitrile R1* (38:62 *V/V*);

Time (min)	Mobile phase A (per cent)	Mobile phase B (per cent)
0 - 1	50	50
1 - 31	50 → 0	50 → 100

Flow rate 1.0 mL/min.
Detection Spectrophotometer at 210 nm.
Injection 10 µL of the test solution and reference solutions (a) and (b).
Identification of impurities Use the chromatogram supplied with *saquinavir for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C and D.
Relative retention With reference to saquinavir (retention time = about 17 min): impurity A = about 0.2;

impurity B = about 0.3; impurity C = about 0.5; impurity D = about 0.9.

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 3, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to saquinavir.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.5; impurity B = 0.5; impurity C = 2.5;

— *impurities A, B, C*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);

— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.03 per cent).

Water (*2.5.12*)

Maximum 1.0 per cent, determined on 0.250 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection 10 µL of the test solution and reference solution (c).

Calculate the percentage content of saquinavir mesilate from the assigned content of *saquinavir mesilate CRS*.

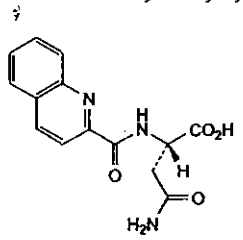
STORAGE

In an airtight container, protected from light.

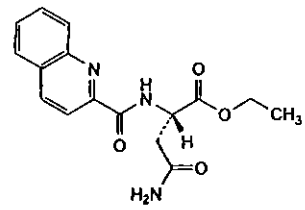
IMPURITIES

Specified impurities *A, B, C.*

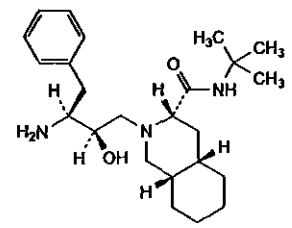
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *D, E, F, G, H.*



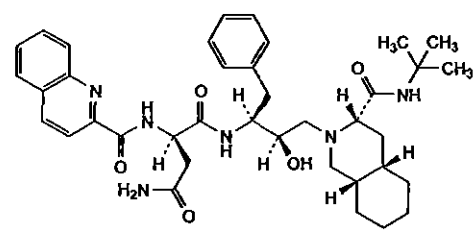
A. (2*S*)-4-amino-4-oxo-2-[(quinolin-2-ylcarbonyl)amino]butanoic acid,



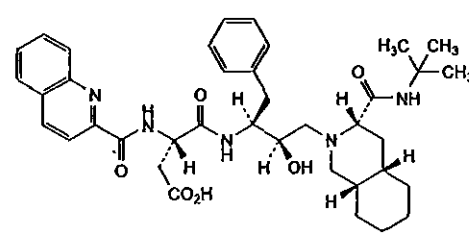
B. ethyl (2*S*)-4-amino-4-oxo-2-[(quinolin-2-ylcarbonyl)amino]butanoate,



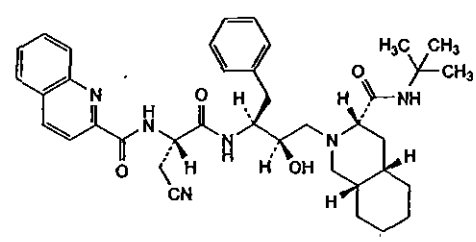
C. (3*S*,4a*S*,8a*S*)-2-[(2*R*,3*S*)-3-amino-2-hydroxy-4-phenylbutyl]-*N*-(1,1-dimethylethyl)decahydroisoquinoline-3-carboxamide,



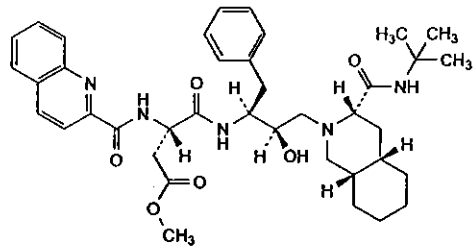
D. (2*R*)-*N*^{1}-[(1*S*,2*R*)-1-benzyl-3-[(3*S*,4a*S*,8a*S*)-3-[(1,1-dimethylethyl)carbamoyl]octahydroisoquinolin-2(1*H*)-yl]-2-hydroxypropyl]-2-[(quinolin-2-ylcarbonyl)amino]butanediamide (2-*epi*-saquinavir),



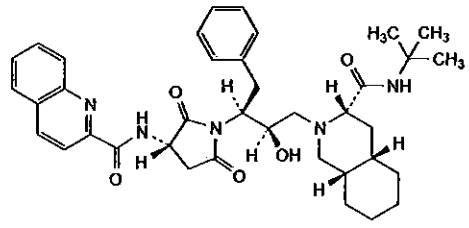
E. (3*S*)-4-[[(1*S*,2*R*)-1-benzyl-3-[(3*S*,4a*S*,8a*S*)-3-[(1,1-dimethylethyl)carbamoyl]octahydroisoquinolin-2(1*H*)-yl]-2-hydroxypropyl]amino]-4-oxo-3-[(quinolin-2-ylcarbonyl)amino]butanoic acid,



F. *N*-[(1*S*)-2-[[(1*S*,2*R*)-1-benzyl-3-[(3*S*,4a*S*,8a*S*)-3-[(1,1-dimethylethyl)carbamoyl]octahydroisoquinolin-2(1*H*)-yl]-2-hydroxypropyl]amino]-1-(cyanomethyl)-2-oxoethyl]quinoline-2-carboxamide,



G. methyl (3*S*)-4-[[(1*S*,2*R*)-1-benzyl-3-[(3*S*,4a*S*,8a*S*)-3-[(1,1-dimethylethyl)carbamoyl]octahydroisoquinolin-2(1*H*)-yl]-2-hydroxypropyl]amino]-4-oxo-3-[(quinolin-2-ylcarbonyl)amino]butanoate,

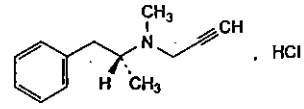


H. *N*-[(3*S*)-1-[(1*S*,2*R*)-1-benzyl-3-[(3*S*,4a*S*,8a*S*)-3-[(1,1-dimethylethyl)carbamoyl]octahydroisoquinolin-2(1*H*)-yl]-2-hydroxypropyl]-2,5-dioxopyrrolidin-3-yl]quinoline-2-carboxamide.

Ph Eur

Selegiline Hydrochloride

(Ph. Eur. monograph 1260)



$C_{13}H_{18}ClN$ 223.7 *14611-52-0*

Action and use
Monoamine oxidase type B inhibitor; treatment of Parkinson's disease.

Preparations
Selegiline Oral Solution
Selegiline Tablets

Ph Eur

DEFINITION
N-Methyl-*N*-[(1*R*)-1-methyl-2-phenylethyl]prop-2-yn-1-amine hydrochloride.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water and in methanol, slightly soluble in acetone and in ethyl acetate.

mp
About 143 °C.

IDENTIFICATION
Carry out either tests A, B, D or tests B, C, D.

A. Specific optical rotation (*2.2.7*): −12.0 to −10.0 (dried substance).
Dissolve 2.00 g in *carbon dioxide-free water R* and dilute to 20.0 mL with the same solvent.

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *selegiline hydrochloride CRS*.

C. Enantiomeric purity (see Tests).

D. It gives reaction (a) of chlorides (*2.3.1*).

TESTS
pH (*2.2.3*)
3.5 to 4.5.
Dissolve 0.20 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Enantiomeric purity
Liquid chromatography (*2.2.29*).
Test solution Dissolve 20.0 mg of the substance to be examined in a mixture of 10 µL of *butylamine R* and 1 mL of *2-propanol R* and dilute to 10.0 mL with the mobile phase.
Reference solution (a) Dissolve 8.0 mg of *(RS)-selegiline hydrochloride CRS* in a mixture of 10 µL of *butylamine R* and 1 mL of *2-propanol R* and dilute to 10.0 mL with the mobile phase.
Reference solution (b) Dilute 0.5 mL of reference solution (a) to 20.0 mL with the mobile phase.
Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *cellulose derivative of silica gel for chiral separation R*.
Mobile phase *2-propanol R, cyclohexane R* (0.2:99.8 *V/V*).
Flow rate 1 mL/min.
Detection Spectrophotometer at 220 nm.
Injection 20 µL.
Relative retention With reference to (*R*)-selegiline (retention time = about 6 min): impurity E = about 0.9.
System suitability Reference solution (a):
— *resolution*: minimum 1.5 between the peaks due to impurity E and (*R*)-selegiline; if necessary, adjust the concentration of 2-propanol in the mobile phase.
Limit:
— *impurity E*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.5 per cent).

Related substances
Liquid chromatography (*2.2.29*).
Butylammonium acetate buffer solution Dilute 4 mL of *butylamine R* in 900 mL of *water R*, adjust to pH 6.5 with *acetic acid R* and dilute to 1000.0 mL with *water R*.
Test solution Dissolve 20 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.
Reference solution (a) Dissolve 50 mg of the substance to be examined and 10 mg of *butyl parahydroxybenzoate R* in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 20.0 mL with the mobile phase.
Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.
Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R1*, butylammonium acetate buffer solution (50:50 *V/V*).

Flow rate 1 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µL.

Run time 1.7 times the retention time of selegiline.

Relative retention With reference to selegiline (retention time = about 14 min): butyl parahydroxybenzoate = about 0.8.

System suitability Reference solution (a):
— *resolution*: minimum 3.0 between the peaks due to butyl parahydroxybenzoate and selegiline.

Limits:
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying at 60 °C at a pressure not exceeding 0.5 kPa for 3 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

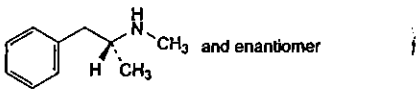
ASSAY
Dissolve 0.180 g in 50 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 22.37 mg of $C_{13}H_{18}ClN$.

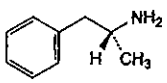
STORAGE
Protected from light.

IMPURITIES
Specified impurities *E.*

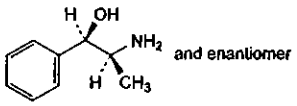
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, G.*



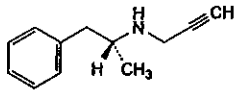
A. (2*RS*)-*N*-methyl-1-phenylpropan-2-amine ((*RS*)-metamfetamine),



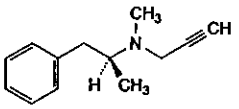
B. (2*R*)-1-phenylpropan-2-amine (amfetamine),



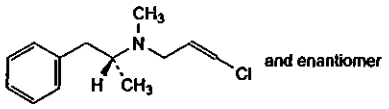
C. (1*RS*,2*SR*)-2-amino-1-phenylpropan-1-ol (phenylpropanolamine),



D. *N*-[(1*R*)-1-methyl-2-phenylethyl]prop-2-yn-1-amine (desmethylselegiline),



E. *N*-methyl-*N*-[(1*S*)-1-methyl-2-phenylethyl]prop-2-yn-1-amine,



G. (2*EZ*)-3-chloro-*N*-methyl-*N*-[(1*RS*)-1-methyl-2-phenylethyl]prop-2-en-1-amine.

Ph Eur

Selenium Sulfide

Selenium Disulphide

(Selenium Disulfide, Ph. Eur. monograph 1147)

SeS_2 143.1 *7488-56-4*

Action and use
Used in treatment of dandruff and seborrhoeic dermatitis of the scalp.

Preparation
Selenium Sulfide Scalp Application

Ph Eur

DEFINITION
Content
52.0 per cent to 55.5 per cent of Se.

CHARACTERS
Appearance
Bright orange or reddish-brown powder.

Solubility
Practically insoluble in water.

IDENTIFICATION
A. Gently boil about 50 mg with 5 mL of *nitric acid R* for 30 min. Dilute to 50 mL with *water R* and filter. To 5 mL of the filtrate add 10 mL of *water R* and 5 g of *urea R*. Heat to boiling, cool and add 1.5 mL of *potassium iodide solution R*. A yellow or orange colour is produced which darkens rapidly on standing. This solution is used in identification test B.

B. Allow the coloured solution obtained under identification A to stand for 10 min and filter through *kieselguhr for chromatography R*. 5 mL of the filtrate gives reaction (a) of sulfates (*2.3.1*).

TESTS

Soluble selenium compounds

Maximum 5 ppm, calculated as Se.

To 10 g add 100 mL of *water R*, mix well, allow to stand for 1 h with frequent shaking and filter. To 10 mL of the filtrate add 2 mL of a 115 g/L solution of *anhydrous formic acid R*, dilute to 50 mL with *water R* and adjust to pH 2.0-3.0 with an 115 g/L solution of *anhydrous formic acid R*. Add 2 mL of a 5 g/L solution of *3,3'-diaminobenzidine tetrahydrochloride R*. Allow to stand for 45 min and then adjust to pH 6.0-7.0 with *dilute ammonia R1*. Shake the solution for 1 min with 10 mL of *toluene R* and allow the phases to separate. The absorbance (*2.2.25*) of the upper layer measured at 420 nm is not greater than that of a standard prepared at the same time and in the same manner beginning at the words "add 2 mL of an 115 g/L solution of *anhydrous formic acid R*" and using 5 mL of *selenium standard solution (1 ppm Se) R* instead of 10 mL of the filtrate.

ASSAY

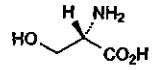
To 0.100 g add 25 mL of *fuming nitric acid R* and heat on a water-bath for 1 h; a small insoluble residue may remain. Cool and dilute to 100.0 mL with *water R*. To 25.0 mL of this solution add 50 mL of *water R* and 5 g of *urea R* and heat to boiling. Cool, add 7 mL of *potassium iodide solution R* and 3 mL of *starch solution R*. Titrate immediately with *0.1 M sodium thiosulfate*. Carry out a blank titration.

1 mL of *0.1 M sodium thiosulfate* is equivalent to 1.974 mg of Se.

Ph Eur

Serine

(Ph. Eur. monograph 0788)



$C_3H_7NO_3$ 105.1 *56-45-1*

Action and use

Amino acid.

Ph Eur

DEFINITION

(2*S*)-2-Amino-3-hydroxypropanoic acid.

Product of fermentation or of protein hydrolysis.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

First identification: A, B.

Second identification: A, C, D.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *serine CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in a 1 per cent *V/V* solution of *hydrochloric acid R* and dilute to 50 mL with the same solution.

Reference solution Dissolve 10 mg of *serine CRS* in a 1 per cent *V/V* solution of *hydrochloric acid R* and dilute to 50 mL with the same solution.

Plate *TLC silica gel plate R.*

Mobile phase *glacial acetic acid R, water R, butanol R* (20:20:60 *V/V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *ninhydrin solution R* and heat at 105 °C for 15 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. To 1 mL of a 10 g/L solution of the substance to be examined in a test tube, add 5 mL of a 20 g/L solution of *sodium periodate R*. Heat on a water-bath and collect the vapour on glass wool moistened with *water R* and inserted in the opening of the test tube. After heating for 5 min, transfer the glass wool to a test tube containing 1 mL of a 15 g/L solution of *chromotropic acid, sodium salt R* and 3 mL of *sulfuric acid R*. Heat on a water-bath for 10 min. A violet-red colour is produced.

TESTS

Solution S

Dissolve 2.5 g in *distilled water R* and dilute to 50 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*)

+ 14.0 to + 16.0 (dried substance).

Dissolve 2.50 g in *dilute hydrochloric acid R* and dilute to 25.0 mL with the same acid.

Ninhydrin-positive substances

Amino acid analysis (*2.2.56*). For analysis, use Method 1.

The concentrations of the test solution and the reference solutions may be adapted according to the sensitivity of the equipment used. The concentrations of all solutions are adjusted so that the system suitability requirements described in general chapter *2.2.46* are fulfilled, keeping the ratios of concentrations between all solutions as described.

Solution A *dilute hydrochloric acid R1* or a sample preparation buffer suitable for the apparatus used.

Test solution Dissolve 30.0 mg of the substance to be examined in solution A and dilute to 50.0 mL with solution A.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with solution A. Dilute 2.0 mL of this solution to 10.0 mL with solution A.

Reference solution (b) Dissolve 30.0 mg of *proline R* in solution A and dilute to 100.0 mL with solution A. Dilute 1.0 mL of the solution to 250.0 mL with solution A.

Reference solution (c) Dilute 6.0 mL of *ammonium standard solution (100 ppm NH_4) R* to 50.0 mL with solution A. Dilute 1.0 mL of this solution to 100.0 mL with solution A.

Reference solution (d) Dissolve 30 mg of *isoleucine R* and 30 mg of *leucine R* in solution A and dilute to 50.0 mL with solution A. Dilute 1.0 mL of the solution to 200.0 mL with solution A.

Blank solution Solution A.

Inject suitable, equal amounts of the test, blank and reference solutions into the amino acid analyser. Run a program suitable for the determination of physiological amino acids.

System suitability Reference solution (d):
— *resolution*: minimum 1.5 between the peaks due to isoleucine and leucine.

Calculation of percentage contents:
— for any ninhydrin-positive substance detected at 570 nm, use the concentration of serine in reference solution (a);
— for any ninhydrin-positive substance detected at 440 nm, use the concentration of proline in reference solution (b); if a peak is above the reporting threshold at both wavelengths, use the result obtained at 570 nm for quantification.

Limits:
— *any ninhydrin-positive substance*: for each impurity, maximum 0.2 per cent;
— *total*: maximum 0.5 per cent;
— *reporting threshold*: 0.05 per cent.

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Chlorides (*2.4.4*)
Maximum 200 ppm.

Dilute 5 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 300 ppm.

Dilute 10 mL of solution S to 15 mL with *distilled water R*.

Ammonium
Amino acid analysis (*2.2.56*) as described in the test for ninhydrin-positive substances with the following modifications.

Injection Test solution, reference solution (c) and blank solution.

Limit:
— *ammonium at 570 nm*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.02 per cent), taking into account the peak due to ammonium in the chromatogram obtained with the blank solution.

Iron (*2.4.9*)
Maximum 10 ppm.

In a separating funnel, dissolve 1.0 g in 10 mL of *dilute hydrochloric acid R*. Shake with 3 quantities, each of 10 mL, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 mL of *water R* and shake for 3 min. Use the aqueous layer.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

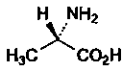
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.100 g in 3 mL of *anhydrous formic acid R*. Add 30 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

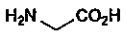
1 mL of *0.1 M perchloric acid* is equivalent to 10.51 mg of $C_3H_7NO_3$.

STORAGE
Protected from light.

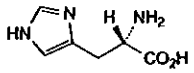
IMPURITIES
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C.*



A. (2*S*)-2-aminopropanoic acid (alanine),



B. 2-aminoacetic acid (glycine),

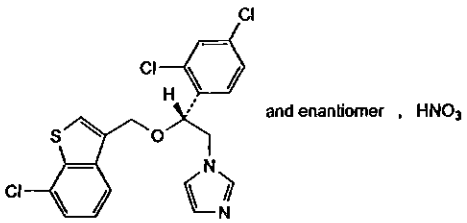


C. (2*S*)-2-amino-3-(imidazol-4-yl)propanoic acid (histidine).

Ph Eur

Sertaconazole Nitrate

(Ph. Eur. monograph 1148)



$C_{20}H_{16}Cl_3N_3O_4S$ 500.8 *99592-39-9*

Action and use
Antifungal.

Ph Eur

DEFINITION
(*RS*)-1-[2-[(7-Chloro-1-benzothiophen-3-yl)methoxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole nitrate.

Content
98.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white powder.

Solubility
Practically insoluble in water, soluble in methanol, sparingly soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

First identification: A, C.

Second identification: A, B, D, E.

A. Melting point (*2.2.14*): 156 °C to 161 °C.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 0.1 g in *methanol R* and dilute to 100 mL with the same solvent. Dilute 10 mL of this solution to 100 mL with *methanol R*.

Spectral range 240-320 nm.

Absorption maxima At 260 nm, 293 nm and 302 nm.

Absorbance ratio A_{302}/A_{293} = 1.16 to 1.28.

C. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Dry the substances at 100-105 °C for 2 h and examine as discs of *potassium bromide R*.

Comparison *sertaconazole nitrate CRS*.

D. Thin-layer chromatography (*2.2.27*).

Solvent mixture *concentrated ammonia R, methanol R* (10:90 *V/V*).

Test solution Dissolve 40 mg of the substance to be examined in the solvent mixture and dilute to 10 mL with the solvent mixture.

Reference solution (a) Dissolve 40 mg of *sertaconazole nitrate CRS* in the solvent mixture and dilute to 10 mL with the solvent mixture.

Reference solution (b) Dissolve 20 mg of *miconazole nitrate CRS* in reference solution (a) and dilute to 5 mL with reference solution (a).

Plate *TLC silica gel G plate R*.

Mobile phase *concentrated ammonia R, toluene R, dioxan R* (1:40:60 *V/V/V*).

Application 5 µL.

Development Over a path of 15 cm.

Drying In a current of air for 15 min.

Detection Expose to iodine vapour for 30 min.

System suitability Reference solution (b):
— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

E. About 1 mg gives the reaction of nitrates (*2.3.1*).

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5 (*2.2.2, Method II*).

Dissolve 0.1 g in *ethanol (96 per cent) R* and dilute to 10 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 10.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dilute 5.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 20.0 mL with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *sertaconazole nitrate CRS* and 5.0 mg of *miconazole nitrate CRS* in the mobile phase and dilute to 20.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 50.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.0 mm;
— *stationary phase*: *nitrile silica gel for chromatography R1* (10 µm).

Mobile phase *acetonitrile R1*, 1.5 g/L solution of *sodium dihydrogen phosphate R* (37:63 *V/V*).

Flow rate 1.6 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µL.

Run time 1.3 times the retention time of sertaconazole.

Retention time Nitrate ion = about 1 min; miconazole = about 17 min; sertaconazole = about 19 min.

System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the peaks due to miconazole and sertaconazole.

Limits:
— *impurities A, B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent);
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent); disregard the peak due to the nitrate ion.

Water (*2.5.12*)
Maximum 1.0 per cent, determined on 0.50 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.400 g in 50 mL of a mixture of equal volumes of *anhydrous acetic acid R* and *methyl ethyl ketone R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

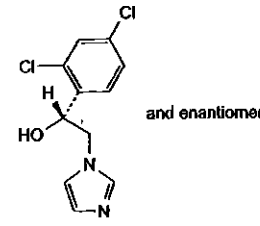
1 mL of *0.1 M perchloric acid* is equivalent to 50.08 mg of $C_{20}H_{16}Cl_3N_3O_4S$.

STORAGE

Protected from light.

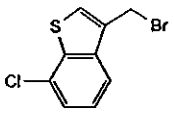
IMPURITIES

Specified impurities *A, B, C.*

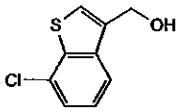


and enantiomer

A. (1*RS*)-1-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-yl) ethanol,



B. 3-(bromomethyl)-7-chloro-1-benzothiophen,

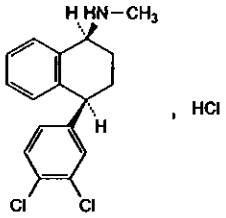


C. (7-chloro-1-benzothiophen-3-yl)methanol.

Ph Eur

Sertraline Hydrochloride

(Ph. Eur. monograph 1705)



$C_{17}H_{18}Cl_3N$ 342.7 *79559-97-0*

Action and use
Selective serotonin reuptake inhibitor; antidepressant.

Preparation
Sertraline Tablets

Ph Eur

DEFINITION
(1*S*,4*S*)-4-(3,4-Dichlorophenyl)-*N*-methyl-1,2,3,4-tetrahydronaphthalen-1-amine hydrochloride.

Content
97.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Slightly soluble in water, sparingly soluble or slightly soluble in anhydrous ethanol, slightly soluble in acetone and in 2-propanol.

It shows polymorphism (*5.9*).

IDENTIFICATION
Carry out either tests A, B, C or tests B, C, D.

A. Specific optical rotation (*2.2.7*): + 38.8 to + 43.0 (anhydrous substance), measured at 25 °C.

Solvent mixture Dilute 1 volume of a 103 g/L solution of *hydrochloric acid R* to 20 volumes with *methanol R*.

Dissolve 0.250 g in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *sertraline hydrochloride CRS*.

If the spectra obtained in the solid state show differences, record new spectra using 10 g/L solutions in *methylene chloride R*.

C. Dissolve 10 mg in 5 mL of *anhydrous ethanol R* and add 5 mL of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

D. Enantiomeric purity (see Tests).

TESTS
Enantiomeric purity
Liquid chromatography (*2.2.29*). *Prepare the test solution immediately before use.*

Solvent mixture *diethylamine R, hexane R, 2-propanol R* (1:40:60 *V/V/V*).

Test solution Dissolve 60.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (a) Dissolve the contents of a vial of *sertraline for system suitability CRS* (containing impurity G) in 1 mL of the solvent mixture.

Reference solution (b) Dilute 0.5 mL of the test solution to 100.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *amylose derivative of silica gel for chiral separation R* (5 µm).

Mobile phase Mix 30 volumes of *hexane R* and 70 volumes of a mixture of 1 volume of *diethylamine R*, 25 volumes of *2-propanol R* and 975 volumes of *hexane R*.

Flow rate 0.4 mL/min.

Detection Spectrophotometer at 275 nm.

Injection 20 µL.

Run time Twice the retention time of sertraline.

Identification of impurities Use the chromatogram supplied with *sertraline for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peak due to impurity G.

Relative retention With reference to sertraline (retention time = about 15 min): impurity G = about 1.3.

System suitability:
— *resolution*: minimum 1.5 between the peaks due to sertraline and impurity G in the chromatogram obtained with reference solution (a);
— *signal-to-noise ratio*: minimum 10 for the peak due to sertraline in the chromatogram obtained with reference solution (b).

Limit:
— *impurity G*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent).

Impurity E
Liquid chromatography (*2.2.29*).

Solvent mixture Mobile phase A, mobile phase B (50:50 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dissolve 5.0 mg of *sertraline impurity E CRS* (mandelic acid) in the solvent mixture and dilute to 25.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 100.0 mL with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *benzoic acid R* and 20 mg of *mandelic acid R* (impurity E) in the solvent mixture and dilute to 50 mL with the solvent mixture. Dilute 1 mL of the solution to 50 mL with the solvent mixture.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography compatible with 100 per cent aqueous mobile phases R* (3 µm).

Mobile phase:
— *mobile phase A*: dissolve 1.0 g of *sodium laurilsulfate R* in 800 mL of *water for chromatography R* and add 200 mL of *acetonitrile for chromatography R*; add 1.0 mL of *phosphoric acid R* and mix;
— *mobile phase B*: dissolve 1.0 g of *sodium laurilsulfate R* in 100 mL of *water for chromatography R* and add 900 mL of *acetonitrile for chromatography R*; add 1.0 mL of *phosphoric acid R* and mix;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 8	60	40
8 - 9	60 → 10	40 → 90
9 - 16	10	90

Flow rate 1 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µL.

Identification of peaks Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity E; use the chromatogram obtained with reference solution (b) to identify the peak due to benzoic acid.

Relative retention With reference to sertraline (retention time = about 18 min): impurity E = about 0.2; benzoic acid = about 0.3.

System suitability Reference solution (b):
— *resolution*: minimum 5.0 between the peaks due to impurity E and benzoic acid.

Limit:
— *impurity E*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent).

Related substances
Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution Introduce 0.250 g of the substance to be examined into a 15 mL stoppered centrifuge tube, add 2.0 mL of *methanol R* and 0.20 mL of a 25 per cent solution of *potassium carbonate R* and mix in a vortex mixer for 30 s. Add 8.0 mL of *methylene chloride R*, stopper the tube and mix in a vortex mixer for 60 s. Add 1 g of *anhydrous sodium sulfate R*, mix well and then centrifuge for about 5 min.

Reference solution (a) Dissolve the contents of a vial of *sertraline for peak identification CRS* (containing impurities A, B, C and F) in 0.2 mL of *methylene chloride R*.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *methylene chloride R*. Dilute 1.0 mL of this solution to 20.0 mL with *methylene chloride R*.

Column:
— *material*: fused silica;
— *size*: *l* = 30 m, Ø = 0.53 mm;
— *stationary phase*: *phenyl(50)methyl(50)polysiloxane R* (film thickness 1.0 µm).

Carrier gas *helium for chromatography R*.

Flow rate 9 mL/min.

Split ratio 1:10.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 1	200
	1 - 31	200 → 260
	31 - 39	260
Injection port		250
Detector		280

Detection Flame ionisation.

Injection 1 µL.

Identification of impurities Use the chromatogram supplied with *sertraline for peak identification CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C and F.

Relative retention With reference to sertraline (retention time = about 24 min): impurity B = about 0.5; impurities C and D = about 0.7; impurity A = about 1.05; impurity F = about 1.1.

System suitability Reference solution (a):
— *peak-to-valley ratio*: minimum 15, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to sertraline.

Limits:
— *sum of impurities C and D*: maximum 0.8 per cent;
— *impurities A, B, F*: for each impurity, maximum 0.2 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 1.5 per cent;
— *reporting threshold*: 0.05 per cent (reference solution (b)).

Water (*2.5.12*)
Maximum 0.5 per cent, determined on 2.00 g.

Sulfated ash (*2.4.14*)
Maximum 0.2 per cent, determined on 1.0 g.

ASSAY
Liquid chromatography (*2.2.29*).

Buffer solution To 28.6 mL of *glacial acetic acid R* slowly add, while stirring and cooling, 34.8 mL of *triethylamine R*, and dilute to 100 mL with *water for chromatography R*. Dilute 10 mL of this solution to 1000 mL with *water for chromatography R*.

Test solution Dissolve 55.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 5.0 mL of the solution to 100.0 mL with the mobile phase.

Reference solution Dissolve 55.0 mg of *sertraline hydrochloride CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 100.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.15 m, Ø = 3.9 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (4 µm);
— *temperature*: 30 °C.

Mobile phase *methanol R*, buffer solution, *acetonitrile R* (15:40:45 *V/V/V*).

Flow rate 1.8 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL.

Run time Twice the retention time of sertraline.

Retention time Sertraline = about 1.9 min.

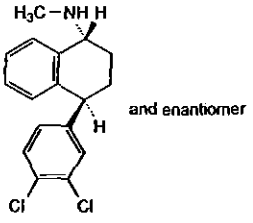
Calculate the percentage content of $C_{17}H_{18}Cl_3N$ taking into account the assigned content of *sertraline hydrochloride CRS*.

STORAGE

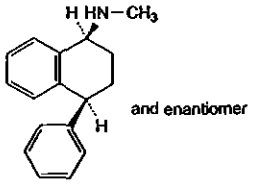
Protected from light.

IMPURITIES

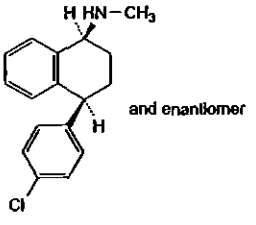
Specified impurities *A, B, C, D, E, F, G.*



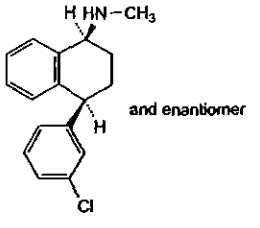
A. (1*RS*,4*SR*)-4-(3,4-dichlorophenyl)-*N*-methyl-1,2,3,4-tetrahydronaphthalen-1-amine,



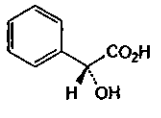
B. (1*RS*,4*RS*)-*N*-methyl-4-phenyl-1,2,3,4-tetrahydronaphthalen-1-amine,



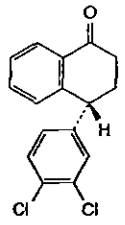
C. (1*RS*,4*RS*)-4-(4-chlorophenyl)-*N*-methyl-1,2,3,4-tetrahydronaphthalen-1-amine,



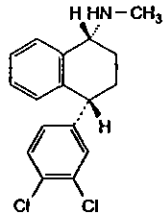
D. (1*RS*,4*RS*)-4-(3-chlorophenyl)-*N*-methyl-1,2,3,4-tetrahydronaphthalen-1-amine,



E. (2*R*)-hydroxyphenylacetic acid ((*R*)-mandelic acid),



F. (4*R*)-4-(3,4-dichlorophenyl)-3,4-dihydronaphthalen-1(2*H*)-one,



G. (1*R*,4*R*)-4-(3,4-dichlorophenyl)-*N*-methyl-1,2,3,4-tetrahydronaphthalen-1-amine (sertraline enantiomer).

Ph Eur

Refined Sesame Oil

Sesame Oil

(Ph. Eur. monograph 0433)

Ph Eur

DEFINITION

Fatty oil obtained from the ripe seeds of *Sesamum indicum* L. by expression or extraction. It is then refined. Improved colour and odour may be obtained by further refining. It may contain a suitable antioxidant.

CHARACTERS

Appearance

Clear, light yellow liquid, almost colourless.

Solubility

Practically insoluble in ethanol (96 per cent), miscible with light petroleum.

Relative density

About 0.919.

Refractive index

About 1.473.

It solidifies to a butter-like mass at about −4 °C.

IDENTIFICATION

First identification: A.

Second identification: B.

A. Composition of triglycerides (see Tests).

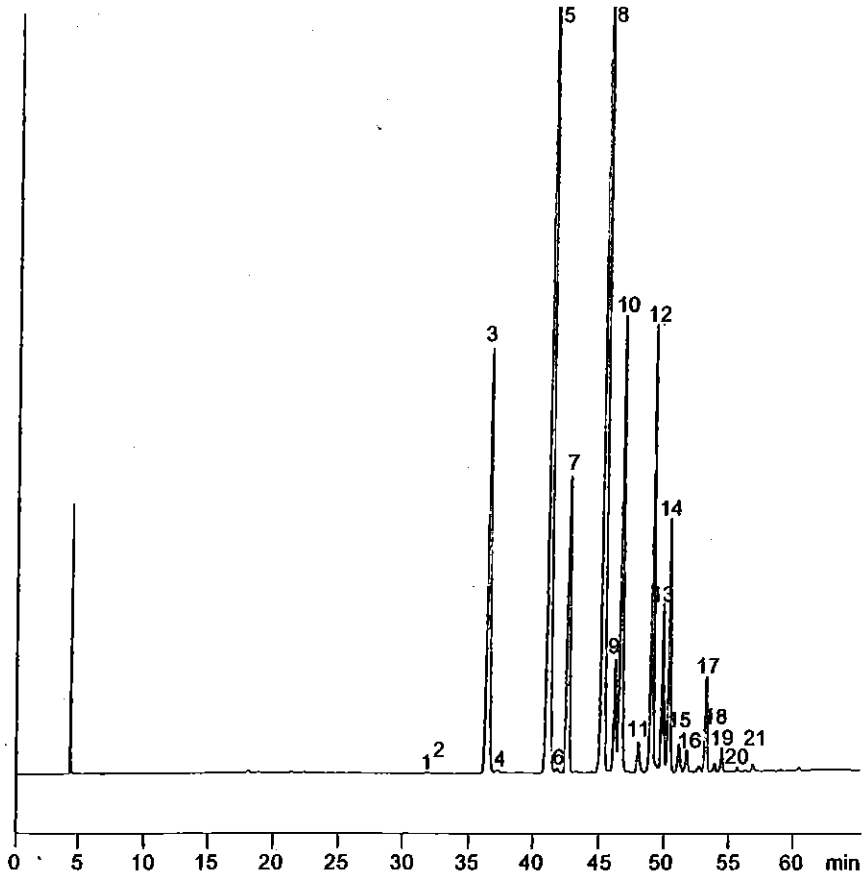
B. Identification of fatty oils by thin-layer chromatography (*2.3.2*).

Results The chromatogram obtained is similar to the corresponding chromatogram shown in Figure 2.3.2-1.

TESTS

Acid value (*2.5.1*)

Maximum 0.5, determined on 10.0 g; maximum 0.3 if intended for use in the manufacture of parenteral preparations.



1. LLLn	4. OLLn	7. PLL	10. POL	13. SOL	16. PPO	19. SSL
2. OLnLn	5. OLL	8. OOL	11. PPL	14. POO	17. SOO	20. PPS
3. LLL	6. OOLn	9. SLL	12. OOO	15. PSL	18. PSO	21. SSO

Figure 0433.-1. – *Chromatogram for the composition of triglycerides in refined sesame oil*

Peroxide value (*2.5.5*)
Maximum 10.0; maximum 5.0 if intended for use in the manufacture of parenteral preparations.

Unsaponifiable matter (*2.5.7*)
Maximum 2.0 per cent, determined on 5.0 g.

Alkaline impurities (*2.4.19*)
It complies with the test for alkaline impurities in fatty oils.

Cottonseed oil
Mix 5 mL in a test-tube with 5 mL of a mixture of equal volumes of pentanol R and a 10 g/L solution of *sulfur R* in *carbon disulfide R*. Warm the mixture carefully until the carbon disulfide is expelled, and immerse the tube to 1/3 of its depth in boiling *saturated sodium chloride solution R*. No reddish colour develops within 15 min.

Composition of triglycerides
Liquid chromatography (*2.2.29*).

Test solution Dilute 50.0 mg of the substance to be examined to 10.0 mL with a mixture of equal volumes of *acetone R* and *methylene chloride R*.

Reference solutions Dissolve 80.0 mg of *triolein R* in a mixture of equal volumes of *acetone R* and *methylene chloride R* and dilute to 50.0 mL with the same mixture of solvents. Prepare 5 reference solutions by dilution of this solution so as to cover concentrations ranging from the disregard limit (0.5 per cent) to the upper limit for OLL (30.0 per cent).

Plot the logarithm of the area of the peak due to triolein against the logarithm of the concentration of triolein in the reference solution.

Column 2 columns coupled in series:
— *size of each column*: l = 0.25 m, Ø = 4 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (4 µm).

Mobile phase:
— *mobile phase A*: *acetone R, methylene chloride R, acetonitrile R* (5:15:80 *V/V/V*);
— *mobile phase B*: *acetone R, acetonitrile R, methylene chloride R* (20:20:60 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	100 → 75	0 → 25
15 - 25	75	25
25 - 70	75 → 0	25 → 100
70 - 75	0 → 100	100 → 0
75 - 80	100	0

Flow rate 1.0 mL/min.

Detection Evaporative light-scattering detector; the following settings have been found to be suitable; if the detector has different setting parameters, adjust the detector settings so as to comply with the system suitability criterion:
— *carrier gas*: *nitrogen R*;
— *flow rate*: 0.7 L/min;
— *evaporator temperature*: 85 °C;
— *nebuliser temperature*: 45 °C.

Injection 20 µL.

Identification of peaks Use the chromatograms obtained with the reference solutions to identify the peak due to triolein; identify the other peaks using the chromatogram shown in Figure 0433.-1. The fatty acids are designated as linolenic (Ln), linoleic (L), oleic (O), palmitic (P) and stearic (S).

System suitability Test solution:
— *resolution*: minimum 1.5 between the peaks due to OOO (triolein) and SOL.

Using the calibration curve obtained with the reference solutions, determine the percentage content of each peak with an area greater than that of the peak corresponding to the disregard limit (0.5 per cent). Assuming that the sum of these percentage contents is 100 per cent, normalise the percentage content of each of the 8 triglycerides specified below.

Composition of triglycerides:
— *LLL*: 7.0 per cent to 19.0 per cent;
— *OLL*: 13.0 per cent to 30.0 per cent;
— *PLL*: 5.0 per cent to 9.0 per cent;
— *OOL*: 12.0 per cent to 23.0 per cent;
— *POL*: 6.0 per cent to 14.0 per cent;
— *OOO*: 5.0 per cent to 16.0 per cent;
— *SOL*: 2.0 per cent to 8.0 per cent;
— *POO*: 2.0 per cent to 10.0 per cent.

Water (*2.5.32*)
Maximum 0.1 per cent, determined on 1.00 g.

STORAGE

In an airtight, well-filled container, protected from light; if intended for use in the manufacture of parenteral preparations store under an inert gas in an airtight container.

When the container has been opened, its contents are to be used as soon as possible. Any part of the contents not used at once is protected by an atmosphere of an inert gas.

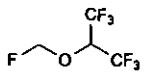
LABELLING

The label states:
— whether the oil is obtained by expression or extraction;
— where applicable, that the substance is suitable for use in the manufacture of parenteral preparations;
— where applicable, the name of the inert gas used.

Ph Eur

Sevoflurane

(Ph. Eur. monograph 2269)



$C_4H_3F_7O$ 200.1 *28523-86-6*

Action and use
General anaesthetic.

Ph Eur

DEFINITION

1,1,1,3,3,3-Hexafluoro-2-(fluoromethoxy)propane.

CHARACTERS

Appearance
Clear, colourless, volatile liquid.

Solubility
Slightly soluble in water, miscible with ethanol (96 per cent).

Relative density
About 1.52.

bp
About 59 °C.

It is non-flammable.

It decomposes in the presence of Lewis acids; this decomposition is inhibited by water in sufficient quantity.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Preparation Examine the substance in the gaseous state or in the liquid state.

Comparison *sevoflurane CRS*.

TESTS

Acidity or alkalinity
Introduce 20.0 mL of the substance to be examined and 20 mL of *carbon dioxide-free water R* into a separating funnel, shake for 3 min and allow to stand. Collect the aqueous upper layer and add 0.2 mL of *bromocresol purple solution R*. Not more than 0.10 mL of *0.01 M sodium hydroxide* or 0.60 mL of *0.01 M hydrochloric acid* is required to change the colour of the indicator.

Refractive index (*2.2.6*)
1.2745 to 1.2760.

Related substances
Gas chromatography (*2.2.28*).

Internal standard *methylal R*.

Test solution Introduce 20.0 mL of the substance to be examined into a vial and seal with a cap and septum. Using a microsyringe, add 5 µL of the internal standard and mix thoroughly.

Reference solution (a) Introduce 2.0 mL of *ethylene chloride R* into a screw-cap vial and immediately seal with a cap and septum. Using a microsyringe, add about 20 µL of the substance to be examined. Record the quantity added, in milligrams, of the substance to be examined (M_2). Then, using a microsyringe, add about 20 µL of the internal standard. Record the quantity added, in milligrams, of the internal standard (M_1).

Reference solution (b) *sevoflurane for system suitability CRS* (containing impurities A and B).

Reference solution (c) Introduce 20.0 mL of *ethylene chloride R* into a vial and seal with a cap and septum. Using a microsyringe, add 20 µL of the substance to be examined and mix thoroughly. Dilute 0.5 mL of this solution to 100.0 mL with *ethylene chloride R*.

Column:
- *material*: fused silica;
- *size*: *l* = 30 m, Ø = 0.32 mm;
- *stationary phase*: *cyanopropyl(3)phenyl(3)methyl(94) polysiloxane R* (film thickness 3 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.0 mL/min.

Split ratio 1:20.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 10	40
	10 - 26	40 → 200
	26 - 40	200
Injection port		200
Detector		225

Detection Flame ionisation.

Injection 2 µL; rinse the syringe with a solution containing *ethylene chloride R* before injection of the reference solutions; rinse the syringe with the substance to be examined before injection of the test solution.

Identification of impurities Use the chromatogram supplied with *sevoflurane for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and B.

Relative retention With reference to sevoflurane (retention time = about 6.6 min): impurity A = about 0.78; impurity B = about 0.83; internal standard = about 1.35.

System suitability Reference solution (b):
- *resolution*: minimum 2.0 between the peaks due to impurities A and B.

Calculate the relative response factor (F_1) for reference solution (a), using the following expression:

$$\frac{M_1 \times R}{M_2}$$

M_1 = mass of the internal standard in reference solution (a), in milligrams;
M_2 = mass of the substance to be examined in reference solution (a), in milligrams;
R = ratio of the area of the peak due to sevoflurane to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (a).

Calculate the content of each impurity in the substance to be examined, in parts per million, using the following expression:

$$\frac{0.859 \times R_1 \times 250}{1.52 \times F_1}$$

0.859 = relative density of the internal standard;
1.52 = relative density of sevoflurane;
R_1 = ratio of the area of the peak due to the impurity to the area of the peak due to the internal standard from the chromatogram obtained with the test solution;
F_1 = relative response factor for reference solution (a).

Limits:
- *impurity A*: maximum 25 ppm;
- *impurity B*: maximum 100 ppm;
- *unspecified impurities*: for each impurity, maximum 100 ppm;
- *total*: maximum 300 ppm;
- *disregard limit*: the area of the peak due to sevoflurane in the chromatogram obtained with reference solution (c) (5 ppm).

Fluorides

Maximum 2 µg/mL.

Potentiometry (*2.2.36, Method I*). *Use plastic utensils throughout this test.*

Buffer solution Dissolve 0.5 g of *sodium citrate R* and 55 g of *sodium chloride R* in 350 mL of *water R*. Carefully add 75 g of *sodium hydroxide R* and shake to dissolve. Cool to room temperature and carefully add 225 mL of *glacial acetic acid R* while stirring. Cool and add 300 mL of *2-propanol R*. Dilute with *water R* to 1000.0 mL. The apparent pH of this solution is between 5.0 and 5.5.

Test solution Introduce 50.0 mL of the substance to be examined and 50.0 mL of *water R* into a separating funnel, shake vigorously for 3 min and allow the layers to separate completely. Dilute 25.0 mL of the upper aqueous layer to 50.0 mL with the buffer solution.

Fluoride standard solution (1000 ppm F) Dissolve 221.0 mg of *sodium fluoride R*, previously dried at 150 °C for 4 h, in *water R*. Add 1.0 mL of *0.01 M sodium hydroxide* and dilute to 100.0 mL with *water R*.

Reference stock solutions Dilute the fluoride standard solution (1000 ppm F) with *water R* to obtain solutions having known concentrations of about 5 µg, 2 µg, 0.5 µg, and 0.2 µg of fluoride per millilitre.

Reference solutions Dilute 25.0 mL of each reference stock solution to 50.0 mL with the buffer solution.

Indicator electrode Fluoride-selective.

Reference electrode Silver-silver chloride.

Apparatus Voltmeter capable of a minimum reproducibility of ± 0.2 mV.

Carry out the measurements on the reference solutions and test solution. To take measurements, transfer the solution under test to a 100 mL beaker containing a polytetrafluoroethylene-coated magnetic stirring bar, and immerse the electrodes. Allow to stir on a magnetic stirrer with an insulated top until equilibrium is attained (about 2-3 min), and record the potential. Rinse the electrodes with the buffer solution and dry, taking care to avoid damaging the crystal of the specific-ion electrode.

Calculate the concentration of fluorides using the calibration curve.

Non-volatile residue

Maximum 100 mg/L.

Transfer 10.0 mL to a tared evaporating dish, evaporate to dryness on a water-bath and dry the residue at 105 °C for 2 h. The residue weighs a maximum of 1.0 mg.

Water (*2.5.12*)

Maximum 0.050 per cent *m/m*, determined on 10.0 mL.

STORAGE

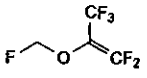
In an airtight, stainless-steel container, protected from light.

IMPURITIES

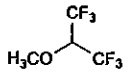
Specified impurities *A, B.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance

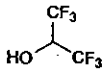
criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) C.



A. 1,1,3,3,3-pentafluoro-2-(fluoromethoxy)prop-1-ene,



B. 1,1,1,3,3,3-hexafluoro-2-methoxypropane,



C. 1,1,1,3,3,3-hexafluoropropan-2-ol.

Ph Eur

Shellac

(Ph. Eur. monograph 1149)

Action and use
Excipient.

Ph Eur

DEFINITION

Purified material obtained from the resinous secretion of the female insect *Kerria lacca* (Kerr) Lindinger (*Laccifer lacca* Kerr). There are 4 types of shellac depending on the nature of the treatment of crude secretion (seedlac): wax-containing shellac, bleached shellac, dewaxed shellac and bleached, dewaxed shellac.

Wax-containing shellac is obtained from seedlac: it is purified by filtration of the molten substance and/or by hot extraction using a suitable solvent.

Bleached shellac is obtained from seedlac by treatment with sodium hypochlorite after dissolution in a suitable alkaline solution, precipitation by dilute acid and drying.

Dewaxed shellac is obtained from wax-containing shellac or seedlac by treatment with a suitable solvent and removal of the insoluble wax by filtering.

Bleached, dewaxed shellac is obtained from wax-containing shellac or seedlac by treatment with sodium hypochlorite after dissolution in a suitable alkaline solution; the insoluble wax is removed by filtration. It is precipitated by dilute acid and dried.

CHARACTERS

Appearance
Brownish-orange or yellow, shining, translucent, hard or brittle, more or less thin flakes (wax-containing shellac and dewaxed shellac), or a creamy white or brownish-yellow powder (bleached shellac and bleached, dewaxed shellac).

Solubility
Practically insoluble in water, gives a more or less opalescent solution (wax containing shellac and bleached shellac) or a clear solution (dewaxed shellac and bleached, dewaxed shellac) in anhydrous ethanol. When warmed it is sparingly soluble or soluble in alkaline solutions.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Heat 0.25 g of the powdered substance (500) (*2.9.12*) on a water-bath with 2 mL of *dilute sodium hydroxide solution R* for 5 min. Cool, add 5 mL of *ethyl acetate R* and slowly, with stirring, 2 mL of *dilute acetic acid R*. Shake and filter the upper layer through *anhydrous sodium sulfate R*.

Reference solution Dissolve 6.0 mg of *aleuritic acid R* in 1.0 mL of *methanol R*, heating slightly if necessary.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *acetic acid R, methanol R, methylene chloride R, ethyl acetate R* (1:8:32:60 *V/V/V/V*).

Application 10 µL, as bands.

Development Twice over a path of 15 cm.

Drying In air.

Detection Spray with *anisaldehyde solution R*, heat at 100-105 °C for 5-10 min and examine in daylight.

Results The chromatogram obtained with the test solution shows several coloured zones, one of which is similar in position and colour to the zone in the chromatogram obtained with the reference solution. Above this zone the chromatogram obtained with the test solution shows a pink zone and below it several violet zones. Below the zone due to aleuritic acid, there is a light blue zone (shellolic acid) accompanied by zones of the same colour but of lower intensity. Other faint grey and violet zones may be visible.

B. Examine the chromatograms obtained in the test for colophony.

Results For wax-containing shellac, in the chromatogram obtained with the test solution, a more or less strong bluish-grey zone is visible, just above the zone due to thymolphthalein in the chromatogram obtained with the reference solution; for dewaxed shellac, no such zone is visible just above the zone due to thymolphthalein in the chromatogram obtained with the reference solution.

TESTS

Acid value (*2.5.1*)
65 to 95 (dried substance).

Examine 1.00 g of the coarsely ground substance. Determine the end-point potentiometrically (*2.2.20*).

Colophony
Thin-layer chromatography (*2.2.27*) as described under identification test A with the following modifications.

Test solution Dissolve 50 mg of the powdered substance (500) (*2.9.12*), with heating, in a mixture of 0.5 mL of *methylene chloride R* and 0.5 mL of *methanol R*.

Reference solution Dissolve 2.0 mg of *thymolphthalein R* in 1.0 mL of *methanol R*.

Detection Examine in ultraviolet light at 254 nm; mark the quenching zones in the chromatogram obtained with the test solution that have similar R_F values to that of the quenching zone due to thymolphthalein in the chromatogram obtained with the reference solution; spray with *anisaldehyde solution R*, heat at 100-105 °C for 5-10 min and examine in daylight.

Results The chromatogram obtained with the reference solution shows a principal zone with a reddish-violet colour (thymolphthalein). None of the quenching zones in the chromatogram obtained with the test solution that have an R_F value similar to the zone due to thymolphthalein in the reference solution show a more or less strong violet or

brownish colour (colophony). Disregard any faint violet zone at this level that does not show quenching before spraying and heating.

Arsenic (*2.4.2, Method A*)
Maximum 3 ppm.

Introduce 0.33 g of the substance to be examined and 5 mL of *sulfuric acid R* into a combustion flask. Carefully add a few millilitres of *strong hydrogen peroxide solution R* and heat to boiling until a clear, colourless solution is obtained. Continue heating to eliminate the water and as much sulfuric acid as possible and dilute to 25 mL with *water R*.

Loss on drying (*2.2.32*)
Maximum 2.0 per cent for unbleached shellac and maximum 6.0 per cent for bleached shellac, determined on 1.000 g of the powdered substance (500) (*2.9.12*) by drying in an oven at 40-45 °C for 24 h.

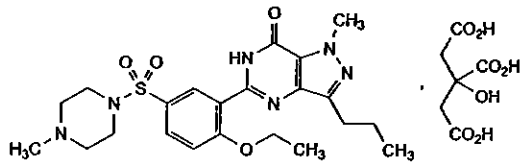
STORAGE
Protected from light. Store bleached shellac and bleached, dewaxed shellac at a temperature not exceeding 15 °C.

LABELLING
The label indicates the type of shellac.

Ph Eur

Sildenafil Citrate

(Ph. Eur. monograph 2270)



$C_{28}H_{38}N_6O_{11}S$ 667 *171599-83-0*

Action and use
Selective inhibitor of cyclic GMP-specific phosphodiesterase (Type V) with vasodilator action; treatment of erectile dysfunction.

Preparations
Sildenafil Tablets
Sildenafil Chewable Tablets
Sildenafil Orodispersible Tablets
Sildenafil Orodispersible Films
Sildenafil Powder for Oral Suspension
Sildenafil Injection

Ph Eur

DEFINITION
5-[2-Ethoxy-5-[(4-methylpiperazin-1-yl)sulfonyl]phenyl]-1-methyl-3-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one dihydrogen 2-hydroxypropane-1,2,3-tricarboxylate.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white, slightly hygroscopic, crystalline powder.

Solubility
Slightly soluble in water and in methanol, practically insoluble in hexane.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *sildenafil citrate CRS.*

TESTS
Impurity E
Thin-layer chromatography (*2.2.27*).

Solvent mixture *concentrated ammonia R, water R, methanol R* (5:25:75 *V/V/V*).

Test solution Dissolve 35.0 mg of the substance to be examined in 2.0 mL of the solvent mixture, with the aid of ultrasound if necessary.

Reference solution (a) Dissolve 7.0 mg of *imidazole CRS* (impurity E) in the solvent mixture and dilute to 20.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dilute 5.0 mL of reference solution (a) to 10.0 mL with the solvent mixture.

Reference solution (c) Mix 1 mL of the test solution and 1 mL of reference solution (a).

Plate *TLC silica gel F_{254} plate R* (2-10 µm).

Mobile phase *concentrated ammonia R, ethanol (96 per cent) R, ethyl acetate R, methylene chloride R* (1:20:30:50 *V/V/V/V*).

Application 10 µL of the test solution and reference solutions (b) and (c) as bands of 6 mm.

Development Over 2/3 of the plate.

Drying At 100 °C for about 15 min.

Detection Expose to iodine vapour until the plate is light brown and examine under ultraviolet light at 254 nm.

Retardation factors Citrate = about 0; impurity E = about 0.25; sildenafil = about 0.4.

System suitability Reference solution (c):
— the chromatogram shows 2 clearly separated zones.

Limit:
— *impurity E*: any zone due to impurity E is not more intense than the principal zone in the chromatogram obtained with reference solution (b) (0.1 per cent).

Related substances
Liquid chromatography (*2.2.29*).

Buffer solution Dissolve 2.72 g of *potassium dihydrogen phosphate R* in 900 mL of *water for chromatography R* and adjust to pH 6.5 with a 120 g/L solution of *potassium hydroxide R* in *water for chromatography R*. Dilute to 1.0 L with *water for chromatography R*.

Solvent mixture *acetonitrile for chromatography R*, mobile phase A (10:90 *V/V*).

Test solution (a) Dissolve 25.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Test solution (b) Dilute 2.0 mL of test solution (a) to 50.0 mL with the solvent mixture.

Reference solution (a) Dissolve 2 mg of *sildenafil impurity A CRS* in test solution (a) and dilute to 10.0 mL with test solution (a). Dilute 1.0 mL of the solution to 20.0 mL with the solvent mixture.

Reference solution (b) Dissolve 5 mg of *sildenafil for peak identification CRS* (containing impurity D) in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (c) Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (d) Dissolve 25.0 mg of *sildenafil citrate CRS* in the solvent mixture and dilute to 50.0 mL with the solvent mixture. Dilute 2.0 mL of the solution to 50.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: *acetonitrile for chromatography R*, buffer solution (20:80 *V/V*);
— *mobile phase B*: buffer solution, *methanol R1*, *acetonitrile for chromatography R* (20:20:60 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	75	25
3 - 26	75 → 30	25 → 70
26 - 38	30	70

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 10 µL of test solution (a) and reference solutions (a), (b) and (c).

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity A; use the chromatogram supplied with *sildenafil for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity D.

Relative retention With reference to sildenafil (retention time = about 16 min): impurity D = about 0.15; impurity A = about 1.25.

System suitability Reference solution (a):
— *resolution*: minimum 5.0 between the peaks due to sildenafil and impurity A.

Calculation of percentage contents:
— *correction factor*: multiply the peak area of impurity D by 0.7;
— for each impurity, use the concentration of sildenafil citrate in reference solution (c).

Limits:
— *impurities A, D*: for each impurity, maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.5 per cent;
— *reporting threshold*: 0.05 per cent; disregard the peak due to citrate.

Water (*2.5.12*)
Maximum 2.5 per cent, determined on 0.200 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.00 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (d).

Calculate the percentage content of $C_{28}H_{38}N_6O_{11}S$ taking into account the assigned content of *sildenafil citrate CRS*.

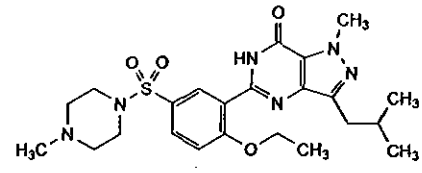
STORAGE

In airtight container.

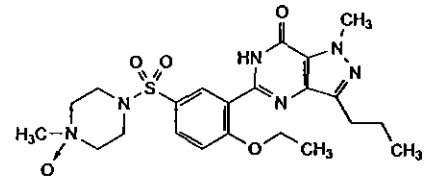
IMPURITIES

Specified impurities *A, D, E.*

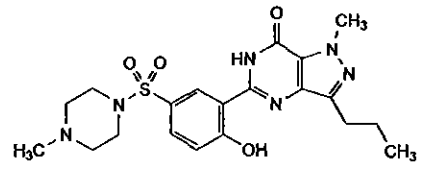
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, F, G.*



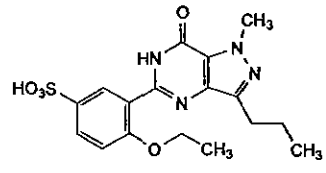
A. 5-[2-ethoxy-5-[(4-methylpiperazin-1-yl)sulfonyl]phenyl]-1-methyl-3-(2-methylpropyl)-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one,



B. 1-methyl-4-[[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6,7-dihydro-1*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]sulfonyl] piperazine 1-oxide,

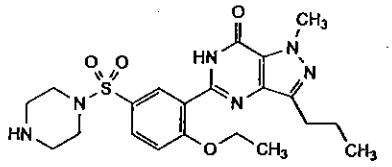


C. 5-[2-hydroxy-5-[(4-methylpiperazin-1-yl)sulfonyl]phenyl]-1-methyl-3-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*] pyrimidin-7-one,

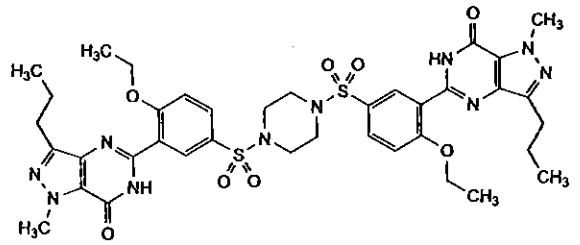


D. 4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6,7-dihydro-1*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)benzenesulfonic acid,

E. 1*H*-imidazole,



F. 5-[2-ethoxy-5-(piperazin-1-ylsulfonyl)phenyl]-1-methyl-3-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one,



G. 5,5′-{piperazin-1,4-diylbis[dioxo-λ^6-sulfanediyl(2-ethoxy-5,1-phenylene)]}bis(1-methyl-3-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one).

Ph Eur

Colloidal Anhydrous Silica

(Ph. Eur. monograph 0434)

SiO_2 · 60.1 *7631-86-9*

Action and use
Excipient.

Ph Eur

DEFINITION

Content
99.0 per cent to 100.5 per cent of SiO_2 (ignited substance).

CHARACTERS

Appearance
White or almost white, light, fine, amorphous powder, with a particle size of about 15 nm.

Solubility
Practically insoluble in water and in mineral acids except hydrofluoric acid. It dissolves in hot solutions of alkali hydroxides.

IDENTIFICATION
About 20 mg gives the reaction of silicates (*2.3.1*).

TESTS

pH (*2.2.3*)
3.5 to 5.5.

Shake 1.0 g with 30 mL of *carbon dioxide-free water R*.

Chlorides (*2.4.4*)
Maximum 250 ppm.

To 1.0 g add a mixture of 20 mL of *dilute nitric acid R* and 30 mL of *water R* and heat on a water-bath for 15 min, shaking frequently. Dilute to 50 mL with *water R* if necessary, filter and cool. Dilute 10 mL of the filtrate to 15 mL with *water R*.

Loss on ignition
Maximum 5.0 per cent, determined on 0.200 g by ignition in a platinum crucible at 900 ± 50 °C for 2 h. Allow to cool in a desiccator before weighing.

ASSAY
To the residue obtained in the test for loss on ignition add 0.2 mL of *sulfuric acid R* and sufficient *ethanol (96 per cent) R* to moisten the residue completely. Add 6 mL of *hydrofluoric acid R* and evaporate to dryness on a hot-plate at 95-105 °C, taking care to avoid loss from sputtering. Wash down the sides of the dish with 6 mL of *hydrofluoric acid R* and evaporate to dryness. Ignite at 900 ± 50 °C, allow to cool in a desiccator and weigh.

The difference between the mass of the final residue and the mass of the residue obtained in the test for loss on ignition gives the amount of SiO_2 in the quantity of the substance to be examined used.

FUNCTIONALITY-RELATED CHARACTERISTICS
This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristic may be relevant for colloidal anhydrous silica used as glidant in tablets and capsules.

Specific surface area (*2.9.26, Method I*)
Determine the specific surface area in the P/P_0 range of 0.05 to 0.30.

Sample outgassing 20 min at 160 °C.

Ph Eur

Colloidal Hydrated Silica

(Ph. Eur. monograph 0738)

63231-67-4

Action and use
Excipient.

Ph Eur

DEFINITION
Colloidal hydrated silica produced by precipitation or gelation process.

Content
98.0 per cent to 100.5 per cent of SiO_2 (M_r 60.1) (ignited substance).

CHARACTERS

Appearance
White or almost white, light, fine, amorphous powder.

Solubility
Practically insoluble in water and in mineral acids, with the exception of hydrofluoric acid. It dissolves in hot solutions of alkali hydroxides.

IDENTIFICATION
About 20 mg gives the reaction of silicates (*2.3.1*).

TESTS

Solution S

To 2.5 g add 50 mL of *hydrochloric acid R* and mix. Heat on a water-bath for 30 min, stirring from time to time. Maintain the original volume by adding *dilute hydrochloric acid R*. Evaporate to dryness. Add to the residue a mixture of 8 mL of *dilute hydrochloric acid R* and 24 mL of *water R*. Heat to boiling and filter under reduced pressure through a sintered-glass filter (16) (*2.1.2*). Wash the residue on the filter with a hot mixture of 3 mL of *dilute hydrochloric acid R* and 9 mL of *water R*. Wash with small quantities of *water R*, combine the filtrate and washings and dilute to 50 mL with *water R*.

pH (*2.2.3*)

4.0 to 7.0.

Suspend 1.0 g in 30 mL of a 75 g/L solution of *potassium chloride R*.

Water-absorption capacity

In a mortar, triturate 5 g with 5 mL of *water R*, added drop by drop. The mixture remains powdery.

Substances soluble in hydrochloric acid

Maximum 2.0 per cent.

In a platinum dish, evaporate to dryness 10.0 mL of solution S and dry to constant mass at 100-105 °C. The mass of the residue is not more than 10 mg.

Chlorides (*2.4.4*)

Maximum 0.1 per cent.

Heat 0.5 g with 50 mL of *water R* on a water-bath for 15 min. Dilute to 100 mL with *water R* and centrifuge at 1500 *g* for 5 min. Dilute 10 mL of the supernatant to 15 mL with *water R*.

Sulfates (*2.4.13*)

Maximum 1 per cent.

Dilute 2 mL of solution S to 100 mL with *distilled water R*.

Iron (*2.4.9*)

Maximum 300 ppm.

To 2 mL of solution S add 28 mL of *water R*.

Loss on ignition

Maximum 20.0 per cent, determined on 0.200 g in a platinum crucible by heating at 100-105 °C for 1 h and then at 900 ± 50 °C for 2 h.

ASSAY

To the residue obtained in the test for loss on ignition add 0.2 mL of *sulfuric acid R* and a quantity of *ethanol (96 per cent) R* sufficient to moisten the residue completely. Add 6 mL of *hydrofluoric acid R* and evaporate to dryness at 95-105 °C, taking care to avoid loss from sputtering. Wash the inside of the dish with 6 mL of *hydrofluoric acid R* and evaporate to dryness again. Ignite at 900 ± 50 °C, allow to cool in a desiccator and weigh. The difference between the mass of the final residue and that of the mass obtained in the test for loss on ignition corresponds to the mass of SiO_2 in the test sample.

Ph Eur

Hydrophobic Colloidal Anhydrous Silica

(Ph. Eur. monograph 2208)

Ph Eur

DEFINITION

Colloidal silicon dioxide partly alkylated for hydrophobation.

Content

99.0 per cent to 101.0 per cent SiO_2 (ignited substance).

CHARACTERS

Appearance

White or almost white, light, fine, amorphous powder, not wettable by water.

Solubility

Practically insoluble in water and mineral acids except hydrofluoric acid. It dissolves slowly in hot solutions of alkali hydroxides.

IDENTIFICATION

A. About 25 mg ignited in a platinum crucible at 900 ± 50 °C for 2 h gives the reaction of silicates (*2.3.1*).

B. Water-dispersible fraction (see Tests).

TESTS

Chlorides (*2.4.4*)

Maximum 250 ppm.

To 1.0 g add 30 mL of *methanol R* and 20 mL of *dilute nitric acid R*. Heat on a water-bath for 15 min stirring frequently. Cool, dilute to 50 mL with *water R* and filter. Dilute 10 mL of the filtrate to 15 mL with *water R*.

Water-dispersible fraction

Maximum 3.0 per cent.

Place 0.400 g in a 500 mL separating funnel, add 100 mL of *water R* and shake for 1 min. Allow to stand for 1 h. Allow 90 mL of the aqueous phase to run out dropwise without filtration into a suitable dish dried at 140 °C and cooled in a desiccator. Evaporate to dryness at 140 °C, starting at a low temperature to avoid splashing. Cool in a desiccator. The residue weighs a maximum of 12 mg.

Loss on ignition

Maximum 6.0 per cent, determined on 0.200 g by ignition in a platinum crucible at 900 ± 50 °C for 2 h. It is advisable to place the crucible in a cold oven and then to heat up the oven. Allow to cool in a desiccator before weighing.

ASSAY

To the residue obtained in the test for loss on ignition add sufficient *ethanol (96 per cent) R* to moisten the residue completely and 0.2 mL of *sulfuric acid R*. Add 6 mL of *hydrofluoric acid R* and evaporate to dryness on a hot-plate at about 100 °C, taking care to avoid loss from sputtering. Wash down the sides of the platinum crucible with 6 mL of *hydrofluoric acid R* and evaporate to dryness. Ignite at 900 ± 50 °C, allow to cool in a desiccator and weigh.

The difference between the mass of the residue obtained in the test for loss on ignition and the mass of the final residue gives the amount of SiO_2 in the quantity of the substance to be examined.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory

part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristic may be relevant for hydrophobic colloidal silica used as glidant in tablets and capsules.

Specific surface area (*2.9.26, Method I*)
Determine the specific surface area in the P/P_0 range of 0.05 to 0.30.

Sample outgassing 20 min at 160 °C.

Ph Eur

Dental-type Silica

(Ph. Eur. monograph 1562)

Action and use
Excipient.

Ph Eur

DEFINITION
Amorphous silica (precipitated, gel or obtained by flame hydrolysis).

Content
94.0 per cent to 100.5 per cent of SiO_2 (ignited substance).

CHARACTERS

Appearance
White or almost white, light, fine, amorphous powder.

Solubility
Practically insoluble in water and in mineral acids.
It dissolves in hydrofluoric acid and hot solutions of alkali hydroxides.

IDENTIFICATION
About 20 mg gives the reaction of silicates (*2.3.1*).

TESTS

Solution S
To 2.5 g add 50 mL of *hydrochloric acid R* and mix. Heat on a water-bath for 30 min, stirring from time to time. Evaporate to dryness. Add to the residue a mixture of 8 mL of *dilute hydrochloric acid R* and 24 mL of *water R*. Heat to boiling and filter under reduced pressure through a sintered-glass filter (16) (*2.1.2*). Wash the residue on the filter with a hot mixture of 3 mL of *dilute hydrochloric acid R* and 9 mL of *water R*. Wash with small quantities of *water R*, combine the washings and the filtrate, and dilute to 50 mL with *water R*.

pH (*2.2.3*)
3.2 to 8.9.

Suspend 5 g in a mixture of 5 mL of a 7.46 g/L solution of *potassium chloride R* and 90 mL of *carbon dioxide-free water R*.

Chlorides
Liquid chromatography (*2.2.29*) as described in the test for sulfates.

Retention time Chlorides = about 4 min.

Limit:
— *chlorides*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.3 per cent).

Sulfates
Liquid chromatography (*2.2.29*).

Test solution To 0.625 g of the substance to be examined add 30 mL of *water R* and boil for 2 h. Allow to cool and quantitatively transfer to a 50 mL graduated flask. Dilute to 50.0 mL with *water R*. Dilute 5.0 mL of the supernatant to 50.0 mL with *water R* and filter through a membrane filter (nominal pore size 0.45 µm).

Reference solution Dissolve 0.50 g of *anhydrous sodium sulfate R* and 0.062 g of *sodium chloride R* in *water R* and dilute to 1000.0 mL with *water R*. Dilute 5.0 mL of the solution to 50.0 mL with *water R*.

Column:
— *material*: non-metallic;
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: suitable anion-exchange resin (30-50 µm).

Mobile phase Dissolve 0.508 g of *sodium carbonate R* and 0.05 g of *sodium hydrogen carbonate R* in *water R* and dilute to 1000 mL with the same solvent.

Flow rate 1.2 mL/min.

Detection Conductivity detector.

Injection 25 µL.

Retention time Sulfates = about 8 min.

Limit:
— *sulfates*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (4.0 per cent, expressed as sodium sulfate).

Iron (*2.4.9*)
Maximum 400 ppm.

Dilute 2 mL of solution S to 40 mL with *water R*.

Loss on ignition
Maximum 25.0 per cent, determined on 0.200 g by heating in a platinum crucible at 100-105 °C for 1 h and then at 1000 ± 50 °C for 2 h.

ASSAY
To the residue obtained in the test for loss on ignition add 0.2 mL of *sulfuric acid R* and a quantity of *ethanol (96 per cent) R* sufficient to moisten the residue completely. Add 6 mL of *hydrofluoric acid R* and evaporate to dryness at 95-105 °C, taking care to avoid loss from sputtering. Wash the inside of the crucible with 6 mL of *hydrofluoric acid R* and evaporate to dryness again. Ignite at 900 ± 50 °C, allow to cool in a desiccator and weigh. The difference between the mass of the final residue and that of the mass obtained in the test for loss on ignition corresponds to the mass of SiO_2 in the test sample.

FUNCTIONALITY-RELATED CHARACTERISTICS
This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are

recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristic may be relevant for dental type silica used as abrasive.

Specific surface area (*2.9.26, Method I*)
Determine the specific surface area in the P/P_0 range of 0.05 to 0.30.

Sample outgasing 60 min at 160 °C.

Ph Eur

Colloidal Silver

Colloidal Silver for External Use
(Ph. Eur. monograph 2281)

Action and use
Antibacterial.

Ph Eur

DEFINITION
Colloidal metallic silver containing protein.

Content
70.0 per cent to 80.0 per cent of Ag (dried substance).

CHARACTERS
Appearance
Green or bluish-black metallic shiny flakes or powder, hygroscopic.

Solubility
Freely soluble or soluble in water, practically insoluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION
A. To 5 mL of the filtrate obtained in the test for alkalinity (see Tests) add 0.05 mL of *copper sulfate solution R* and 1 mL of *dilute sodium hydroxide solution R*. Shake. A violet colour appears within 15 min.

B. To 1 mL of solution S (see Tests), add 2 mL of *sodium chloride solution R*. A precipitate is formed which dissolves in an excess of water.

C. Ignite 0.05 g of the substance to be examined. Dissolve the residue in 10 mL of *nitric acid R*. The filtrate gives the reaction of silver (*2.3.1*).

TESTS
Solution S
Dissolve 1.25 g of the substance to be examined in *carbon dioxide-free water R* and dilute to 50.0 mL with the same solvent. Allow to stand for 5 min then shake vigorously. Filter through a tared sintered-glass filter (16) (*2.1.2*) after 30 min. Keep the residue for the test for water-insoluble substances.

Alkalinity
To 40.0 mL of solution S add 10.0 mL of *0.05 M sulfuric acid* and 2.0 g of *anhydrous sodium sulfate R*. Shake and filter several times if necessary. To 25.0 mL of the clear and colourless solution add 0.1 mL of *phenolphthalein solution R*. Not less than 1.5 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

Silver ions
To 0.50 g of the substance to be examined add 5 mL of *anhydrous ethanol R*. Shake for 1 min, filter and add 2 mL of *hydrochloric acid R* to the filtrate. No precipitate is formed.

Sensitivity to electrolytes
Dissolve 0.1 g of the substance to be examined in 100 mL of *water R*. Transfer a part of the solution into a test tube. When viewed horizontally the solution appears clear and reddish-brown. When viewed vertically, the solution appears turbid with a greenish-brown fluorescence. To 5 mL of the solution add 5 mL of a 0.50 g/L solution of *sodium chloride R* and mix by shaking for 1 min. When viewed horizontally the solution remains clear and reddish-brown.

Water-insoluble substances
Maximum 1.0 per cent.

Wash the residue obtained on the filter during preparation of solution S 5 times with 10 mL of *water R*. Dry the filter to constant mass at 100-105 °C. The residue weighs a maximum of 12.5 mg.

Loss on drying (*2.2.32*)
Maximum 8.0 per cent, determined on 0.500 g by drying in an oven at 80 °C.

ASSAY
Ignite 0.200 g of the substance to be examined at 650 ± 50 °C until the residue is white. Allow to cool, add 10 mL of a mixture of equal volumes of *nitric acid R* and *water R* and boil for 1 min. Transfer the contents of the crucible to a flask and titrate with *0.1 M ammonium thiocyanate* using 50 mg of *ferric sulfate R* as indicator, until a reddish-brown colour appears.

1 mL of *0.1 M ammonium thiocyanate* is equivalent to 10.79 mg of Ag.

STORAGE
In an airtight container.

Ph Eur

Silver Nitrate

(Ph. Eur. monograph 0009)

$AgNO_3$ 169.9 *7761-88-8*

Action and use
Antiseptic.

Preparations
Silver Nitrate Eye Drops
Silver Nitrate Solution
Silver Nitrate Sterile Solution

Ph Eur

DEFINITION
Content
99.0 per cent to 100.5 per cent.

CHARACTERS
Appearance
White or almost white, crystalline powder or transparent, colourless crystals.

Solubility
Very soluble in water, soluble in ethanol (96 per cent).

IDENTIFICATION
A. 10 mg gives the reaction of nitrates (*2.3.1*).

B. 10 mg gives the reaction of silver (*2.3.1*).

TESTS

Solution S

Dissolve 2.0 g in *water R* and dilute to 50 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity

To 2 mL of solution S add 0.1 mL of *bromocresol green solution R*. The solution is blue. To 2 mL of solution S add 0.1 mL of *phenol red solution R*. The solution is yellow.

Foreign salts

Maximum 0.3 per cent.

To 30 mL of solution S, add 7.5 mL of *dilute hydrochloric acid R*, shake vigorously, heat for 5 min on a water-bath and filter. Evaporate 20 mL of the filtrate to dryness on a water-bath and dry at 100-105 °C. The residue weighs a maximum of 2 mg.

Aluminium, lead, copper and bismuth

Dissolve 1.0 g in a mixture of 4 mL of *concentrated ammonia R* and 6 mL of *water R*. The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

ASSAY

Dissolve 0.300 g in 50 mL of *water R*, add 2 mL of *dilute nitric acid R* and 2 mL of *ferric ammonium sulfate solution R2*. Titrate with *0.1 M ammonium thiocyanate* until a reddish-yellow colour is obtained.

1 mL of *0.1 M ammonium thiocyanate* is equivalent to 16.99 mg of $AgNO_3$.

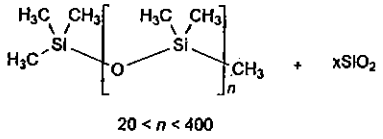
STORAGE

In a non-metallic container, protected from light.

Ph Eur

Simeticone

(Ph. Eur. monograph 1470)



8050-81-5

Action and use

Silicon dioxide analogue; defoaming agent.

Ph Eur

DEFINITION

Mixture of α-trimethylsilyl-ω-methylpoly[oxy(dimethylsilanediyl)] and silicon dioxide.

Simeticone is prepared by incorporation of 4 per cent to 7 per cent silica into poly(dimethylsiloxane) with a degree of polymerisation between 20 and 400.

Content

90.5 per cent to 99.0 per cent of poly(dimethylsiloxane).

PRODUCTION

Poly(dimethylsiloxane) is obtained by hydrolysis and polycondensation of dichlorodimethylsilane and chlorotrimethylsilane and the silica is modified at the surface by incorporation of methylsilyl groups.

CHARACTERS

Appearance

Viscous, greyish-white, opalescent liquid.

Solubility

Practically insoluble in water, very slightly soluble or practically insoluble in anhydrous ethanol, practically insoluble in methanol, partly miscible with ethyl acetate, with methylene chloride, with methyl ethyl ketone and with toluene.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Thin films between plates of *sodium chloride R*.

Absorption maxima At 2964 cm^{-1}, 2905 cm^{-1}, 1412 cm^{-1}, 1260 cm^{-1} and 1020 cm^{-1}.

B. Heat 0.5 g in a test-tube over a small flame until white fumes begin to appear. Invert the tube over a 2nd tube containing 1 mL of a 1 g/L solution of *chromotropic acid, sodium salt R* in *sulfuric acid R* so that the fumes reach the solution. Shake the 2nd tube for about 10 s and heat on a water-bath for 5 min. The solution is violet.

C. The residue obtained in the test for silica under Assay gives the reaction of silicates (*2.3.1*).

TESTS

Acidity

To 2.0 g add 25 mL of a mixture of equal volumes of *anhydrous ethanol R* and *ether R*, previously neutralised to 0.2 mL of *bromothymol blue solution R1*, and shake. Not more than 3.0 mL of *0.01 M sodium hydroxide* is required to change the colour of the solution to blue.

Defoaming activity

Foaming solution Dissolve 5.0 g of *docusate sodium R* in 1 L of *water R*, warm to 50 °C if necessary.

Defoaming solution To 50 mL of *methyl ethyl ketone R* add 0.250 g of the substance to be examined, warm to not more than 50 °C with shaking.

Into a 250 mL cylindrical tube about 5 cm in diameter introduce 100 mL of foaming solution and 1 mL of defoaming solution. Close tightly and fix the tube on a suitable oscillating shaker that complies with the following conditions:

— 250-300 oscillations per minute;
— angle of oscillation of about 10°;
— oscillation radius of about 10 cm.

Shake for 10 s and record the time between the end of the shaking and the instant the 1st portion of foam-free liquid surface appears.

This duration is not longer than 15 s.

Mineral oils

Place 2.0 g in a test-tube and examine in ultraviolet light at 365 nm. The fluorescence is not more intense than that of a solution containing 0.1 ppm of *quinine sulfate R* in *0.005 M sulfuric acid* examined in the same conditions.

Phenylated compounds

The corrected absorbance (*2.2.25*) is not greater than 0.2.

Test solution Dissolve 5.0 g with shaking in 10.0 mL of *cyclohexane R*.

Spectral range 200-350 nm.

Calculate the corrected absorbance using the following expression:

$$B - C$$

B = absorbance at the absorption maximum between 250 nm and 270 nm;
C = absorbance at 300 nm.

Volatile matter
Maximum 1.0 per cent, determined on 1.00 g by heating in an oven at 150 °C for 2 h. Carry out the test using a dish 60 mm in diameter and 10 mm deep.

ASSAY

Silica
Heat not less than 20.0 mg to 800 °C increasing the temperature by 20 °C/min under a current of *nitrogen R* at a flow rate of 200 mL/min and weigh the residue (silica).

Poly(dimethylsiloxane)
Infrared absorption spectrophotometry (*2.2.24*).

Test solution Place about 50 mg (*E*) in a screw-capped 125 mL cylindrical tube, add 25.0 mL of *toluene R*, swirl manually to disperse and add 50 mL of *dilute hydrochloric acid R*, close the tube and place on a vortex mixer; shake for 5 min. Transfer the contents of the tube to a separating funnel, allow to settle and transfer 5 mL of the upper layer to a screw-capped test-tube containing 0.5 g of *anhydrous sodium sulfate R*. Cap and shake vigorously manually. Centrifuge to obtain a clear solution.

Reference solution Introduce about 0.20 g of *dimeticone CRS* (poly(dimethylsiloxane)) into 100.0 mL of *toluene R*. Prepare the reference solution in the same way as for the test solution, using 25.0 mL of the dimeticone solution obtained above.

Blank solution Shake 10 mL of *toluene R* with 1 g of *anhydrous sodium sulfate R*. Centrifuge the resulting suspension.

Record the infrared absorption spectra for the test solution and the reference solution in 0.5 mm cells, from 1330 cm^{-1} to 1180 cm^{-1}. Determine the absorbance of the band at 1260 cm^{-1}.

Calculate the percentage content of poly(dimethylsiloxane) using the following expression:

$$\frac{25 \times C \times A_M \times 100}{A_E \times E}$$

A_M = absorbance of the test solution;
A_E = absorbance of the reference solution;
C = concentration of the reference solution, in milligrams per millilitre;
E = mass of the substance to be examined, in milligrams.

FUNCTIONALITY-RELATED CHARACTERISTICS
This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristic may be relevant for simeticone used as defoaming agent.

Defoaming activity
(see Tests).

Ph Eur

Simeticone for Oral Use

Action and use
Silicon dioxide analogue; defoaming agent.

Preparations
Simeticone Suspension for Infants

When activated dimethicone is prescribed or demanded, Simeticone for Oral Use shall be dispensed or supplied.

DEFINITION
Simeticone for Oral Use contains not less than 4.5% and not more than 8.0% w/w of silica, SiO_2, and not less than 91.0% and not more than 97.0% w/w of Dimeticone 1000. It is prepared by the addition of finely powdered silica to Dimeticone 1000.

CHARACTERISTICS
A viscous, grey, translucent liquid.

Immiscible with *water* and with *ethanol (96%)*; almost completely soluble in *toluene*, leaving a residue of silica.

IDENTIFICATION
A. The *infrared absorption spectrum* of the supernatant liquid obtained by centrifugation, Appendix II A, is concordant with the *reference spectrum* of dimeticone *(RS 381)*.

B. Ignite; dense white fumes are evolved leaving a white residue which is insoluble in *hydrochloric acid*.

TESTS

Acidity
Dissolve 15.0 g in a mixture of 15 mL of *toluene* and 15 mL of *butan-1-ol*, previously neutralised to a 0.5% w/v solution of *bromophenol blue* in *ethanol (96%)*, and titrate with *0.1M ethanolic potassium hydroxide VS* using the bromophenol blue solution as indicator. Not more than 0.15 mL is required to change the colour of the solution.

Refractive index
Of the supernatant liquid obtained in Identification test A, 1.4050 to 1.4080, Appendix V E.

Viscosity
Of the supernatant liquid obtained in Identification test A, 950 to 1050 $mm^2 s^{-1}$, Appendix V H, Method I, using a U-tube viscometer (size G).

Weight per mL
Of the supernatant liquid obtained in Identification test A, 0.965 to 0.980 g, Appendix V G.

ASSAY

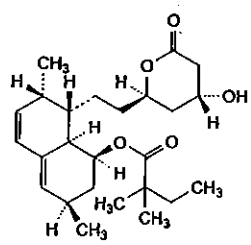
For silica
To 1 g add 50 mL of *toluene*, mix well and filter through an ignited silica crucible. Wash the residue thoroughly with *toluene*, dry at 105° and ignite to constant weight at 500°.

For dimeticone
Place 40 mg in a stoppered centrifuge tube, add 20 mL of *toluene* and shake for 20 minutes. Filter and record the *infrared absorption spectrum*, Appendix II A, of a 0.5-mm layer of the filtrate over the range 1330 to 1180 cm^{-1} (7.52 to 8.47 µm). Measure the absorbance of the CH_3Si stretching

band at the maximum at 1261 cm^{-1} (7.93 µm). Repeat the operation using *dimeticone EPCRS* in place of the preparation being examined.

Simvastatin

(Ph. Eur. monograph 1563)



$C_{25}H_{38}O_5$ 418.6 *79902-63-9*

Action and use
HMG Co-A reductase inhibitor; lipid-regulating drug.

Preparations
Simvastatin Tablets
Simvastatin Oral Suspension

Ph Eur

DEFINITION
(1*S*,3*R*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-Hydroxy-6-oxooxan-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate.

Content
97.0 per cent to 102.0 per cent (dried substance).

A suitable antioxidant may be added.

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, very soluble in methylene chloride, freely soluble in ethanol (96 per cent).

IDENTIFICATION
A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *simvastatin CRS.*

TESTS
Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_7 (*2.2.2, Method II*).

Dissolve 0.2 g in *methanol R* and dilute to 20 mL with the same solvent.

Specific optical rotation (*2.2.7*)
+ 285 to + 300 (dried substance).

Dissolve 0.125 g in *acetonitrile R* and dilute to 25.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*) *Prepare the solutions immediately before use.*

Solvent mixture *acetonitrile R, water R* (50:50 *V/V*).

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

Test solution (b) Dilute 1.0 mL of test solution (a) to 10.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 10.0 mg of *simvastatin CRS* in the solvent mixture and dilute to 5.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 2 mg of *simvastatin for system suitability CRS* (containing impurities A, B, C, D, E, F, G, I and J) in 1 mL of the solvent mixture.

Reference solution (d) Dissolve 4.0 mg of *simvastatin impurity K CRS* in the solvent mixture and dilute to 100.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 5.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.15 m, Ø = 2.1 mm;
— *stationary phase*: *end-capped extra-dense bonded octadecylsilyl silica gel for chromatography R* (3.5 µm);
— *temperature*: 35 °C.

Mobile phase:
— *mobile phase A*: *acetonitrile R1*, 0.1 per cent *V/V* solution of *phosphoric acid R* (40:60 *V/V*);
— *mobile phase B*: 0.1 per cent *V/V* solution of *phosphoric acid R, acetonitrile R1* (5:95 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 4	100	0
4 - 5	100 → 80	0 → 20
5 - 33	80 → 60	20 → 40
33 - 34	60 → 0	40 → 100
34 - 45	0	100

Flow rate 0.4 mL/min.

Detection Spectrophotometer at 238 nm and, for impurity K, at 200 nm.

Autosampler Set at 8 °C.

Injection 5 µL of test solution (a) and reference solutions (a), (c) and (d).

Identification of impurities Use the chromatogram supplied with *simvastatin for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, C, D, E, F, G, I and J; use the chromatogram obtained with reference solution (d) to identify the peak due to impurity K.

Relative retention With reference to simvastatin (retention time = about 19 min): impurity I = about 0.67; impurity A = about 0.69; impurity E = about 0.81; impurity F = about 0.83; impurity G = about 0.9; impurity K = about 1.2; impurity B = about 1.69; impurity J = about 1.74; impurity C = about 1.76; impurity D = about 2.3.

System suitability Reference solution (c):
— *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity F and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity E; minimum 1.5, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity J.

Calculation of percentage contents:
— for impurity K, use the concentration of impurity K in reference solution (d) and the peak areas recorded at 200 nm;
— for impurities other than K, use the concentration of simvastatin in reference solution (a) and the peak areas recorded at 238 nm.

Limits:
— *impurities E, F*: for each impurity, maximum 0.5 per cent;
— *sum of impurities A and I*: maximum 0.4 per cent;
— *impurity D*: maximum 0.4 per cent;
— *impurity K at 200 nm*: maximum 0.4 per cent;
— *impurities B, C*: for each impurity, maximum 0.3 per cent;
— *impurities G, J*: for each impurity, maximum 0.2 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 3.0 per cent;
— *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in a desiccator under high vacuum at 60 °C for 3 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Mobile phase:

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 14	70	30
14 - 15	70 → 0	30 → 100
15 - 20	0	100

Injection Test solution (b) and reference solution (b).
Calculate the percentage content of $C_{25}H_{38}O_5$ taking into account the assigned content of *simvastatin CRS*.

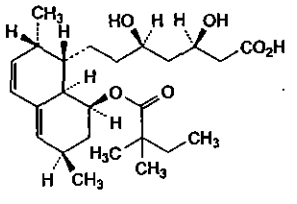
STORAGE

Protected from light. If no antioxidant is present, store under nitrogen, in an airtight container.

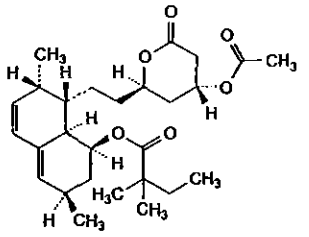
IMPURITIES

Specified impurities *A, B, C, D, E, F, G, I, J, K.*

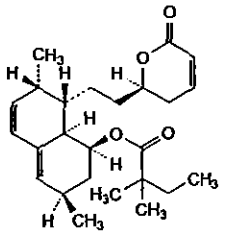
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *H, M, N.*



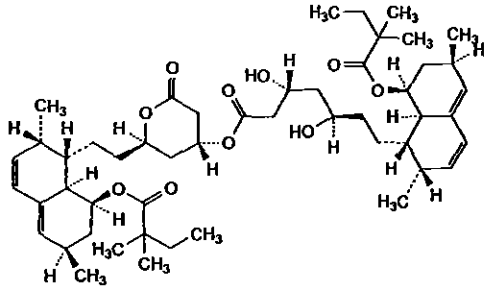
A. (3*R*,5*R*)-7-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-8-[(2,2-dimethylbutanoyl)oxy]-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]-3,5-dihydroxyheptanoic acid (tenivastatin),



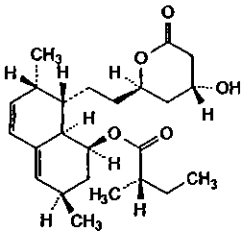
B. (1*S*,3*R*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-(acetyloxy)-6-oxooxan-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate,



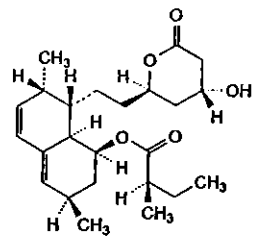
C. (1*S*,3*R*,7*S*,8*S*,8a*R*)-3,7-dimethyl-8-[2-[(2*R*)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl]ethyl]-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate,



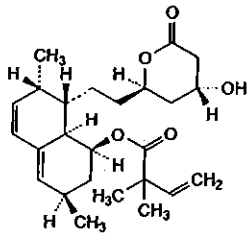
D. (2*R*,4*R*)-2-[2-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-8-[(2,2-dimethylbutanoyl)oxy]-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]ethyl]-6-oxooxan-4-yl (3*R*,5*R*)-7-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-8-[(2,2-dimethylbutanoyl)oxy]-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]-3,5-dihydroxyheptanoate,



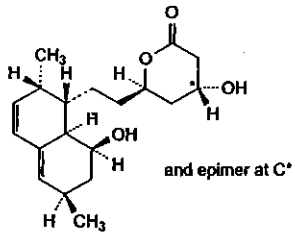
E. (1*S*,3*R*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-hydroxy-6-oxooxan-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl (2*S*)-2-methylbutanoate (lovastatin),



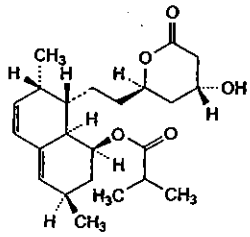
F. (1*S*,3*R*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-hydroxy-6-oxooxan-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl (2*R*)-2-methylbutanoate (epilovastatin),



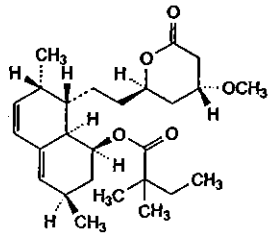
G. (1*S*,3*R*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-hydroxy-6-oxooxan-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbut-3-enoate,



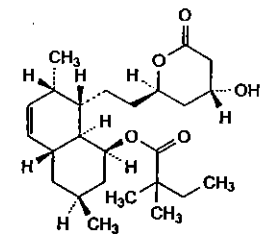
H. (4*RS*,6*R*)-4-hydroxy-6-[2-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-8-hydroxy-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]ethyl]oxan-2-one,



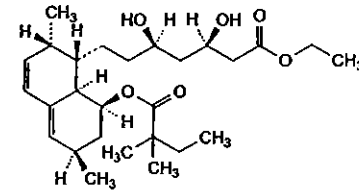
I. (1*S*,3*R*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-hydroxy-6-oxooxan-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2-methylpropanoate,



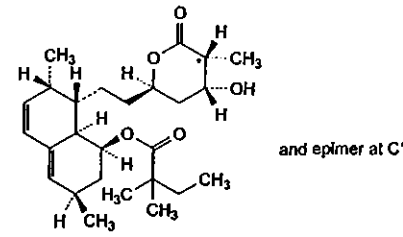
J. (1*S*,3*R*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-methoxy-6-oxooxan-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate,



K. (1*S*,3*S*,4a*R*,7*S*,8*S*,8a*S*)-8-[2-[(2*R*,4*R*)-4-hydroxy-6-oxooxan-2-yl]ethyl]-3,7-dimethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalen-1-yl 2,2-dimethylbutanoate,



M. ethyl (3*R*,5*R*)-7-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-8-[(2,2-dimethylbutanoyl)oxy]-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]-3,5-dihydroxyheptanoate,

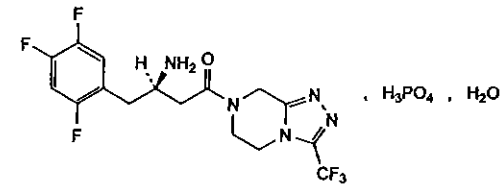


N. (1*S*,3*R*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*,5*RS*)-4-hydroxy-5-methyl-6-oxooxan-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate.

Ph Eur

Sitagliptin Phosphate Monohydrate

(Ph. Eur. monograph 2778)



$C_{16}H_{18}F_6N_5O_5P,H_2O$ 523.3 *654671-77-9*

Action and use
Dipeptidylpeptidase-4 inhibitor; treatment of diabetes mellitus.

Preparation
Sitagliptin Tablets

Ph Eur

DEFINITION
(3*R*)-3-Amino-1-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-(2,4,5-trifluorophenyl)butan-1-one phosphate monohydrate.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Soluble in water, very slightly soluble in anhydrous ethanol, practically insoluble in heptane.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *sitagliptin phosphate monohydrate CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

B. Enantiomeric purity (see Tests).

C. Dissolve 0.200 g in *water R* and dilute to 5.0 mL with the same solvent. The solution gives reaction (a) of phosphates (*2.3.1*).

TESTS

Enantiomeric purity
Liquid chromatography (*2.2.29*): use the normalisation procedure.

Solvent mixture *water R, methanol R* (10:90 *V/V*).

Test solution Dissolve 80 mg of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 8 mg of *sitagliptin containing impurity A CRS* in 1 mL of the solvent mixture.

Column:
— *size:* l = 0.25 m, Ø = 4.6 mm;
— *stationary phase:* *amylose derivative of silica gel for chiral separation R* (5 µm);
— *temperature:* 35 °C.

Mobile phase *water for chromatography R, diethylamine R, heptane R, anhydrous ethanol R* (1:1:400:600 *V/V/V/V*).

Flow rate 0.8 mL/min.

Detection Spectrophotometer at 268 nm.

Injection 10 µL.

Run time 1.6 times the retention time of sitagliptin.

Identification of impurities Use the chromatogram supplied with *sitagliptin containing impurity A CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity A.

Relative retention With reference to sitagliptin (retention time = about 15 min): impurity A = about 0.9.

System suitability Reference solution (b):
— *resolution*: minimum 1.5 between the peaks due to impurity A and sitagliptin.

Limit:
— *impurity A*: maximum 0.5 per cent;
— *reporting threshold*: 0.1 per cent (reference solution (a)).

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R*, 0.1 per cent *V/V* solution of *phosphoric acid R* (5:95 *V/V*).

Test solution Dissolve 25.0 mg of the substance to be examined in the solvent mixture and dilute to 250.0 mL with the solvent mixture.

Reference solution (a) Dissolve 25.0 mg of *sitagliptin phosphate monohydrate CRS* in the solvent mixture and dilute to 250.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) In order to prepare the fumarate adduct *in situ*, heat 1 mL of *water R*, 1 mg of *sodium stearyl fumarate R* and 10 mg of the substance to be examined in a tightly closed vial at 80 °C for about 30 h. Dilute to 100 mL with the solvent mixture and stir for 1 h. Centrifuge a portion of the solution and use the clear supernatant.

Column:
— *size:* l = 0.15 m, Ø = 4.6 mm;
— *stationary phase:* *end-capped cyanosilyl silica gel for chromatography R* (5 µm);
— *temperature:* 30 °C.

Mobile phase Mix 15 volumes of *acetonitrile R1* and 85 volumes of a 1.36 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 2.0 with *phosphoric acid R.*

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 205 nm.

Injection 20 µL of the test solution and reference solutions (b) and (c).

Run time 5.5 times the retention time of sitagliptin.

Relative retention With reference to sitagliptin (retention time = about 5.5 min): fumarate adduct = about 1.2.

System suitability Reference solution (c):
— *resolution*: minimum 1.5 between the peaks due to sitagliptin and the fumarate adduct.

Calculation of percentage contents:
— for each impurity, use the concentration of sitagliptin phosphate monohydrate in reference solution (b).

Limits:
— *unspecified impurities:* for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.5 per cent;
— *reporting threshold*: 0.05 per cent.

Water (*2.5.12*)
3.3 per cent to 3.7 per cent, determined on 0.300 g.

Sulfated ash (*2.4.14*)
Maximum 0.2 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (a).

Run time Twice the retention time of sitagliptin.

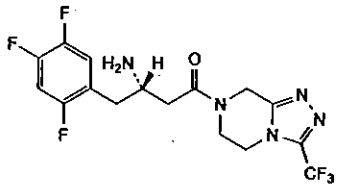
Calculate the percentage content of $C_{16}H_{18}F_6N_5O_5P$ taking into account the assigned content of *sitagliptin phosphate monohydrate CRS.*

IMPURITIES

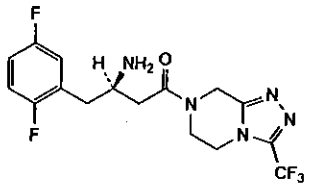
Specified impurities *A.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general

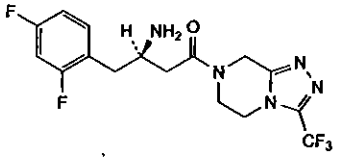
monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) B, C.



A. (3*S*)-3-amino-1-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-(2,4,5-trifluorophenyl)butan-1-one,



B. (3*R*)-3-amino-4-(2,5-difluorophenyl)-1-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]butan-1-one,



C. (3*R*)-3-amino-4-(2,4-difluorophenyl)-1-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]butan-1-one.

Ph Eur

Soft Soap

Preparation
Soap Spirit

DEFINITION
Soft Soap is soap made by the interaction of potassium hydroxide or sodium hydroxide with a suitable vegetable oil or oils or with fatty acids derived there from. It yields not less than 44.0% of fatty acids. It may be coloured with chlorophyll or not more than 0.015% of a suitable green soap dye.

CHARACTERISTICS
A yellowish white to green or brown, unctuous substance. Soluble in *water* and in *ethanol (96%)*.

TESTS

Chlorides and other ethanol-insoluble substances
Dissolve 5 g in 100 mL of hot *ethanol (96%)* previously neutralised to *phenolphthalein solution R1*, filter through a dried and tared filter, wash the residue thoroughly with hot neutralised *ethanol (96%)* and dry to constant weight at 105°. The residue weighs not more than 0.15 g.

Free fatty acid or alkali hydroxide
Boil 250 mL of *ethanol (96%)* to remove carbon dioxide, add 0.5 mL of *phenolphthalein solution R1*, allow to cool to 70° and neutralise, if necessary, with *0.1M sodium hydroxide VS* or *0.05M sulfuric acid VS*. To 100 mL of the neutral ethanol add 10 g of the substance being examined and dissolve it as quickly as possible by heating under a reflux condenser. Cool to 70° and, if the solution is not pink, titrate at 70° with *0.1M sodium hydroxide VS*; not more than 0.2 mL is required. If the solution is pink add, in a thin stream, 5 mL of hot *barium chloride solution* previously neutralised to *phenolphthalein solution R1*, mix thoroughly and titrate with *0.1M hydrochloric acid VS* until the pink colour disappears; not more than 1.0 mL is required.

Total free alkali
To 100 mL of the neutral ethanol prepared as described in the test for Free fatty acid or alkali hydroxide add 10 g of the substance being examined and dissolve it as quickly as possible by heating under a reflux condenser. Add immediately 3 mL of *0.5M sulfuric acid VS* and boil under a reflux condenser on a water bath for at least 10 minutes. If the solution is not pink, cool to 70° and titrate with *1M sodium hydroxide VS* until a pink colour is produced. The volume of *0.5M sulfuric acid VS* neutralised by the substance being examined is not more than 1.0 mL.

Unsaponifiable matter and unsaponified neutral fat
Dissolve 5 g in 80 mL of a mixture of 50 mL of *ethanol (96%)* and 100 mL of *water*, without heating more than is necessary, and transfer to a separating funnel, washing the vessel with the remaining 70 mL of the mixture. Extract with 100 mL of *ether* while still slightly warm, run off the ethanolic soap layer into a second separating funnel and extract with 50 mL of *ether*. Repeat the extraction with 50 mL of *ether* and pour the three ether extracts into a separating funnel containing 20 mL of *water*. Rotate the separating funnel without violent shaking and, after allowing the liquids to separate, run off the water. Repeat the washing with *water* in the same manner until the separated washings are not more than faintly turbid when acidified. Wash the ether solution twice by shaking vigorously with 20 mL of *0.5M potassium hydroxide*, each washing with alkali being immediately followed by washing with 20 mL of *water*, shaking vigorously each time. Acidify the last alkali washing after separation and, if the liquid becomes turbid, repeat the washing with *0.5M potassium hydroxide* and *water* until the alkali washing remains clear on acidification. Finally wash with successive 20 mL quantities of *water* until the washings do not give a pink colour with *phenolphthalein solution R1*. Transfer the ether solution to a tared flask and remove the ether. When nearly all the ether has evaporated, add 3 mL of *acetone*. With the aid of a gentle current of air remove the solvent completely from the flask, which is preferably almost entirely immersed in boiling *water*, held obliquely and rotated. Repeat the last operation until the weight of the residue is constant. The residue weighs not more than 40 mg.

CHARACTERISTICS OF THE FATTY ACIDS OBTAINED IN THE ASSAY

Acid value
Not more than 205, Appendix X B, when determined on 2 to 3 g of the fatty acids, using *0.5M potassium hydroxide VS* and substituting 2.805 for 5.610 in the formula.

Iodine value
Not less than 83 (*iodine monochloride method*), Appendix X E.

Solidifying point
Not above 31°, Appendix V B, with the following modifications. Where the determination is made at 15° to 20° the 1000 mL beaker and cooling liquid need not be used. Where the room temperature falls outside this range the 1000 mL beaker should contain water maintained at 15° to 20° and the level of this water should not be below the level of the sample in the inner tube.

Transfer about 15 mL of the melted fatty acids to the inner test tube. Before the temperature of the fatty acids drops to a point 10° above their expected solidifying point, begin agitation in a vertical manner at a rate of 100 complete up and down motions per minute, the stirrer moving through a vertical distance of about 38 mm. Continue stirring in this manner until the temperature has remained constant for 30 seconds or has begun to rise within 30 seconds of ceasing to fall. Discontinue stirring immediately and lift the stirrer out of the sample. Observe the rise in temperature; the highest temperature reached after cessation of stirring is the solidifying point of the fatty acids. When reading the thermometer avoid all undue vibration as this will cause the temperature to drop before reaching the maximum.

Resin
Mix 0.5 mL of the melted fatty acids in a test tube with 2 mL of *acetic anhydride*, warm, shake until clear and cool to 15.5°. Transfer one drop of this solution to a white porcelain tile, place one drop of a cold mixture of equal volumes of *sulfuric acid* and *water* adjacent to it and gently bring the drops together with a glass rod. No transient violet colour is produced.

ASSAY
Dissolve 30 g in 100 mL of *water*, transfer to a separating funnel, acidify with 1M *sulfuric acid* and extract with successive quantities of 50, 40 and 30 mL of *ether*. Mix the ether solutions in a separating funnel and wash with *water* until the washings are free from mineral acid. Transfer the ether solution to a tared flask, remove the ether and dry the residue of fatty acids to constant weight at 80°.

Soda Lime

8006-28-8

Action and use
Used to absorb carbon dioxide.

DEFINITION
Soda Lime is a mixture of sodium hydroxide, or sodium hydroxide and potassium hydroxide, with calcium hydroxide.

CHARACTERISTICS
White or greyish white granules, or it may be coloured with an indicator to show when its absorptive capacity is exhausted. It absorbs about 20% of its weight of carbon dioxide.

Partially soluble in *water*; almost completely soluble in 1M *acetic acid*.

IDENTIFICATION
A. When moistened with *hydrochloric acid* and introduced on a platinum wire into a flame, imparts a yellow colour to the flame.

B. A solution in 1M *acetic acid* yields reaction C characteristic of *calcium salts*, Appendix VI.

C. A suspension in *water* is strongly alkaline to *litmus paper*.

TESTS
Hardness of granules
Shake 200 g on a sieve no. 2000 for 3 minutes using a mechanical sieve shaker that reproduces in a uniform manner the circular and tapping motion given to sieves in manual use and has a frequency of oscillation of 282 to 288 cycles per minute. Place 50 g of the retained material in a hardness pan 20 cm in diameter having a concave brass bottom, 7.9 mm thick at the circumference, 3.2 mm thick at the centre and with an inside spherical radius of curvature of 109 cm. Add 15 steel balls, 7.9 mm in diameter, and shake on the mechanical sieve shaker for 30 minutes. Remove the steel balls, transfer the contents of the pan to a sieve no. 2000 and again shake on the mechanical sieve shaker for 3 minutes. The material retained by the sieve weighs not less than 37.5 g.

Size of granules
Shake 500 g on a perforated plate of nominal pore size 6.70 mm; not more than 5 g is retained. Then shake on a sieve no. 4750; not more than 50 g is retained. Shake the unretained material on a sieve no. 1400; not more than 20 g passes through. Shake the unretained material on a sieve no. 600; not more than 7.5 g passes through.

Loss on drying
When dried to constant weight at 105°, loses 14.0 to 21.0% of its weight. Use 1 g.

Moisture absorption
Place 10 g in an open glass dish about 50 mm in diameter and 30 mm high in a desiccator over *sulfuric acid (14%)* and allow it to remain for 24 hours. The increase in weight is not more than 7.5%.

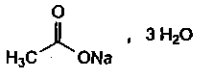
Carbon dioxide absorption
The activity is not less than 120 minutes when determined by the following method.

Use a vertically-clamped tube of glass or other suitable transparent material about 25 cm long and 29 to 31 mm internal diameter with closely fitting rubber bungs at each end; the bungs are bored to receive polythene or glass tubing of about 8 mm external diameter, the tubing being flush with the inner ends of the bungs. With the lower bung in position, place sufficient nylon mesh support on top of the bung to produce a bed of mesh about 10 cm deep and press a closely fitting disc of stainless steel gauze of nominal mesh aperture about 500 µm on top of the nylon mesh so that its surface is at right angles to the axis of the tube. Introduce 59.8 to 60.2 g of the substance being examined onto the steel gauze in three portions, tamping lightly after the addition of each portion. Place a second disc of steel gauze on top, followed by a sufficient quantity of nylon mesh such that the soda lime is kept consolidated by slight pressure when the second bung has been inserted. The exit tube is connected to a condenser, consisting of two 50 mL separating funnels, leading to a drying tube packed with *anhydrous calcium chloride* and then to a carbon dioxide analyser sufficiently sensitive to detect 0.2% v/v of carbon dioxide.
A katharometer, calibrated for carbon dioxide and preferably used in conjunction with a chart recorder, is suitable.

Using the gas analyser in accordance with the manufacturer's instructions, accurately determine the carbon dioxide content, p, as a percentage v/v, of a nominal 5% carbon dioxide mixture, the balance gas being oxygen, air or nitrogen as appropriate to the type of gas analyser being used. Suitable compressed gas mixtures are available commercially. Assemble the apparatus described above and pass the gas mixture downwards into the absorption tube at a rate of 900 cm^3 per minute until such time that the gas analyser shows the content of carbon dioxide in the effluent gas to have risen to 0.2% v/v. Steps should be taken to vent the effluent gas if an oxygen-carbon dioxide mixture is being used. Record the time taken, t, in minutes. The *activity* of the soda lime is given, in minutes, by the expression $tp/5$.

Sodium Acetate Trihydrate

(Ph. Eur. monograph 0411)



$C_2H_3NaO_2,3H_2O$ 136.1 *6131-90-4*

Action and use
Used in solutions for dialysis; excipient.

Preparation
Sodium Acetate Sterile Concentrate

Ph Eur

DEFINITION
Sodium acetate trihydrate (sodium ethanoate trihydrate).

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder or colourless crystals.

Solubility
Very soluble in water, soluble in ethanol (96 per cent).

IDENTIFICATION
A. 1 mL of solution S (see Tests) gives reaction (b) of acetates (*2.3.1*).

B. 1 mL of solution S gives reaction (a) of sodium (*2.3.1*).

C. Loss on drying (see Tests).

TESTS
Solution S
Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)
7.5 to 9.0.

Dilute 5 mL of solution S to 10 mL with *carbon dioxide-free water R*.

Reducing substances
Dissolve 5.0 g in 50 mL of *water R*, then add 5 mL of *dilute sulfuric acid R* and 0.5 mL of a 0.32 g/L solution of *potassium permanganate R*. The pink colour persists for at least 1 h. Prepare a blank in the same manner but without the substance to be examined.

Chlorides (*2.4.4*)
Maximum 200 ppm.

Dilute 2.5 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 200 ppm.

Dilute 7.5 mL of solution S to 15 mL with *distilled water R*.

Aluminium (*2.4.17*)
Maximum 0.2 ppm, if intended for use in the manufacture of dialysis solutions.

Prescribed solution Dissolve 20 g in 100 mL of *water R* and adjust to pH 6.0 with a 103 g/L solution of *hydrochloric acid R* (about 10 mL).

Reference solution Mix 2 mL of *aluminium standard solution (2 ppm Al) R*, 10 mL of *acetate buffer solution pH 6.0 R* and 98 mL of *water R*.

Blank solution Mix 10 mL of *acetate buffer solution pH 6.0 R* and 100 mL of *water R*.

Calcium and magnesium
Maximum 50 ppm, calculated as Ca.

To 200 mL of *water R* add 10 mL of *ammonium chloride buffer solution pH 10.0 R*, 0.1 g of *mordant black 11 triturate R*, 2.0 mL of *0.05 M zinc chloride* and, dropwise, *0.02 M sodium edetate* until the colour changes from violet to blue. Add to the solution 10.0 g of the substance to be examined and shake to dissolve. Titrate with *0.02 M sodium edetate* until the blue colour is restored. Not more than 0.65 mL of *0.02 M sodium edetate* is required.

Iron (*2.4.9*)
Maximum 10 ppm, determined on solution S.

Loss on drying (*2.2.32*)
39.0 per cent to 40.5 per cent, determined on 1.000 g by drying in an oven at 130 °C. Introduce the substance to be examined into the oven while the latter is cold.

ASSAY
Dissolve 0.250 g in 50 mL of *anhydrous acetic acid R*, add 5 mL of *acetic anhydride R*, mix and allow to stand for 30 min. Using 0.3 mL of *naphtholbenzein solution R* as indicator, titrate with *0.1 M perchloric acid* until a green colour is obtained.

1 mL of *0.1 M perchloric acid* is equivalent to 8.20 mg of $C_2H_3NaO_2$.

STORAGE
In an airtight container.

LABELLING
The label states, where applicable, that the substance is suitable for use in the manufacture of dialysis solutions.

Ph Eur

Sodium Acid Citrate

Disodium Hydrogen Citrate

$C_6H_6Na_2O_7,1½H_2O$ 263.1 *144-33-2*

Action and use
Anticoagulant.

DEFINITION
Sodium Acid Citrate contains not less than 98.0% and not more than 104.0% of $C_6H_6Na_2O_7,1½\ H_2O$.

CHARACTERISTICS
A white powder.

Freely soluble in *water*; practically insoluble in *ethanol (96%)*.

IDENTIFICATION
Yields the reactions characteristic of *sodium salts* and of *citrates*, Appendix VI.

TESTS
Acidity
pH of a 3% w/v solution, 4.9 to 5.2, Appendix V L.

Arsenic
0.50 g dissolved in 25 mL of *water* complies with the *limit test for arsenic*, Appendix VII (2 ppm).

Chloride
Dissolve 1.0 g in 100 mL of *water*. 15 mL of the resulting solution complies with the *limit test for chlorides*, Appendix VII (330 ppm).

Oxalate
Dissolve 1.0 g in 4 mL of *water*, add 3 mL of *hydrochloric acid* and 1 g of granulated *zinc* and heat on a water bath for 1 minute. Allow to stand for 2 minutes, decant the liquid into a test tube containing 0.25 mL of a 1% w/v solution of *phenylhydrazine hydrochloride* and heat to boiling. Cool rapidly, transfer to a graduated measuring cylinder, add an equal volume of *hydrochloric acid* and 0.25 mL of *potassium hexacyanoferrate(III) solution*, shake and allow to stand for 30 minutes. Any red colour produced is not more intense than that produced by treating in the same manner 4 mL of a 0.005% w/v solution of *oxalic acid* (150 ppm, calculated as anhydrous oxalic acid).

Sulfate
Dissolve 0.50 g in 57 mL of *water* and add 3 mL of 2M *hydrochloric acid*. 15 mL of the resulting solution complies with the *limit test for sulfates*, Appendix VII (0.12%).

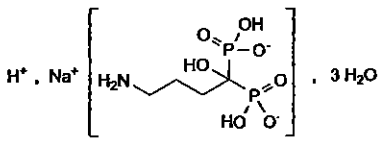
Readily carbonisable substances
Heat 1.0 g, in powder, with 10 mL of *sulfuric acid* for 30 minutes in a water bath protected from light. Not more than a pale brown colour is produced.

ASSAY
Heat 2 g until carbonised, cool and boil the residue with 50 mL each of *water* and *0.5M hydrochloric acid VS*. Filter, wash the filter with *water* and titrate the excess of acid in the filtrate and washings with *0.5M sodium hydroxide VS* using *methyl orange solution* as indicator. Each mL of *0.5M hydrochloric acid VS* is equivalent to 65.78 mg of $C_6H_6Na_2O_7,1½H_2O$.

Sodium Alendronate Trihydrate

Sodium Alendronate

(Ph. Eur. monograph 1564)



$C_4H_{12}NNaO_7P_2,3H_2O$ 325.1 *121268-17-5*

Preparation
Alendronic Acid and Colecalciferol Tablets

Ph Eur

DEFINITION
Monosodium trihydrogen (4-amino-1-hydroxybutylidene) bisphosphonate trihydrate.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Soluble in water, practically insoluble in methanol and in methylene chloride.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *sodium alendronate trihydrate CRS*.
B. Loss on drying (see Tests).
C. It gives reaction (a) of sodium (*2.3.1*).

TESTS
Solution S
Dissolve 0.5 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50 mL with the same solvent.

pH (*2.2.3*)
4.0 to 5.0 for solution S.

Related substances
Liquid chromatography (*2.2.29*): use the normalisation procedure.

Solution A Dissolve 29.4 g of *sodium citrate R* in *water R* and dilute to 1.0 L with the same solvent.

Solution B Dissolve 19.1 g of *disodium tetraborate R* in *water R* and dilute to 1.0 L with the same solvent.

Solution C *Prepare immediately before use.* Dissolve 0.200 g of *(9-fluorenyl)methyl chloroformate R* in *acetonitrile R* and dilute to 50.0 mL with the same solvent.

Buffer solution Dissolve 2.84 g of *anhydrous disodium hydrogen phosphate R* and 5.88 g of *sodium citrate R* in 1.9 L of *water R*, adjust to pH 8.0 with either *phosphoric acid R* or a 42 g/L solution of *sodium hydroxide R* and dilute to 2.0 L with *water R*.

Test solution Dissolve 30 mg of the substance to be examined in solution A and dilute to 50 mL with solution A. Transfer 5 mL of the solution to a 50 mL polypropylene screw-cap centrifuge tube containing 5 mL of solution B. Add 5 mL of *acetonitrile R* and 5 mL of solution C. Shake for 45 s and allow to stand at room temperature for 30 min. Add 20 mL of *methylene chloride R* and shake vigorously for 1 min. Centrifuge for 5-10 min and use the clear upper layer.

Reference solution (a) Dissolve 15 mg of *4-aminobutanoic acid R* (impurity A) in solution A and dilute to 100 mL with solution A. Dilute 10 mL of the solution to 50 mL with solution A. Take 5 mL of this solution and proceed as described for the test solution, starting from 'to a 50 mL polypropylene screw-cap centrifuge tube'.

Reference solution (b) Dissolve 3 mg of *sodium alendronate for system suitability CRS* (containing impurity D) in solution A and dilute to 5 mL with solution A. Take this solution and proceed as described for the test solution, starting from 'to a 50 mL polypropylene screw-cap centrifuge tube'.

Blank solution Take 5 mL of solution A and proceed as described for the test solution, starting from 'to a 50 mL polypropylene screw-cap centrifuge tube'.

Column:
— *size*: l = 0.25 m, Ø = 4.1 mm;
— *stationary phase*: *styrene-divinylbenzene copolymer R* (10 µm);
— *temperature*: 45 °C.

Mobile phase:
— *mobile phase A*: *acetonitrile R*, buffer solution (15:85 *V/V*);
— *mobile phase B*: buffer solution, *acetonitrile R* (30:70 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	100	0
3 - 18	100 → 50	0 → 50
18 - 28	50 → 0	50 → 100

Flow rate 1.8 mL/min.

Detection Spectrophotometer at 266 nm.

Injection 20 µL of the test solution, reference solutions (a), (b) and the blank solution.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity A; use the chromatogram supplied with *sodium alendronate for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity D.

Relative retention With reference to alendronate (retention time = about 7 min): impurity D = about 1.4; impurity A = about 1.9.

System suitability Reference solution (b):
— *resolution*: minimum 5.0 between the peaks due to alendronate and impurity D.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity A by 0.4;
— *impurity A*: maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.5 per cent;
— *reporting threshold*: 0.05 per cent.

Impurities B and C

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 25.0 mL with the same solvent.

Reference solution (a) Dissolve 50.0 mg of *sodium alendronate trihydrate CRS* in *water R* and dilute to 25.0 mL with the same solvent.

Reference solution (b) Dissolve 3.0 g of *phosphoric acid R* in *water R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of the solution to 100.0 mL with *water R*.

Reference solution (c) Dissolve 2.5 g of *phosphorous acid R* in *water R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of the solution to 100.0 mL with *water R*.

Reference solution (d) Mix 2.0 mL of reference solution (b) with 2.0 mL of reference solution (c) and dilute to 50.0 mL with *water R*.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *anion-exchange resin R1* (7 µm);
— *temperature*: 35 °C.

Mobile phase Mix 0.2 mL of *anhydrous formic acid R* with 1 L of *water R*; adjust to pH 3.5 with *2 M sodium hydroxide R*.

Flow rate 1.2 mL/min.

Detection Differential refractometer.

Injection 100 µL of the test solution and reference solutions (b), (c) and (d).

Run time Twice the retention time of alendronate.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity B; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity C.

Relative retention With reference to alendronate (retention time = about 16 min): impurity B = about 1.3; impurity C = about 1.6.

Limits:
— *impurities B, C*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.5 per cent).

Loss on drying (*2.2.32*)

16.1 per cent to 17.1 per cent, determined on 1.000 g by drying in an oven at 140-145 °C.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for impurities B and C with the following modification.

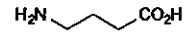
Injection Test solution and reference solution (a).

Calculate the percentage content of $C_4H_{12}NNaO_7P_2$ taking into account the assigned content of *sodium alendronate trihydrate CRS*.

IMPURITIES

Specified impurities *A, B, C.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *D.*



A. 4-aminobutanoic acid (γ-aminobutyric acid),
B. phosphate,
C. phosphite,
D. unknown structure.

Ph Eur

Sodium Alginate

(Ph. Eur. monograph 0625)

Action and use

Excipient.

Preparations

Alginate Raft-forming Oral Suspension

Compound Alginate Antacid Oral Suspension

Ph Eur

DEFINITION

Sodium alginate consists mainly of the sodium salt of alginic acid, which is a mixture of polyuronic acids $[(C_6H_8O_6)_n]$ composed of units of D-mannuronic acid and L-guluronic acid. Sodium alginate is obtained mainly from algae belonging to the Phaeophyceae.

CHARACTERS

Appearance

White or pale yellowish-brown powder.

Solubility

Slowly soluble in water forming a viscous, colloidal solution, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Dissolve 0.2 g with shaking in 20 mL of *water R*. To 5 mL of this solution add 1 mL of *calcium chloride solution R*. A voluminous gelatinous mass is formed.

B. To 10 mL of the solution prepared in identification test A add 1 mL of *dilute sulfuric acid R*. A gelatinous mass is formed.

C. To 5 mg add 5 mL of *water R*, 1 mL of a freshly prepared 10 g/L solution of *1,3-dihydroxynaphthalene R* in *ethanol (96 per cent) R* and 5 mL of *hydrochloric acid R*. Boil for 3 min, cool, add 5 mL of *water R*, and shake with 15 mL of *di-isopropyl ether R*. Carry out a blank test. The upper layer obtained with the substance to be examined exhibits a deeper bluish-red colour than that obtained with the blank.

D. Sulfated ash (see Tests). The residue obtained, dissolved in 2 mL of *water R*, gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S

Dissolve 0.10 g in *water R* with constant stirring, dilute to 30 mL with the same solvent and allow to stand for 1 h.

Appearance of solution

The solution is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than intensity 6 of the range of reference solutions of the most appropriate colour (*2.2.2, Method II*).

Dilute 1 mL of solution S to 10 mL with *water R*.

Chlorides

Maximum 1.0 per cent.

To 2.50 g add 50 mL of *dilute nitric acid R*, shake for 1 h and dilute to 100.0 mL with *dilute nitric acid R*. Filter. To 50.0 mL of the filtrate add 10.0 mL of *0.1 M silver nitrate* and 5 mL of *toluene R*. Titrate with *0.1 M ammonium thiocyanate*, using 2 mL of *ferric ammonium sulfate solution R2* as indicator and shaking vigorously towards the end point.

1 mL of *0.1 M silver nitrate* is equivalent to 3.545 mg of Cl.

Calcium

Maximum 1.5 per cent.

Atomic absorption spectrometry (*2.2.23, Method II*).

Test solution Dissolve 0.10 g in 50 mL of *dilute ammonia R2*, heating on a water-bath. Allow to cool and dilute to 100.0 mL with *distilled water R* (solution (a)). Dilute 3.0 mL of solution (a) to 100.0 mL with *distilled water R*.

Reference solutions Prepare 3 reference solutions in the same manner as the test solution but add 0.75 mL, 1.0 mL and 1.5 mL respectively of *calcium standard solution (100 ppm Ca) R* to the 3.0 mL of solution (a).

Set the zero of the instrument using a mixture of 1.5 volumes of *dilute ammonia R2* and 98.5 volumes of *distilled water R*.

Source Calcium hollow-cathode lamp.

Wavelength 422.7 nm.

Atomisation device Air-acetylene flame.

Loss on drying (*2.2.32*)

Maximum 15.0 per cent, determined on 0.1000 g by drying in an oven at 105 °C for 4 h.

Sulfated ash (*2.4.14*)

30.0 per cent to 36.0 per cent (dried substance), determined on 0.1000 g.

Microbial contamination

TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*).

TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

Absence of *Escherichia coli* (*2.6.13*).

Absence of *Salmonella* (*2.6.13*).

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristic may be relevant for sodium alginate used as viscosity-increasing agent or binder.

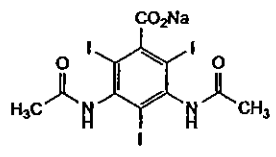
Apparent viscosity (*2.2.10*)

Carry out the test on a 10 g/L solution (dried substance). Determine the dynamic viscosity at 20 °C using a rotating viscometer at 20 r/min.

Ph Eur

Sodium Amidotrizoate

(Ph. Eur. monograph 1150)



$C_{11}H_8I_3N_2NaO_4$ 636 *737-31-5*

Action and use

Iodinated contrast medium.

Ph Eur

DEFINITION

Sodium amidotrizoate contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of sodium 3,5-bis(acetylamino)-2,4,6-tri-iodobenzoate, calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white powder, freely soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in acetone.

It melts at about 261 °C with decomposition.

IDENTIFICATION

First identification: A, D.

Second identification: B, C, D.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *sodium amidotrizoate CRS*. Dry both the substance to be examined and the reference substance at 100 °C to 105 °C for 3 h.

B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (b).

C. Heat 50 mg gently in a small porcelain dish over a naked flame. Violet vapour is evolved.

D. It gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S

Dissolve 10 g in *carbon dioxide-free water R* and dilute to 20 mL with the same solvent.

Appearance of solution

Dilute 1 mL of solution S to 10 mL with *water R*. The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)

The pH of solution S is 7.5 to 9.5.

Related substances

Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel GF_{254} plate R. Prepare the solutions in subdued light and develop the chromatograms protected from light.*

Test solution (a) Dissolve 0.50 g of the substance to be examined in a 3 per cent *V/V* solution of *ammonia R* in *methanol R* and dilute to 10 mL with the same solution.

Test solution (b) Dilute 1 mL of test solution (a) to 10 mL with a 3 per cent *V/V* solution of *ammonia R* in *methanol R*.

Reference solution (a) Dilute 1 mL of test solution (b) to 50 mL with a 3 per cent *V/V* solution of *ammonia R* in *methanol R*.

Reference solution (b) Dissolve 50 mg of *sodium amidotrizoate CRS* in a 3 per cent *V/V* solution of *ammonia R* in *methanol R* and dilute to 10 mL with the same solution.

Apply separately to the plate 2 µL of each solution. Develop over a path of 15 cm using a mixture of 20 volumes of *anhydrous formic acid R*, 25 volumes of *methyl ethyl ketone R* and 60 volumes of *toluene R*. Allow the plate to dry until the solvents have evaporated and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.2 per cent).

Free aromatic amines

Maintain the solutions and reagents in iced water, protected from light To 0.50 g in a 50 mL volumetric flask add 15 mL of *water R*. Shake and add 1 mL of *dilute sodium hydroxide solution R*. Cool in iced water, add 5 mL of a freshly prepared 5 g/L solution of *sodium nitrite R* and 12 mL of *dilute hydrochloric acid R*. Shake gently and allow to stand for exactly 2 min after adding the hydrochloric acid. Add 10 mL of a 20 g/L solution of *ammonium sulfamate R*. Allow to stand for 5 min, shaking frequently, and add 0.15 mL of a 100 g/L solution of *α-naphthol R* in *ethanol (96 per cent) R*. Shake and allow to stand for 5 min. Add 3.5 mL of *buffer solution pH 10.9 R*, mix and dilute to 50.0 mL with *water R*. The absorbance (*2.2.25*), measured within 20 min at 485 nm using as the compensation liquid a solution prepared at the same time and in the same manner but omitting the substance to be examined, is not greater than 0.30.

Free iodine and iodides

Not more than 50 ppm. Dissolve 1.0 g in *distilled water R* and dilute to 10 mL with the same solvent. Add dropwise *dilute nitric acid R* until the precipitation is complete, then add 3 mL of *dilute nitric acid R*. Filter and wash the precipitate with 5 mL of *water R*. Collect the filtrate and washings. Add 1 mL of *strong hydrogen peroxide solution R* and 1 mL of *methylene chloride R*. Shake. The lower layer is not more intensely coloured than a reference solution prepared simultaneously and in the same manner, using a mixture of 5 mL of *iodide standard solution (10 ppm I) R*, 3 mL of *dilute nitric acid R* and 15 mL of *water R*.

Water (*2.5.12*)

Not more than 11.0 per cent, determined on 0.400 g by the semi-micro determination of water.

ASSAY

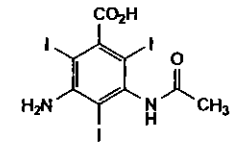
To 0.150 g in a 250 mL round-bottomed flask add 5 mL of *strong sodium hydroxide solution R*, 20 mL of *water R*, 1 g of *zinc powder R* and a few glass beads. Boil under a reflux condenser for 30 min. Allow to cool and rinse the condenser with 20 mL of *water R*, adding the rinsings to the flask. Filter through a sintered-glass filter (*2.1.2*) and wash the filter with several quantities of *water R*. Collect the filtrate and washings. Add 40 mL of *dilute sulfuric acid R* and titrate immediately with *0.1 M silver nitrate*. Determine the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M silver nitrate* is equivalent to 21.20 mg of $C_{11}H_8I_3N_2NaO_4$.

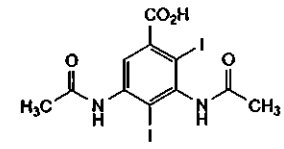
STORAGE

Store protected from light.

IMPURITIES



A. 3-acetylamino-5-amino-2,4,6-tri-iodobenzoic acid,

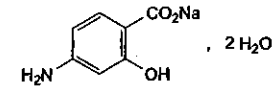


B. 3,5-bis(acetylamino)-2,4-di-iodobenzoic acid.

Ph Eur

Sodium Aminosalicylate Dihydrate

(Ph. Eur. monograph 1993)



$C_7H_6NNaO_3,2H_2O$ 211.1 *6018-19-5*

Action and use

Antituberculosis drug.

Ph Eur

DEFINITION

Sodium 4-amino-2-hydroxybenzoate dihydrate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or crystals, slightly hygroscopic.

Solubility

Freely soluble in water, sparingly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

First identification: A, E.

Second identification: B, C, D, E.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *sodium aminosalicylate dihydrate CRS.*

B. Introduce 0.3 g into a porcelain crucible. Cautiously heat on a small flame until vapour is evolved. Cover the crucible with a watch glass and collect the white sublimate. The melting point (*2.2.14*) of the sublimate is 120 °C to 124 °C.

C. To 0.1 mL of solution S (see Tests) add 5 mL of *water R* and 0.1 mL of *ferric chloride solution R1*. A reddish-brown colour develops.

D. 2 mL of solution S gives the reaction of primary aromatic amines (*2.3.1*).

E. 0.5 mL of solution S gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S

Dissolve 0.50 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent.

Appearance of solution

The freshly prepared solution is clear (*2.2.1*) and not more intensely coloured than reference solution B_5 (*2.2.2, Method II*).

Dissolve 2.5 g in *water R* and dilute to 25 mL with the same solvent.

pH (*2.2.3*)

6.5 to 8.5 for solution S.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 50.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *water R*. Dilute 1.0 mL of this solution to 20.0 mL with *water R*.

Reference solution (b) Dissolve 5 mg of *3-aminophenol R* (impurity A) and 5 mg of *mesalazine R* (impurity B) in *water R* and dilute to 100 mL with the same solvent. Dilute 1 mL of the solution to 5 mL with *water R*.

Column:
- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: base-deactivated end-capped octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A*: dissolve 2.2 g of *perchloric acid R* and 1.0 g of *phosphoric acid R* in *water for chromatography R* and dilute to 1000 mL with the same solvent;
- *mobile phase B*: dissolve 1.7 g of *perchloric acid R* and 1.0 g of *phosphoric acid R* in *acetonitrile for chromatography R* and dilute to 1000 mL with the same solvent;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	100	0
15 - 30	100 → 40	0 → 60

Flow rate 1.25 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µL.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and B.

Relative retention With reference to 4-aminosalicylate (retention time = about 17 min): impurity A = about 0.31; impurity B = about 0.39.

System suitability Reference solution (b):
- *resolution*: minimum 4.0 between the peaks due to impurities A and B.

Calculation of percentage contents:
- *correction factor*: multiply the peak area of impurity A by 0.62;
- for each impurity, use the concentration of sodium aminosalicylate dihydrate in reference solution (a).

Limits:
- *impurity A*: maximum 0.15 per cent;
- *unspecified impurities*: for each impurity, maximum 0.05 per cent;
- *total*: maximum 0.2 per cent;
- *reporting threshold*: 0.03 per cent.

Loss on drying (*2.2.32*)

16.0 per cent to 17.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.150 g in 20 mL of *water R*. Add 10 mL of a 500 g/L solution of *sodium bromide R* and 25 mL of *glacial acetic acid R*. Add 5 mL of *0.1 M sodium nitrite* rapidly and continue the titration with the same titrant, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M sodium nitrite* is equivalent to 17.51 mg of $C_7H_6NNaO_3$.

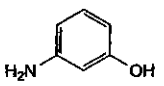
STORAGE

In an airtight container, protected from light. If the substance is sterile, the container is also sterile and tamper-evident.

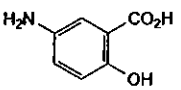
IMPURITIES

Specified impurities *A.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B.*



A. 3-aminophenol,

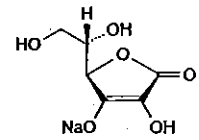


B. 5-amino-2-hydroxybenzoic acid (mesalazine).

Ph Eur

Sodium Ascorbate

(Ph. Eur. monograph 1791)



$C_6H_7NaO_6$ 198.1 *134-03-2*

Action and use
Excipient.

Ph Eur

DEFINITION

Sodium (2*R*)-2-[(1*S*)-1,2-dihydroxyethyl]-4-hydroxy-5-oxo-2,5-dihydrofuran-3-olate.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or yellowish, crystalline powder or crystals.

Solubility
Freely soluble in water, sparingly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *sodium ascorbate CRS.*

C. To 1 mL of solution S (see Tests) add 0.2 mL of *dilute nitric acid R* and 0.2 mL of *silver nitrate solution R2*. A grey precipitate is formed.

D. 1 mL of solution S gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S
Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 or BY_6 (*2.2.2, Method II*); examine the colour immediately after preparation of the solution.

pH (*2.2.3*)
7.0 to 8.0 determined on freshly prepared solution S.

Specific optical rotation (*2.2.7*)
+ 103 to + 108 (dried substance), determined on freshly prepared solution S.

Impurity E
Maximum 0.2 per cent.

Test solution Dissolve 0.25 g in 5 mL of *water R*. Add 1 mL of *dilute acetic acid R* and 0.5 mL of *calcium chloride solution R*.

Reference solution Dissolve 70 mg of *oxalic acid R* (dihydrate of impurity E) in *water R* and dilute to 500 mL with the same solvent; to 5 mL of the solution add 1 mL of *dilute acetic acid R* and 0.5 mL of *calcium chloride solution R*.

Allow the solutions to stand for 1 h. Any opalescence in the test solution is not more intense than that in the reference solution.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Phosphate buffer solution Dissolve 6.8 g of *potassium dihydrogen phosphate R* in *water for chromatography R* and dilute to about 175 mL with the same solvent. Filter through a membrane filter (nominal pore size 0.45 µm) and dilute to 1000 mL with *water for chromatography R*.

Test solution Dissolve 0.500 g of the substance to be examined in the phosphate buffer solution and dilute to 10.0 mL with the phosphate buffer solution.

Reference solution (a) Dissolve 10.0 mg of *ascorbic acid impurity C CRS* (impurity C) in the mobile phase and dilute to 5.0 mL with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *ascorbic acid impurity D CRS* (impurity D) and 5.0 mg of *ascorbic acid CRS* in the mobile phase, add 2.5 mL of reference solution (a) and dilute to 100.0 mL with the mobile phase.

Reference solution (c) Dilute 1 mL of the test solution to 200 mL with the mobile phase. Mix 1 mL of this solution and 1 mL of reference solution (a).

Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: aminopropylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 45 °C.

Mobile phase Phosphate buffer solution, *acetonitrile R1* (25:75 *V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µL of the test solution and reference solutions (b) and (c).

Run time 2.5 times the retention time of ascorbic acid.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities C and D.

Relative retention With reference to ascorbic acid (retention time = about 11 min): impurity D = about 0.4; impurity C = about 1.7.

System suitability:
— *resolution*: minimum 3.0 between the peaks due to ascorbic acid and impurity C in the chromatogram obtained with reference solution (c);
— *signal-to-noise ratio*: minimum 20 for the peak due to impurity C in the chromatogram obtained with reference solution (b).

Limits:
— *impurities C, D*: for each impurity, not more than 1.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the peak due to ascorbic acid in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *sum of impurities other than C and D*: not more than twice the area of the peak due to ascorbic acid in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *disregard limit*: 0.5 times the area of the peak due to ascorbic acid in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulfates (*2.4.13*)
Maximum 150 ppm.

To 10 mL of solution S add 2 mL of *hydrochloric acid R1* and dilute to 15 mL with *distilled water R*.

Copper
Maximum 5 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Dissolve 2.0 g in *0.1 M nitric acid* and dilute to 25.0 mL with the same acid.

Reference solutions Prepare the reference solutions (0.2 ppm, 0.4 ppm and 0.6 ppm) using *copper standard solution (10 ppm Cu) R*, diluting with *0.1 M nitric acid*.

Source Copper hollow-cathode lamp.

Wavelength 324.8 nm.

Atomisation device Air-acetylene flame.

Iron
Maximum 2 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Dissolve 5.0 g in *0.1 M nitric acid* and dilute to 25.0 mL with the same acid.

Reference solutions Prepare the reference solutions (0.2 ppm, 0.4 ppm and 0.6 ppm) using *iron standard solution (20 ppm Fe) R*, diluting with *0.1 M nitric acid*.

Source Iron hollow-cathode lamp.

Wavelength 248.3 nm.

Atomisation device Air-acetylene flame.

Loss on drying (*2.2.32*)
Maximum 0.25 per cent, determined on 1.000 g by drying in an oven at 105 °C.

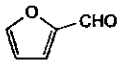
ASSAY
Dissolve 80 mg in a mixture of 10 mL of *dilute sulfuric acid R* and 80 mL of *carbon dioxide-free water R*. Add 1 mL of *starch solution R*. Titrate with *0.05 M iodine* until a persistent violet-blue colour is obtained.

1 mL of *0.05 M iodine* is equivalent to 9.91 mg of $C_6H_7NaO_6$.

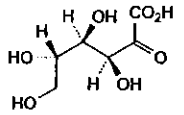
STORAGE
In a non-metallic container, protected from light.

IMPURITIES
Specified impurities *C, D, E.*

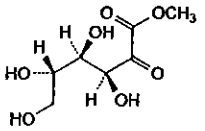
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, F, G, H.*



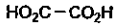
A. furan-2-carbaldehyde,



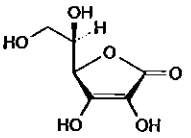
C. L-*xylo*-hex-2-ulosonic acid (L-sorbosonic acid),



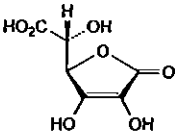
D. methyl L-*xylo*-hex-2-ulosonate (methyl L-sorbosonate),



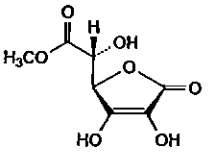
E. oxalic acid,



F. (5*R*)-5-[(1*R*)-1,2-dihydroxyethyl]-3,4-dihydroxyfuran-2(5*H*)-one,



G. (*R*)-[(2*R*)-3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl]hydroxyacetic acid,



H. methyl (*R*)-[(2*R*)-3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl]hydroxyacetate.

Ph Eur

Sodium Aurothiomalate

(Ph. Eur. monograph 1994)

Action and use
Used in the treatment of rheumatoid arthritis.

Preparation
Sodium Aurothiomalate Injection

Ph Eur

DEFINITION
Mixture of monosodium and disodium salts of (2*RS*)-2-(aurosulfanyl)butanedioic acid.

Content
— *gold* (Au; A_r 197.0): 44.5 per cent to 46.0 per cent (dried substance);
— *sodium* (Na; A_r 22.99): 10.8 per cent to 11.8 per cent (dried substance).

CHARACTERS

Appearance

Fine, pale yellow, hygroscopic powder.

Solubility

Very soluble in water, practically insoluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

A. Dissolve 20 mg in *water R* and dilute to 2 mL with the same solvent. Add 2 mL of *strong hydrogen peroxide solution R* and 1 mL of *sodium hydroxide solution R*. Carefully heat to boiling and boil for 30 s. A precipitate is produced that appears brownish-black by reflected light and bluish-green by transmitted light.

B. To 20 mg add 2 mL of *water R*. The solution gives reaction (a) of sodium (*2.3.1*).

C. Ignite 100 mg, dissolve the residue in *hydrochloric acid R* and dilute to 10 mL with the same acid. Allow to stand. 5 mL of the clear supernatant gives reaction (a) of sulfates (*2.3.1*).

TESTS

Appearance of solution

Dissolve 1.0 g in *water R* and dilute to 10 mL with the same solvent. Filter, seal in an ampoule and heat at 100 °C for 1 h. Cool and dilute the contents of the ampoule to 100 mL with *water R*. The solution remains clear and is not more intensely coloured than a 0.100 g/L solution of *potassium ferricyanide R*.

pH (*2.2.3*)

6.0 to 7.0.

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 0.200 g of the substance to be examined in *water R* and dilute to 100.0 mL with the same solvent.

Reference solution (a) Dissolve 10.0 mg of *fumaric acid R* and 100.0 mg of *thiomalic acid R* in *water R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of this solution to 25.0 mL with *water R*.

Reference solution (b) Dissolve 12.0 mg of *thiomalic acid R* in *water R* and dilute to 100.0 mL with the same solvent.

Reference solution (c) Dissolve 10.0 mg of *maleic acid R* in *water R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of this solution to 25.0 mL with *water R*.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 90 volumes of a 10.5 g/L solution of *phosphoric acid R*, 100 volumes of *methanol R2* and 810 volumes of *water R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 205 nm.

Injection 10 µL.

Run time Twice the retention time of impurity C.

Relative retention With reference to impurity C (retention time = about 8 min): impurity A = about 0.4; impurity B = about 0.6. Aurothiomalate does not elute under the chromatographic conditions described.

System suitability Reference solution (a):

— *resolution*: minimum 5.0 between the peaks due to impurities B and C.

Limits:

— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (6.0 per cent).

Glycerol

Maximum 8.0 per cent.

Test solution Dissolve 0.50 g of the substance to be examined in *water R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dilute 0.80 g of *glycerol R* to 100.0 mL with *water R*.

Reference solution (b) To 2.5 mL of reference solution (a) add 7.5 mL of *water R*.

Reference solution (c) To 5.0 mL of reference solution (a) add 5.0 mL of *water R*.

Reference solution (d) To 7.5 mL of reference solution (a) add 2.5 mL of *water R*.

Blank solution 10 mL of *water R*.

To the test solution, reference solutions (b), (c) and (d) and the blank solution, add 2.5 mL of a freshly prepared 235 g/L solution of *sodium hydroxide R* and mix. Add dropwise in 0.2 mL increments a 38.0 g/L solution of *cupric chloride R*, shaking vigorously after each addition, until the solutions become slightly turbid. Then add 0.2 mL of the 38.0 g/L solution of *cupric chloride R*. Stopper and shake vigorously for 1 min. Dilute to 25.0 mL with *water R* and mix. Centrifuge for 2 min. Measure the absorbance (*2.2.25*) of the supernatant solution of a 1 cm layer at 635 nm. Use the solution prepared from the blank solution as the compensation liquid. Draw a calibration curve and calculate the content of glycerol in the sample.

Loss on drying (*2.2.32*)

Maximum 2.0 per cent, determined on 1.000 g by drying in a desiccator at a pressure not exceeding 0.7 kPa for 24 h.

ASSAY

Gold

Heat 0.2 g with 10 mL of *sulfuric acid R* and continue to boil gently until a clear, pale yellow liquid is produced. Cool, add about 1 mL of *nitric acid R* dropwise and boil for 1 h. Cool, dilute to 70 mL with *water R*, boil for 5 min and filter. Wash the residue of gold with *water R* at 60 °C. Dry and ignite at a temperature of at least 600 °C for 3 h. Weigh the residue and calculate the percentage content of Au.

Sodium

Evaporate to dryness the filtrate and washings obtained in the assay for gold, moisten with *sulfuric acid R* and ignite at 600 ± 50 °C for 3 h.

1.000 g of residue is equivalent to 0.324 g of Na.

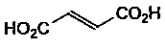
STORAGE

In an airtight container.

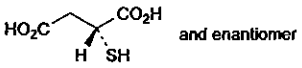
IMPURITIES

Specified impurities *A, B, C.*

A. (*Z*)-butenedioic acid (maleic acid),



B. (*E*)-butenedioic acid (fumaric acid),

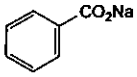


C. (2*RS*)-2-sulfanylbutanedioic acid (thiomalic acid).

Ph Eur

Sodium Benzoate

(Ph. Eur. monograph 0123)



$C_7H_5NaO_2$ 144.1 *532-32-1*

Action and use
Excipient; treatment of hyperammonaemia due to urea cycle disorders; treatment of non-ketotic hyperglycinaemia.

Preparations
Sodium Benzoate Oral Solution

Sodium Benzoate Sterile Concentrate

Ph Eur

DEFINITION
Sodium benzenecarboxylate.

Content
99.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline or granular powder or flakes, slightly hygroscopic.

Solubility
Freely soluble in water, sparingly soluble in ethanol (90 per cent *V/V*).

IDENTIFICATION
A. It gives reactions (b) and (c) of benzoates (*2.3.1*).

B. It gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S
Dissolve 10.0 g in *carbon dioxide-free water R* and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Acidity or alkalinity
To 10 mL of solution S add 10 mL of *carbon dioxide-free water R* and 0.2 mL of *phenolphthalein solution R*. Not more than 0.2 mL of *0.1 M sodium hydroxide* or *0.1 M hydrochloric acid* is required to change the colour of the indicator.

Halogenated compounds
Maximum 200 ppm for ionised chlorine and maximum 300 ppm for total chlorine.

All glassware used must be chloride-free and may be prepared by soaking overnight in a 500 g/L solution of nitric acid R, rinsed with water R and stored full of water R. It is recommended that glassware be reserved exclusively for this test.

Test solution To 20.0 mL of solution S add 5 mL of *water R* and dilute to 50.0 mL with *ethanol (96 per cent) R*.

Determination of ionised chlorine

In three 25 mL volumetric flasks, prepare the following solutions.

Solution (a) To 4.0 mL of the test solution add 3 mL of *dilute sodium hydroxide solution R* and 3 mL of *ethanol (96 per cent) R*. This solution is used to prepare solution A.

Solution (b) To 3 mL of *dilute sodium hydroxide solution R* add 2 mL of *water R* and 5 mL of *ethanol (96 per cent) R*. This solution is used to prepare solution B.

Solution (c) To 4.0 mL of *chloride standard solution (8 ppm Cl) R* add 6.0 mL of *water R*. This solution is used to prepare solution C.

In a fourth 25 mL volumetric flask, place 10 mL of *water R*. To each flask add 5 mL of *ferric ammonium sulfate solution R5*, mix and add dropwise and with swirling 2 mL of *nitric acid R* and 5 mL of *mercuric thiocyanate solution R*. Shake. Dilute the contents of each flask to 25.0 mL with *water R* and allow the solutions to stand in a water-bath at 20 °C for 15 min. Measure at 460 nm in a 2 cm cell the absorbance (*2.2.25*) of solution A using solution B as the compensation liquid, and the absorbance of solution C using the solution obtained with 10 mL of *water R* as the compensation liquid. The absorbance of solution A is not greater than that of solution C.

Determination of total chlorine

Solution (a) To 10.0 mL of the test solution add 7.5 mL of *dilute sodium hydroxide solution R* and 0.125 g of *nickel-aluminium alloy R* and heat on a water-bath for 10 min. Allow to cool to room temperature, filter into a 25 mL volumetric flask and wash the filter with 3 quantities, each of 2 mL, of *ethanol (96 per cent) R* (a slight precipitate may form that disappears on acidification). Dilute the filtrate and washings to 25.0 mL with *water R*. This solution is used to prepare solution A.

Solution (b) In the same manner, prepare a similar solution replacing the test solution by a mixture of 5 mL of *ethanol (96 per cent) R* and 5 mL of *water R*. This solution is used to prepare solution B.

Solution (c) To 6.0 mL of *chloride standard solution (8 ppm Cl) R* add 4.0 mL of *water R*. This solution is used to prepare solution C.

In four 25 mL volumetric flasks, place separately 10 mL of solution (a), 10 mL of solution (b), 10 mL of solution (c) and 10 mL of *water R*. To each flask add 5 mL of *ferric ammonium sulfate solution R5*, mix and add dropwise and with swirling 2 mL of *nitric acid R* and 5 mL of *mercuric thiocyanate solution R*. Shake. Dilute the contents of each flask to 25.0 mL with *water R* and allow the solutions to stand in a water-bath at 20 °C for 15 min. Measure at 460 nm in a 2 cm cell the absorbance (*2.2.25*) of solution A using solution B as the compensation liquid, and the absorbance of solution C using the solution obtained with 10 mL of *water R* as the compensation liquid. The absorbance of solution A is not greater than that of solution C.

Loss on drying (*2.2.32*)
Maximum 2.0 per cent, determined on 1.00 g by drying in an oven at 105 °C.

ASSAY
Dissolve 0.250 g in 20 mL of *anhydrous acetic acid R*, heating to 50 °C if necessary. Cool. Using 0.05 mL of *naphtholbenzein solution R* as indicator, titrate with *0.1 M perchloric acid* until a green colour is obtained.

1 mL of *0.1 M perchloric acid* is equivalent to 14.41 mg of $C_7H_5NaO_2$.

Ph Eur

Sodium Bicarbonate

(Sodium Hydrogen Carbonate, Ph. Eur. monograph 0195)

$NaHCO_3$ 84.0 *144-55-8*

Action and use
Antacid; used in treatment of electrolyte deficiency.

Preparations
Sodium Bicarbonate Ear Drops
Sodium Bicarbonate Eye Lotion
Sodium Bicarbonate Infusion
Sodium Bicarbonate Oral Solution
Compound Sodium Bicarbonate Tablets

Ph Eur

DEFINITION
Content
99.0 per cent to 101.0 per cent.

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Soluble in water, practically insoluble in ethanol (96 per cent).

When heated in the dry state or in solution, it gradually changes into sodium carbonate.

IDENTIFICATION
A. To 5 mL of solution S (see Tests) add 0.1 mL of *phenolphthalein solution R*. A pale pink colour is produced. Heat; gas is evolved and the solution becomes red.

B. It gives the reaction of carbonates and bicarbonates (*2.3.1*).

C. Solution S gives reaction (a) of sodium (*2.3.1*).

TESTS
Solution S
Dissolve 5.0 g in 90 mL of *carbon dioxide-free water R* and dilute to 100.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Carbonates
The pH (*2.2.3*) of freshly prepared solution S is not greater than 8.6.

Chlorides (*2.4.4*)
Maximum 150 ppm.

To 7 mL of solution S add 2 mL of *nitric acid R* and dilute to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 150 ppm.

To a suspension of 1.0 g in 10 mL of *distilled water R* add *hydrochloric acid R* until neutral and about 1 mL in excess. Dilute to 15 mL with *distilled water R*.

Ammonium (*2.4.1*)
Maximum 20 ppm.

Dilute 10 mL of solution S to 15 mL with *water R*. Prepare the standard using a mixture of 5 mL of *water R* and 10 mL of *ammonium standard solution (1 ppm NH_4) R*.

Calcium (*2.4.3*)
Maximum 100 ppm.

To a suspension of 1.0 g in 10 mL of *distilled water R* add *hydrochloric acid R* until neutral and dilute to 15 mL with *distilled water R*.

Iron (*2.4.9*)
Maximum 20 ppm.

Dissolve 0.5 g in 5 mL of *dilute hydrochloric acid R* and dilute to 10 mL with *water R*.

ASSAY
Dissolve 0.750 g in 50 mL of *carbon dioxide-free water R*. Titrate with *1 M hydrochloric acid*, determining the end-point potentiometrically (*2.2.20*). Read the volume added at the 2nd point of inflection, or at the point of inflection if only 1 point is detected.

1 mL of *1 M hydrochloric acid* is equivalent to 84.0 mg of $NaHCO_3$.

Ph Eur

Sodium Bromide

(Ph. Eur. monograph 0190)

NaBr 102.9 *7647-15-6*

Ph Eur

DEFINITION
Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, granular powder or small, colourless, transparent or opaque crystals, slightly hygroscopic.

Solubility
Freely soluble in water, soluble in ethanol (96 per cent).

IDENTIFICATION
A. It gives reaction (a) of bromides (*2.3.1*).

B. Solution S (see Tests) gives reaction (a) of sodium (*2.3.1*).

TESTS
Solution S
Dissolve 10.0 g in *carbon dioxide-free water R* and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity
To 10 mL of solution S add 0.1 mL of *bromothymol blue solution R1*. Not more than 0.5 mL of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Bromates
To 10 mL of solution S add 1 mL of *starch solution R*, 0.1 mL of a 100 g/L solution of *potassium iodide R* and 0.25 mL of *0.5 M sulfuric acid* and allow to stand protected from light for 5 min. No blue or violet colour develops.

Chlorides and sulfates
Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 0.400 g of the substance to be examined in 50 mL of *water for chromatography R* and dilute to 100.0 mL with the same solvent.

Test solution (b) Dilute 25.0 mL of test solution (a) to 50.0 mL with *water for chromatography R*.

Reference solution (a) To 25.0 mL of test solution (a) add 1.0 mL of *sulfate standard solution (10 ppm SO_4) R* and 12.0 mL of *chloride standard solution (50 ppm Cl) R* and dilute to 50.0 mL with *water for chromatography R*.

Reference solution (b) Dilute 10 mL of test solution (a) to 100 mL with *water for chromatography R*. To 2 mL of this solution add 8 mL of *chloride standard solution (50 ppm Cl) R* and dilute to 20 mL with *water for chromatography R*.

Blank solution *water for chromatography R*.

Column:
— *size*: *l* = 0.25 m, Ø = 2 mm;
— *stationary phase*: *strongly basic anion-exchange resin for chromatography R2* (13 µm).

Mobile phase Dissolve 0.600 g of *potassium hydroxide R* in *water for chromatography R* and dilute to 1000 mL with the same solvent.

Flow rate 0.4 mL/min.

Detection Conductivity detector equipped with a suitable ion suppressor.

Injection 50 µL of test solution (b), reference solutions (a) and (b) and the blank solution.

Run time 2.5 times the retention time of bromide.

Retention time Chloride = about 5 min; bromide = about 8 min; sulfate = about 16 min.

System suitability Reference solution (b):
— *resolution*: minimum 8.0 between the peaks due to chloride and bromide.

Calculation of percentage contents:
— for chlorides, use the concentration of chloride in reference solution (a); correct the area of the peak due to chloride in the chromatogram obtained with reference solution (a) by subtracting the area of the peak due to chloride in the chromatogram obtained with test solution (b);
— for sulfates, use the concentration of sulfate in reference solution (a); correct the area of the peak due to sulfate in the chromatogram obtained with reference solution (a) by subtracting the area of the peak due to sulfate in the chromatogram obtained with test solution (b).

Limits:
— *chlorides*: maximum 0.6 per cent;
— *sulfates*: maximum 0.01 per cent.

Iodides
To 5 mL of solution S add 0.15 mL of *ferric chloride solution R1* and 2 mL of *methylene chloride R*. Shake and allow to separate. The lower layer is colourless (*2.2.2, Method I*).

Iron (*2.4.9*)
Maximum 20 ppm.

Dilute 5 mL of solution S to 10 mL with *water R*.

Magnesium and alkaline-earth metals (*2.4.7*)
Maximum 200 ppm, calculated as Ca.

10.0 g complies with the test for magnesium and alkaline-earth metals. The volume of *0.01 M sodium edetate* used does not exceed 5.0 mL.

Loss on drying (*2.2.32*)
Maximum 3.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

ASSAY
Dissolve 85.0 mg in *water R*, add 5 mL of *dilute nitric acid R* and dilute to 50 mL with *water R*. Titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M silver nitrate* is equivalent to 10.29 mg of NaBr.

Calculate the percentage content of NaBr using the following expression:

$$a - 2.902\ b$$

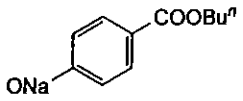
a = percentage content of NaBr and NaCl obtained in the assay and calculated as NaBr;
b = percentage content of Cl obtained in the test for chlorides.

STORAGE
In an airtight container.

Ph Eur

Sodium Butyl Hydroxybenzoate

Sodium Butylparaben



$C_{11}H_{13}NaO_3$ 216.2 *36457-20-2*

Action and use
Antimicrobial preservative.

DEFINITION
Sodium Butyl Hydroxybenzoate is the sodium salt of butyl 4-hydroxybenzoate. It contains not less than 99.0% and not more than 102.0% of $C_{11}H_{13}NaO_3$, calculated with reference to the anhydrous substance.

CHARACTERISTICS
A white powder; hygroscopic.

Freely soluble in *water* and in *ethanol (96%)*.

IDENTIFICATION
A. Dissolve 0.5 g in 5 mL of *water* and acidify to *litmus paper* with *hydrochloric acid*. A white precipitate is produced. Wash the precipitate with *water* and dry. The *infrared absorption spectrum* of the precipitate, Appendix II A, is concordant with the *reference spectrum* of butyl hydroxybenzoate *(RS 036)*.

B. The residue on ignition yields the reactions characteristic of *sodium salts*, Appendix VI.

TESTS
Alkalinity
pH of a 0.1% w/v solution, 9.5 to 10.5, Appendix V L.

Clarity of solution
Dissolve 1.0 g in 10 mL of *water*. The solution is *clear*, Appendix IV A.

Chloride
Dissolve 1.0 g in 100 mL of *water*, add 1 mL of *nitric acid* and filter. 15 mL of the filtrate complies with the *limit test for chlorides*, Appendix VII (330 ppm).

Sulfate
Dissolve 0.50 g in 40 mL of *water*, add 3.5 mL of 2M *hydrochloric acid*, dilute to 60 mL with *water* and filter. 15 mL of the filtrate complies with the *limit test for sulfates*, Appendix VII (0.12%).

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute a 2.0% w/v solution of the substance being examined in *water* with an equal volume of *acetone*.

(2) Dilute 1 volume of solution (1) to 25 volumes with a mixture of equal volumes of *acetone* and *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating silica gel F_{254} the surface of which has been modified with chemically-bonded octadecylsilyl groups (Whatman KC18F plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 2 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of *glacial acetic acid*, 30 volumes of *water* and 70 volumes of *methanol*.

LIMITS

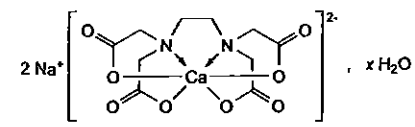
Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Water
Not more than 5.0% w/w, Appendix IX C. Use 1 g.

ASSAY
Gently boil 0.1 g under a reflux condenser with 25 mL of 1.25M *sodium hydroxide* for 30 minutes. Allow to cool, add 25 mL of *0.0333M potassium bromate VS*, 5 mL of a 12.5% w/v solution of *potassium bromide* and 10 mL of *hydrochloric acid* and immediately stopper the flask. Shake for 15 minutes and allow to stand for 15 minutes. Add 25 mL of *dilute potassium iodide solution* and shake vigorously. Titrate the liberated iodine with *0.1M sodium thiosulfate VS* using *starch mucilage*, added towards the end of the titration, as indicator. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of potassium bromate required. The volume of *0.0333M potassium bromate VS* used is equivalent to half of the volume of *0.1M sodium thiosulfate VS* required for the titration. Each mL of *0.0333M potassium bromate VS* is equivalent to 7.207 mg of $C_{11}H_{13}NaO_3$.

Sodium Calcium Edetate

(Ph. Eur. monograph 0231)



$C_{10}H_{12}CaN_2Na_2O_8,xH_2O$ 374.3 *62-33-9*
(anhydrous substance)

Action and use
Chelating agent.

Preparation
Sodium Calcium Edetate Infusion

Ph Eur

DEFINITION
Disodium [(ethylenedinitrilo)tetraacetato]calciate(2−).

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

It contains a variable quantity of water of crystallisation.

CHARACTERS

Appearance
White or almost white, hygroscopic powder.

Solubility
Freely soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION
First identification: A, C, D.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *sodium calcium edetate CRS.*

B. Dissolve 2 g in 10 mL of *water R*, add 6 mL of *lead nitrate solution R*, shake and add 3 mL of *potassium iodide solution R*. No yellow precipitate is formed. Make alkaline to *red litmus paper R* by the addition of *dilute ammonia R2* and add 3 mL of *ammonium oxalate solution R*. A white precipitate is formed.

C. Ignite. The residue gives reaction (b) of calcium (*2.3.1*).

D. Dissolve 0.5 g in 10 mL of *water R* and add 10 mL of *potassium pyroantimonate solution R*. A white, crystalline precipitate is formed. The formation of the precipitate is accelerated by rubbing the wall of the tube with a glass rod.

TESTS

Solution S
Dissolve 5.0 g in *water R* and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)
6.5 to 8.0.

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent.

Impurity A
Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Solvent mixture Dissolve 10.0 g of *ferric sulfate pentahydrate R* in 20 mL of *0.5 M sulfuric acid* and add

780 mL of *water R*. Adjust to pH 2.0 with *1 M sodium hydroxide* and dilute to 1000 mL with *water R*.

Test solution Dissolve 0.100 g of the substance to be examined in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

Reference solution Dissolve 40.0 mg of *nitrilotriacetic acid R* (impurity A) in the solvent mixture and dilute to 100.0 mL with the solvent mixture. To 1.0 mL of this solution add 0.1 mL of the test solution and dilute to 100.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.10 m, Ø = 4.6 mm;
— *stationary phase*: spherical *graphitised carbon for chromatography R1* (5 µm) with a specific surface area of 120 m^2/g and a pore size of 25 nm.

Mobile phase Dissolve 50.0 mg of *ferric sulfate pentahydrate R* in 50 mL of *0.5 M sulfuric acid* and add 750 mL of *water R*; adjust to pH 1.5 with *0.5 M sulfuric acid* or *1 M sodium hydroxide*, add 20 mL of *ethylene glycol R* and dilute to 1000 mL with *water R*.

Flow rate 1 mL/min.

Detection Spectrophotometer at 273 nm.

Injection 20 µL; filter the solutions and inject immediately.

Run time 4 times the retention time of the iron complex of impurity A.

Retention time Iron complex of impurity A = about 5 min; iron complex of edetic acid = about 10 min.

System suitability Reference solution:
— *resolution*: minimum 7 between the peaks due to the iron complex of impurity A and the iron complex of edetic acid;
— *signal-to-noise ratio*: minimum 50 for the peak due to impurity A.

Limit:
— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.1 per cent).

Disodium edetate
Maximum 1.0 per cent.

Dissolve 5.0 g in 250 mL of *water R*. Add 10 mL of *ammonium chloride buffer solution pH 10.0 R* and about 50 mg of *mordant black 11 triturate R*. Not more than 1.5 mL of 0.1 M magnesium chloride is required to change the colour of the indicator to violet.

Chlorides
Maximum 0.1 per cent.

Dissolve 0.7 g in *water R* and dilute to 20 mL with the same solvent. Add 30 mL of *dilute nitric acid R*, allow to stand for 30 min and filter. Dilute 10 mL of the filtrate to 50 mL with *water R*. Use this solution as the test solution. Prepare the reference solution using 0.40 mL of *0.01 M hydrochloric acid*, add 6 mL of *dilute nitric acid R* and dilute to 50 mL with *water R*. Filter both solutions if necessary. Add 1 mL of *silver nitrate solution R2* to the test solution and to the reference solution and mix. After standing for 5 min protected from light, any opalescence in the test solution is not more intense than that in the reference solution.

Iron (*2.4.9*)
Maximum 80 ppm.

Dilute 2.5 mL of solution S to 10 mL with *water R*. Add 0.25 g of *calcium chloride R* to the test solution and the standard before the addition of the *thioglycollic acid R*.

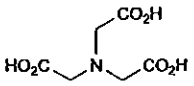
Water (*2.5.12*)
5.0 per cent to 13.0 per cent, determined on 0.200 g.

ASSAY
Dissolve 0.500 g in *water R* and dilute to 200 mL with the same solvent. To 20.0 mL of this solution, add 80 mL of *water R* and adjust to pH 2 with *dilute nitric acid R*. Titrate with *0.01 M bismuth nitrate*, using 0.1 mL of a 1 g/L solution of *xylenol orange R* as indicator. The colour of the solution changes from yellow to red.

1 mL of *0.01 M bismuth nitrate* is equivalent to 3.74 mg of $C_{10}H_{12}CaN_2Na_2O_8$.

STORAGE
In an airtight container, protected from light.

IMPURITIES
Specified impurities *A*.



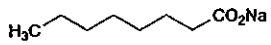
A. nitrilotriacetic acid.

Ph Eur

Sodium Caprylate

Sodium Octanoate

(Ph. Eur. monograph 1471)



$C_8H_{15}NaO_2$ 166.2 *1984-06-1*

Action and use
Excipient.

Ph Eur

DEFINITION
Sodium octanoate.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Very soluble or freely soluble in water, freely soluble in acetic acid, sparingly soluble in ethanol (96 per cent), practically insoluble in acetone.

IDENTIFICATION
A. Examine the chromatograms obtained in the test for related substances.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

B. To 0.2 mL of solution S (see Tests) add 0.3 mL of *water R*. The solution gives reaction (b) of sodium (*2.3.1*).

TESTS

Solution S
Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)
8.0 to 10.5 for solution S.

Related substances
Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution Dissolve 0.116 g in *water R* and dilute to 5 mL with the same solvent. Add 1 mL of a 2.8 per cent *V/V* solution of *sulfuric acid R* and shake with 10 mL of *ethyl acetate R*. Separate the organic layer and dry over *anhydrous sodium sulfate R*.

Reference solution (a) Dissolve 0.10 g of *caprylic acid CRS* in *ethyl acetate R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dilute 1 mL of the test solution to 100 mL with *ethyl acetate R*. Dilute 5 mL of this solution to 50 mL with *ethyl acetate R*.

Column:
— *material*: fused silica;
— *size*: l = 30 m, Ø = 0.25 mm;
— *stationary phase*: *macrogol 20 000 2-nitroterephthalate R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.5 mL/min.

Split ratio 1:100.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 1	100
	1 - 25	100 → 220
	25 - 35	220
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 1 µL.

System suitability Reference solution (b):
— *signal-to-noise ratio*: minimum 5 for the principal peak.

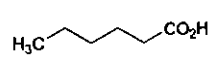
Limits:
— *any impurity*: for each impurity, maximum 0.3 per cent;
— *total*: maximum 0.5 per cent;
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.12*)
Maximum 3.0 per cent, determined on 1.000 g.

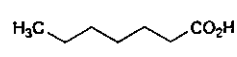
ASSAY
Dissolve 0.150 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 16.62 mg of $C_8H_{15}NaO_2$.

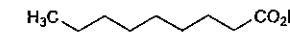
IMPURITIES



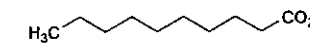
A. hexanoic acid,



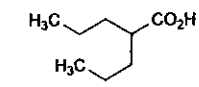
B. heptanoic acid,



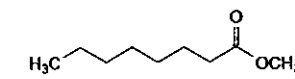
C. nonanoic acid,



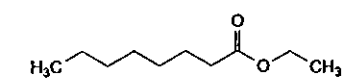
D. decanoic acid,



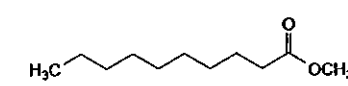
E. 2-propylpentanoic acid (valproic acid),



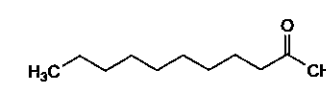
F. methyl octanoate,



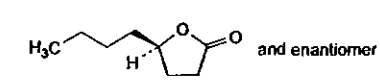
G. ethyl octanoate,



H. methyl decanoate,



I. undecan-2-one,



J. (*RS*)-5-butyltetrahydrofuran-2-one (γ-hydroxyoctanoic acid lactone).

Ph Eur

Sodium Carbonate

Anhydrous Sodium Carbonate

(Ph. Eur. monograph 0773)

Na_2CO_3 106.0 *497-19-8*

Ph Eur

DEFINITION

Content
99.5 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, slightly granular powder, hygroscopic.

Solubility
Freely soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION
A. Dissolve 1 g in *water R* and dilute to 10 mL with the same solvent. The solution is strongly alkaline (*2.2.4*).

B. The solution prepared for identification test A gives the reaction of carbonates (*2.3.1*).

C. The solution prepared for identification test A gives reaction (a) of sodium (*2.3.1*).

D. Loss on drying (see Tests).

TESTS

Solution S

Dissolve 2.0 g in portions in a mixture of 5 mL of *hydrochloric acid R* and 25 mL of *distilled water R*. Heat to boiling and cool. Add *dilute sodium hydroxide solution R* until the solution is neutral and dilute to 50 mL with *distilled water R*.

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method I*).

Dissolve 2.0 g in 10 mL of *water R*.

Alkali hydroxides and bicarbonates

Dissolve 0.4 g in 20 mL of *water R*. Add 20 mL of *barium chloride solution R1* and filter. To 10 mL of the filtrate add 0.1 mL of *phenolphthalein solution R*. The solution does not become red. Boil the remainder of the filtrate for 2 min. The solution remains clear (*2.2.1*).

Chlorides (*2.4.4*)

Maximum 125 ppm.

Dissolve 0.4 g in *water R*, add 4 mL of *dilute nitric acid R* and dilute to 15 mL with *water R*.

Sulfates (*2.4.13*)

Maximum 250 ppm, determined on solution S.

Iron (*2.4.9*)

Maximum 50 ppm.

Dilute 5 mL of solution S to 10 mL with *water R*.

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 300 ± 15 °C.

ASSAY

Dissolve 0.400 g in 50 mL of *carbon dioxide-free water R*. Titrate with *1 M hydrochloric acid*, determining the end-point potentiometrically (*2.2.20*). Read the volume added at the 2nd point of inflection.

1 mL of *1 M hydrochloric acid* is equivalent to 52.99 mg of Na_2CO_3.

STORAGE

In an airtight container.

Ph Eur

Sodium Carbonate Decahydrate

(Ph. Eur. monograph 0191)

$Na_2CO_3,10H_2O$ 286.1 *6132-02-1*

Ph Eur

DEFINITION

Content

36.7 per cent to 40.0 per cent of Na_2CO_3.

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless, transparent crystals, efflorescent.

Solubility

Freely soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Dissolve 1 g in *water R* and dilute to 10 mL with the same solvent. The solution is strongly alkaline (*2.2.4*).

B. The solution prepared for identification test A gives the reaction of carbonates (*2.3.1*).

C. The solution prepared for identification test A gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S

Dissolve 5.0 g in portions in a mixture of 5 mL of *hydrochloric acid R* and 25 mL of *distilled water R*. Heat the solution to boiling and cool. Add *dilute sodium hydroxide solution R* until the solution is neutral and dilute to 50 mL with *distilled water R*.

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method I*).

Dissolve 4.0 g in 10 mL of *water R*.

Alkali hydroxides and bicarbonates

Dissolve 1.0 g in 20 mL of *water R*, add 20 mL of *barium chloride solution R1* and filter. To 10 mL of the filtrate add 0.1 mL of *phenolphthalein solution R*. The solution does not become red. Heat the remainder of the filtrate to boiling for 2 min. The solution remains clear (*2.2.1*).

Chlorides (*2.4.4*)

Maximum 50 ppm.

Dissolve 1.0 g in *water R*, add 4 mL of *dilute nitric acid R* and dilute to 15 mL with *water R*.

Sulfates (*2.4.13*)

Maximum 100 ppm, determined on solution S.

Iron (*2.4.9*)

Maximum 20 ppm.

Dilute 5 mL of solution S to 10 mL with *water R*.

ASSAY

Dissolve 1.000 g in 50 mL of *carbon dioxide-free water R*. Titrate with *1 M hydrochloric acid*, determining the end-point potentiometrically (*2.2.20*). Read the volume added at the 2nd point of inflection.

1 mL of *1 M hydrochloric acid* is equivalent to 52.99 mg of Na_2CO_3.

STORAGE

In an airtight container.

Ph Eur

Sodium Carbonate Monohydrate

(Ph. Eur. monograph 0192)

Na_2CO_3,H_2O 124.0 *5968-11-6*

Ph Eur

DEFINITION

Content

83.0 per cent to 87.5 per cent of Na_2CO_3.

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Dissolve 1 g in *water R* and dilute to 10 mL with the same solvent. The solution is strongly alkaline (*2.2.4*).

B. The solution prepared for identification test A gives the reaction of carbonates (*2.3.1*).

C. The solution prepared for identification test A gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S

Dissolve 2.0 g in portions in a mixture of 5 mL of *hydrochloric acid R* and 25 mL of *distilled water R*. Heat the solution to boiling and cool. Add *dilute sodium hydroxide solution R* until the solution is neutral and dilute to 50 mL with *distilled water R*.

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method I*).

Dissolve 2.0 g in 10 mL of *water R*.

Alkali hydroxides and bicarbonates

Dissolve 0.4 g in 20 mL of *water R*, add 20 mL of *barium chloride solution R1* and filter. To 10 mL of the filtrate add 0.1 mL of *phenolphthalein solution R*. The solution does not become red. Heat the remainder of the filtrate to boiling for 2 min. The solution remains clear (*2.2.1*).

Chlorides (*2.4.4*)

Maximum 125 ppm.

Dissolve 0.4 g in *water R*, add 4 mL of *dilute nitric acid R* and dilute to 15 mL with *water R*.

Sulfates (*2.4.13*)

Maximum 250 ppm, determined on solution S.

Iron (*2.4.9*)

Maximum 50 ppm.

Dilute 5 mL of solution S to 10 mL with *water R*.

ASSAY

Dissolve 0.500 g in 50 mL of *carbon dioxide-free water R*. Titrate with *1 M hydrochloric acid*, determining the end-point potentiometrically (*2.2.20*). Read the volume added at the 2nd point of inflection.

1 mL of *1 M hydrochloric acid* is equivalent to 52.99 mg of Na_2CO_3.

STORAGE

In an airtight container.

Ph Eur

Sodium Cetostearyl Sulfate

Sodium Cetostearyl Sulphate

(Ph. Eur. monograph 0847)

Action and use

Excipient.

Ph Eur

DEFINITION

Mixture of sodium cetyl sulfate (sodium hexadecyl sulfate, $C_{16}H_{33}NaO_4S$; M_r 344.5) and sodium stearyl sulfate (sodium octadecyl sulfate, $C_{18}H_{37}NaO_4S$; M_r 372.5). A suitable buffer may be added.

Content

— *sodium cetostearyl sulfate*: minimum 90.0 per cent (anhydrous substance);

— *sodium cetyl sulfate*: minimum 40.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or pale yellow, amorphous or crystalline powder.

Solubility

Soluble in hot water giving an opalescent solution, practically insoluble in cold water, partly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: B, D, F.

Second identification: A, C, D, E, F.

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 50 mg of the substance to be examined in 10 mL of *ethanol (70 per cent V/V) R*, heating on a water-bath.

Reference solution Dissolve 50 mg of *sodium cetostearyl sulfate CRS* in 10 mL of *ethanol (70 per cent V/V) R*, heating on a water-bath.

Plate *TLC octadecylsilyl silica gel F_{254} plate R.*

Mobile phase *water R, acetone R, methanol R* (20:40:40 *V/V/V*).

Application 10 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with a 50 g/L solution of *phosphomolybdic acid R* in *ethanol (96 per cent) R*; heat at 120 °C until spots appear (about 5 min).

Results The principal spots in the chromatogram obtained with the test solution are similar in position and colour to the principal spots in the chromatogram obtained with the reference solution.

B. Examine the chromatograms obtained in the assay.

Results The 2 principal peaks in the chromatogram obtained with test solution (b) are similar in retention time to the 2 principal peaks in the chromatograms obtained with reference solutions (a) and (b).

C. Dissolve 0.1 g in 10 mL of *water R* and shake. A foam is formed.

D. It gives a yellow colour to a non-luminous flame.

E. To 0.1 mL of the solution prepared for identification test C add 0.1 mL of a 1 g/L solution of *methylene blue R* and 2 mL of *dilute sulfuric acid R*. Add 2 mL of *methylene chloride R* and shake. The methylene chloride layer has an intense blue colour.

F. Mix about 10 mg with 10 mL of *anhydrous ethanol R*. Heat to boiling on a water-bath, shaking frequently. Filter immediately and evaporate to dryness. Dissolve the residue in 7 mL of *water R*, add 3 mL of *dilute hydrochloric acid R* and evaporate the solution to half its volume. Allow to cool. Filter. To the filtrate add 1 mL of *barium chloride solution R1*. A white, crystalline precipitate is formed.

TESTS

Acidity or alkalinity

Dissolve 0.5 g with heating in a mixture of 10 mL of *water R* and 15 mL of *ethanol (90 per cent V/V) R*. Add 0.1 mL of *phenolphthalein solution R1*. The solution is colourless. Add 0.1 mL of *0.1 M sodium hydroxide*. The solution is red.

Sodium chloride and sodium sulfate

Maximum 8.0 per cent for the sum of the percentage contents.

Sodium chloride Dissolve 5.00 g in 50 mL of *water R*, add *dilute nitric acid R* dropwise until the solution is neutral to *blue litmus paper R*. Titrate with *0.1 M silver nitrate* determining the end-point potentiometrically (*2.2.20*).
1 mL of *0.1 M silver nitrate* is equivalent to 5.844 mg of NaCl.

Sodium sulfate Dissolve 0.500 g in 20 mL of *water R*, warming gently if necessary, and add 1 mL of a 0.5 g/L solution of *dithizone R* in *acetone R*. If the solution is red, add *1 M nitric acid* dropwise until a bluish-green colour is obtained. Add 2.0 mL of *dichloroacetic acid solution R* and 80 mL of *acetone R*. Titrate with *0.01 M lead nitrate* until a persistent orange-red colour is obtained.
1 mL of *0.01 M lead nitrate* is equivalent to 1.420 mg of Na_2SO_4.

Free cetostearyl alcohol
Maximum 4.0 per cent.

From the chromatogram obtained with test solution (a) in the assay, calculate the percentage content of free cetostearyl alcohol in the substance to be examined using the following expression and taking into account the assigned content of the chemical reference substances:

$$\frac{A_1}{A_2} \times \frac{m_2}{m_1} \times \frac{2}{100} \times 100$$

A_1 = sum of the areas of the peaks due to cetyl alcohol and stearyl alcohol in the chromatogram obtained with test solution (a);
A_2 = area of the peak due to the internal standard in the chromatogram obtained with test solution (a);
m_1 = mass of the substance to be examined in test solution (a), in milligrams;
m_2 = mass of the internal standard in the internal standard solution, in milligrams.

Water (*2.5.12*)
Maximum 1.5 per cent, determined on 1.00 g.

ASSAY
Gas chromatography (*2.2.28*).

Internal standard solution Dissolve 0.200 g of *1-nonadecanol CRS* in *anhydrous ethanol R* and dilute to 100.0 mL with the same solvent.

Test solution (a) Dissolve 0.300 g of the substance to be examined in 50.0 mL of *anhydrous ethanol R* and add 2.0 mL of the internal standard solution and 48.0 mL of *water R*. Shake with 4 quantities, each of 25 mL, of *pentane R*, adding *sodium chloride R*, if necessary, to facilitate the separation of the layers. Combine the upper layers. Reserve the lower layer for the preparation of test solution (b). Wash the combined upper layers with 2 quantities, each of 30 mL, of *water R*, dry over *anhydrous sodium sulfate R* and filter.

Test solution (b) Transfer 25.0 mL of the lower layer obtained in the preparation of test solution (a) to a 200 mL flask that can be fitted with a reflux condenser. Add 10.0 mL of the internal standard solution and 20 mL of *hydrochloric acid R*. Boil under a reflux condenser for 2 h. Allow to cool and shake with 4 quantities, each of 20 mL, of *pentane R*. Combine the upper layers, wash with 2 quantities, each of 20 mL, of *water R*, dry over *anhydrous sodium sulfate R* and filter.

Reference solution (a) Dissolve 0.100 g of *cetyl alcohol CRS* in 25.0 mL of the internal standard solution. Add 25 mL of *water R* and shake with 4 quantities, each of 25 mL, of *pentane R*, adding *sodium chloride R*, if necessary, to facilitate the separation of the layers. Combine the upper layers, wash with 2 quantities, each of 30 mL, of *water R*, dry over *anhydrous sodium sulfate R* and filter.

Reference solution (b) Dissolve 0.100 g of *stearyl alcohol CRS* in 25.0 mL of the internal standard solution. Add 25 mL of *water R* and shake with 4 quantities, each of 25 mL, of *pentane R*, adding *sodium chloride R*, if necessary, to facilitate the separation of the layers. Combine the upper layers, wash with 2 quantities, each of 30 mL, of *water R*, dry over *anhydrous sodium sulfate R* and filter.

Column:
— *material*: fused silica;
— *size*: l = 25 m, Ø = 0.25 mm;
— *stationary phase*: *methylpolysiloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R.*

Flow rate 1 mL/min.

Split ratio 1:100.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 20	150 → 250
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 1 µL.

Elution order Cetyl alcohol, stearyl alcohol, 1-nonadecanol.

Calculate the percentage content of sodium cetyl sulfate in the substance to be examined using the following expression and taking into account the assigned content of *cetyl alcohol CRS*:

$$A_x \times \frac{A_2}{A_1} \times \frac{m_{x,y}}{A_{x,y}} \times \frac{1}{m} \times 100 \times 4 \times \frac{1}{2.5} \times F_x$$

A_x = area of the peak due to cetyl alcohol in the chromatogram obtained with test solution (b);
$A_{x,y}$ = area of the peak due to *cetyl alcohol CRS* in the chromatogram obtained with reference solution (a);
A_1 = area of the peak due to the internal standard in the chromatogram obtained with test solution (b);
A_2 = area of the peak due to the internal standard in the chromatogram obtained with reference solution (a);
F_x = conversion factor from cetyl alcohol to sodium cetyl sulfate (1.421);
m = mass of the substance to be examined in test solution (a), in milligrams;
$m_{x,y}$ = mass of *cetyl alcohol CRS* in reference solution (a), in milligrams.

Calculate the percentage content of sodium stearyl sulfate in the substance to be examined using the following expression and taking into account the assigned content of *stearyl alcohol CRS*:

$$A_z \times \frac{A_3}{A_1} \times \frac{m_{z,y}}{A_{z,y}} \times \frac{1}{m} \times 100 \times 4 \times \frac{1}{2.5} \times F_z$$

A_z = area of the peak due to stearyl alcohol in the chromatogram obtained with test solution (b);
$A_{z,y}$ = area of the peak due to *stearyl alcohol CRS* in the chromatogram obtained with reference solution (b);
A_1 = area of the peak due to the internal standard in the chromatogram obtained with test solution (b);
A_3 = area of the peak due to the internal standard in the chromatogram obtained with reference solution (b);
F_z = conversion factor from stearyl alcohol to sodium stearyl sulfate (1.377);
m = mass of the substance to be examined in test solution (a), in milligrams;
$m_{z,y}$ = mass of *stearyl alcohol CRS* in reference solution (b), in milligrams.

The percentage content of sodium cetostearyl sulfate corresponds to the sum of the percentage contents of sodium cetyl sulfate and sodium stearyl sulfate.

LABELLING
The label states, where appropriate, the name and concentration of any added buffer.

Ph Eur

Sodium Chloride[1]

(Ph. Eur. monograph 0193)

NaCl 58.44 *7647-14-5*

Action and use
Used in treatment of electrolyte deficiency.

Preparations
Compound Glucose, Sodium Chloride and Sodium Citrate Oral Solution

Compound Sodium Chloride Mouthwash

Magnesium Sulfate, Potassium Chloride and Sodium Chloride Infusion

Oral Rehydration Salts

Potassium Chloride and Sodium Chloride Infusion

Potassium Chloride, Sodium Chloride and Glucose Infusion

Sodium Chloride Eye Drops

Sodium Chloride Eye Lotion

Sodium Chloride Eye Ointment

Sodium Chloride and Glucose Infusion

Sodium Chloride Infusion

Sodium Chloride Irrigation Solution

Sodium Chloride Nebuliser Solution

Sodium Chloride Oral Solution

Sodium Chloride Solution

Sodium Chloride Soluble Tablets

Ph Eur

DEFINITION
Content
99.0 per cent to 100.5 per cent (dried substance).

♦CHARACTERS
Appearance
White or almost white, crystalline powder or colourless crystals or white or almost white pearls.

Solubility
Freely soluble in water, practically insoluble in anhydrous ethanol.♦

IDENTIFICATION
A. It gives reaction (a) of chlorides (*2.3.1*).

B. It gives reaction (a) of sodium (*2.3.1*).

TESTS
◊*If the substance is in the form of pearls, crush before use.*◊

Solution S
Dissolve 20.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100.0 mL with the same solvent.

♦Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).♦

Acidity or alkalinity
To 20 mL of solution S add 0.1 mL of *bromothymol blue solution R1*. Not more than 0.5 mL of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Bromides
Maximum 100 ppm.

To 0.5 mL of solution S add 4.0 mL of *water R*, 2.0 mL of *phenol red solution R2* and 1.0 mL of a 0.1 g/L solution of *chloramine R* and mix immediately. After exactly 2 min, add 0.15 mL of *0.1 M sodium thiosulfate*, mix and dilute to 10.0 mL with *water R*. The absorbance (*2.2.25*) of the solution measured at 590 nm, using *water R* as the compensation liquid, is not greater than that of a standard prepared at the same time and in the same manner, using 5.0 mL of a 3.0 mg/L solution of *potassium bromide R*.

Ferrocyanides
Dissolve 2.0 g in 6 mL of *water R*. Add 0.5 mL of a mixture of 5 mL of a 10 g/L solution of *ferric ammonium sulfate R* in a 2.5 g/L solution of *sulfuric acid R* and 95 mL of a 10 g/L solution of *ferrous sulfate R*. No blue colour develops within 10 min.

Iodides
Moisten 5 g by the dropwise addition of a freshly prepared mixture of 0.15 mL of *sodium nitrite solution R*, 2 mL of *0.5 M sulfuric acid*, 25 mL of *iodide-free starch solution R* and 25 mL of *water R*. After 5 min, examine in daylight.
The mixture shows no blue colour.

Nitrites
To 10 mL of solution S add 10 mL of *water R*.
The absorbance (*2.2.25*) is not greater than 0.01 at 354 nm.

Phosphates (*2.4.11*)
Maximum 25 ppm.

Dilute 2 mL of solution S to 100 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 200 ppm.

Dilute 7.5 mL of solution S to 30 mL with *distilled water R*.

Aluminium (*2.4.17*)
Maximum 0.2 ppm, if intended for use in the manufacture of peritoneal dialysis solutions, haemodialysis solutions or haemofiltration solutions.

Prescribed solution Dissolve 20.0 g in 100 mL of *water R* and add 10 mL of *acetate buffer solution pH 6.0 R*.

Reference solution Mix 2 mL of *aluminium standard solution (2 ppm Al) R*, 10 mL of *acetate buffer solution pH 6.0 R* and 98 mL of *water R*.

Blank solution Mix 10 mL of *acetate buffer solution pH 6.0 R* and 100 mL of *water R*.

♦Arsenic (*2.4.2, Method A*)
Maximum 1 ppm, determined on 5 mL of solution S.♦

Barium
To 5 mL of solution S add 5 mL of *distilled water R* and 2 mL of *dilute sulfuric acid R*. After 2 h, any opalescence in the solution is not more intense than that in a mixture of 5 mL of solution S and 7 mL of *distilled water R*.

Iron (*2.4.9*)
Maximum 2 ppm, determined on solution S.

Prepare the standard using a mixture of 4 mL of *iron standard solution (1 ppm Fe) R* and 6 mL of *water R*.

[1] *This monograph has undergone pharmacopoeial harmonisation. See chapter 5.8 Pharmacopoeial harmonisation.*

Magnesium and alkaline-earth metals (*2.4.7*)
Maximum 100 ppm, calculated as Ca and determined on 10.0 g.

Use 0.150 g of *mordant black 11 triturate R*. The volume of *0.01 M sodium edetate* used is not more than 2.5 mL.

Potassium
Maximum 500 ppm, if intended for use in the manufacture of parenteral preparations or haemodialysis, haemofiltration or peritoneal dialysis solutions.

Atomic emission spectrometry (*2.2.22, Method I*).

Test solution Dissolve 1.00 g in *water R* and dilute to 100.0 mL with the same solvent.

Reference solutions Dissolve 1.144 g of *potassium chloride R*, previously dried at 100-105 °C for 3 h, in *water R* and dilute to 1000.0 mL with the same solvent (600 µg of K per millilitre). Dilute as required.

Wavelength 766.5 nm.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

♦Bacterial endotoxins (*2.6.14*)
Less than 5 IU/g, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for removal of bacterial endotoxins.♦

ASSAY
Dissolve 50.0 mg in *water R* and dilute to 50 mL with the same solvent. Titrate with *0.1 M silver nitrate* determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M silver nitrate* is equivalent to 5.844 mg of NaCl.

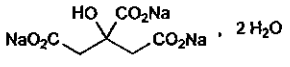
♦LABELLING
The label states:
— where applicable, that the substance is suitable for use in the manufacture of parenteral preparations;
— where applicable, that the substance is suitable for use in the manufacture of peritoneal dialysis solutions, haemodialysis solutions or haemofiltration solutions.♦

Ph Eur

Sodium Citrate

Trisodium Citrate

(Ph. Eur. monograph 0412)



$C_6H_5Na_3O_7,2H_2O$ 294.1 *6132-04-3*

Action and use
Systemic alkalinising substance.

Preparations
Compound Glucose, Sodium Chloride and Sodium Citrate Oral Solution

Sodium Citrate Eye Drops

Sodium Citrate Irrigation Solution

Ph Eur

DEFINITION
Trisodium 2-hydroxypropane-1,2,3-tricarboxylate dihydrate.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder or white or almost white, granular crystals, slightly deliquescent in moist air.

Solubility
Freely soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION
A. To 1 mL of solution S (see Tests) add 4 mL of *water R*. The solution gives the reaction of citrates (*2.3.1*).

B. 1 mL of solution S gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S
Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity
To 10 mL of solution S add 0.1 mL of *phenolphthalein solution R*. Not more than 0.2 mL of *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Readily carbonisable substances
To 0.20 g of the powdered substance to be examined add 10 mL of *sulfuric acid R* and heat in a water-bath at 90 ± 1 °C for 60 min. Cool rapidly. The solution is not more intensely coloured than reference solution Y_2 or GY_2 (*2.2.2, Method II*).

Chlorides (*2.4.4*)
Maximum 50 ppm.

Dilute 10 mL of solution S to 15 mL with *water R*.

Oxalates
Maximum 300 ppm.

Dissolve 0.50 g in 4 mL of *water R*, add 3 mL of *hydrochloric acid R* and 1 g of *zinc R* in granules and heat on a water-bath for 1 min. Allow to stand for 2 min, decant the liquid into a test-tube containing 0.25 mL of a 10 g/L solution of *phenylhydrazine hydrochloride R* and heat to boiling. Cool rapidly, transfer to a graduated cylinder and add an equal volume of *hydrochloric acid R* and 0.25 mL of *potassium ferricyanide solution R*. Shake and allow to stand for 30 min. Any pink colour in the solution is not more intense than that in a standard prepared at the same time in the same manner using 4 mL of a 50 mg/L solution of *oxalic acid R*.

Sulfates (*2.4.13*)
Maximum 150 ppm.

To 10 mL of solution S add 2 mL of *hydrochloric acid R1* and dilute to 15 mL with *distilled water R*.

Water (*2.5.12*)
11.0 per cent to 13.0 per cent, determined on 0.300 g.
Use as the solvent a mixture of 20 mL of *methanol R*, 30 mL of *formamide R* and 5 g of *salicylic acid R*.

Pyrogens (*2.6.8*)
If intended for use in the manufacture of large-volume parenteral preparations, the competent authority may require that it comply with the test for pyrogens. Inject per kilogram of the rabbit's mass 10 mL of a freshly prepared solution in *water for injections R* containing per millilitre 10.0 mg of the substance to be examined and 7.5 mg of pyrogen-free *calcium chloride R*.

ASSAY

Dissolve 0.150 g in 20 mL of *anhydrous acetic acid R*, heating to about 50 °C. Allow to cool. Titrate with *0.1 M perchloric acid*, using 0.25 mL of *naphtholbenzein solution R* as indicator until a green colour is obtained.

1 mL of *0.1 M perchloric acid* is equivalent to 8.602 mg of $C_6H_5Na_3O_7$.

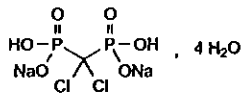
STORAGE

In an airtight container.

Ph Eur

Sodium Clodronate Tetrahydrate

(Clodronate Disodium Tetrahydrate, Ph. Eur. monograph 1777)



$CH_2Cl_2Na_2O_6P_2,4H_2O$ 360.9

Ph Eur

DEFINITION

Disodium (dichloromethylene)bis(hydrogen phosphonate) tetrahydrate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, practically insoluble in ethanol (96 per cent), slightly soluble in methanol.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *clodronate disodium tetrahydrate CRS.*

B. Dissolve 0.5 g in 10 mL of *water R*. The solution gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)

3.0 to 4.5, for solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.125 g of the substance to be examined in 30 mL of *water R*, sonicate for 10 min and dilute to 50.0 mL with *water R* (*test stock solution*). Dilute 10.0 mL of the test stock solution to 20.0 mL with *water R*.

Reference solution (a) Dilute 1.0 mL of the test solution to 10.0 mL with *water R*. Dilute 1.0 mL of this solution to 50.0 mL with *water R*.

Reference solution (b) Dissolve 1 mg of *clodronate impurity D CRS* in 10 mL of *water R*, sonicate for 10 min and dilute to 20.0 mL with *water R*. Mix 2.0 mL of this solution with 10.0 mL of the test stock solution and dilute to 20.0 mL with *water R*.

Reference solution (c) Dilute 1.0 mL of a 0.3 g/L solution of *phosphoric acid R* (impurity B) to 100.0 mL with *water R*.

Precolumn:
- *size*: *l* = 0.05 m, Ø = 4 mm;
- *stationary phase*: *anion-exchange resin R*;
- *particle size*: 9 µm.

Column:
- *size*: *l* = 0.25 m, Ø = 4 mm;
- *stationary phase*: *anion-exchange resin R*;
- *particle size*: 9 µm.

Mobile phase:
- *mobile phase A*: 0.21 g/L solution of *sodium hydroxide R* in *carbon dioxide-free water R*; close immediately, mix and use under helium pressure;
- *mobile phase B*: 4.2 g/L solution of *sodium hydroxide R* in *carbon dioxide-free water R*; close immediately, mix and use under helium pressure;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	90 → 60	10 → 40
10 - 22	60 → 50	40 → 50
22 - 23	50 → 20	50 → 80
23 - 25	20	80

Flow rate 1 mL/min.

Detection Conductivity detector. Use a self-regenerating anion suppressor.

Injection 20 µL.

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peak due to impurity B.

Relative retention With reference to clodronate (retention time = about 13 min): impurities A and B = about 0.7; impurity D = about 1.1.

System suitability Reference solution (b):
- *peak-to-valley ratio*: minimum 3, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to clodronate.

Limits:
- *sum of impurities A and B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
- *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)

18.5 per cent to 21.0 per cent, determined on 0.100 g.

ASSAY

Dissolve 0.140 g in 10 mL of *water R*. Add 10 mL of *strong sodium hydroxide solution R* and some glass beads. Boil until the solution is completely decolourised (about 10 min). Cool

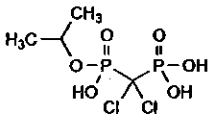
in an ice-bath and add 30 mL of *water R* and 10 mL of *nitric acid R*. Titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M silver nitrate* is equivalent to 14.44 mg of $CH_2Cl_2Na_2O_6P_2$.

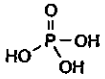
IMPURITIES

Specified impurities *A, B.*

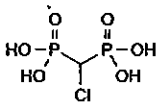
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *D.*



A. [dichloro[hydroxy(1-methylethoxy)phosphinoyl]methyl] phosphonic acid,



B. phosphoric acid,

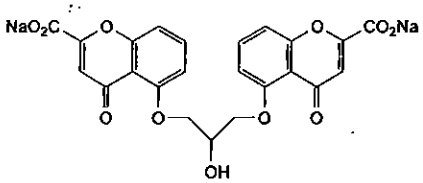


D. (chloromethylene)bis(phosphonic acid).

Ph Eur

Sodium Cromoglicate

(Ph. Eur. monograph 0562)



$C_{23}H_{14}Na_2O_{11}$ 512.3 *15826-37-6*

Action and use

Prophylaxis of allergic conditions.

Preparation

Sodium Cromoglicate Eye Drops

Ph Eur

DEFINITION

Disodium 5,5′-[(2-hydroxypropane-1,3-diyl)bis(oxy)]bis(4-oxo-4*H*-1-benzopyran-2-carboxylate).

Content

98.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, hygroscopic, crystalline powder.

Solubility

Soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 10.0 mg in *phosphate buffer solution pH 7.4 R* and dilute to 100.0 mL with the same buffer solution. Dilute 10.0 mL of the solution to 100.0 mL with *phosphate buffer solution pH 7.4 R*.

Spectral range 230-350 nm.

Absorption maxima 239 nm and 327 nm.

Absorbance ratio A_{327}/A_{239} = 0.25 to 0.30.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *sodium cromoglicate CRS.*

C. Dissolve about 5 mg in 0.5 mL of *methanol R*. Add 3 mL of a 5 g/L solution of *4-aminoantipyrine R* in *methanol R* containing 1 per cent *V/V* of *hydrochloric acid R*. Allow to stand for 5 min. An intense yellow colour develops.

D. It gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

Acidity or alkalinity

To 10 mL of solution S add 0.1 mL of *phenolphthalein solution R*. The solution is colourless. Add 0.2 mL of a 0.40 g/L solution of *sodium hydroxide R*. The solution is pink. Add 0.4 mL of a 1.03 g/L solution of *hydrochloric acid R*. The solution is colourless. Add 0.25 mL of *methyl red solution R*. The solution is red.

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture *water R, acetonitrile R* (40:60 *V/V*).

Test solution Dissolve 0.100 g of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 7 mg of *sodium cromoglicate for system suitability CRS* (containing impurity C) in the solvent mixture and dilute to 10 mL with the solvent mixture.

Column:

— *size: l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase: base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:

— *mobile phase A: acetonitrile R*, 10 g/L solution of *tetrabutylammonium hydrogen sulfate R* (5:95 *V/V*);

— *mobile phase B: acetonitrile R*, 10 g/L solution of *tetrabutylammonium hydrogen sulfate R* (50:50 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	100 → 0	0 → 100
15 - 20	0	100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 330 nm.

Injection 10 µL.

Identification of impurities Use the chromatogram supplied with *sodium cromoglicate for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity C.

Relative retention With reference to cromoglicate (retention time = about 11 min): impurity C = about 1.1.

System suitability Reference solution (b):
— *resolution*: minimum 5.0 between the peaks due to cromoglicate and impurity C.

Limits:
— *impurity C*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Oxalates

Maximum 0.35 per cent.

Dissolve 0.10 g in 20 mL of *water R*, add 5.0 mL of *iron salicylate solution R* and dilute to 50.0 mL with *water R*. Measure the absorbance (*2.2.25*) at 480 nm. The absorbance is not less than that of a reference solution prepared in the same manner using 0.35 mg of *oxalic acid R* instead of the substance to be examined.

Water (*2.5.12*)

Maximum 10.0 per cent, determined on 0.250 g. Use as the solvent a mixture of 10 mL of *anhydrous methanol R* and 20 mL of *formamide R*.

ASSAY

Dissolve 0.200 g with heating in a mixture of 5 mL of *2-propanol R* and 25 mL of *ethylene glycol R*. Cool and add a mixture of 6 mL of *tetrahydrofuran R* and 24 mL of *acetonitrile R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 25.62 mg of $C_{23}H_{14}Na_2O_{11}$.

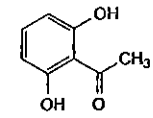
STORAGE

In an airtight container, protected from light.

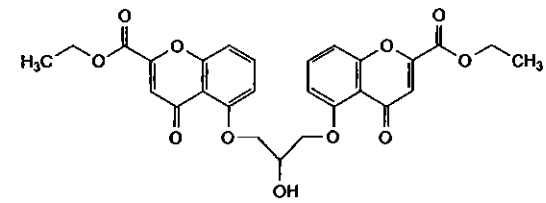
IMPURITIES

Specified impurities *C.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B.*



A. 1-(2,6-dihydroxyphenyl)ethan-1-one,



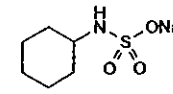
B. diethyl 5,5′-[(2-hydroxypropane-1,3-diyl)bis(oxy)]bis(4-oxo-4*H*-1-benzopyran-2-carboxylate),

C. unknown structure.

Ph Eur

Sodium Cyclamate

(Ph. Eur. monograph 0774)



$C_6H_{12}NNaO_3S$ 201.2 *139-05-9*

Action and use

Sweetening agent.

Ph Eur

DEFINITION

Sodium *N*-cyclohexylsulfamate.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: A, E.

Second identification: B, C, D, E.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *sodium cyclamate CRS.*

B. Examine the chromatograms obtained in the test for impurity A.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. To 1 mL of solution S (see Tests), add 1 mL of *water R* and 2 mL of *silver nitrate solution R1*, then shake. A white, crystalline precipitate is formed.

D. To 1 mL of solution S add 5 mL of *water R*, 2 mL of *dilute hydrochloric acid R* and 4 mL of *barium chloride solution R1* and mix. The solution is clear. Add 2 mL of

sodium nitrite solution R. A voluminous white precipitate is formed and gas is given off.

E. A mixture of 1 mL of solution S and 1 mL of *water R* gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S

Dissolve 5 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)

5.5 to 7.5 for solution S.

Absorbance (*2.2.25*)

Maximum 0.10, determined at 270 nm on solution S.

Impurity A

Thin-layer chromatography (*2.2.27*).

Test solution (a) Solution S.

Test solution (b) Dilute 1 mL of test solution (a) to 10 mL with *water R*.

Reference solution (a) Dissolve 0.10 g of *sodium cyclamate CRS* in *water R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 10 mg of *sulfamic acid R* (impurity A) in *water R* and dilute to 100 mL with the same solvent.

Plate *TLC silica gel G plate R*.

Mobile phase *concentrated ammonia R, water R, ethyl acetate R, propanol R* (10:10:20:70 *V/V/V/V*).

Application 2 µL.

Development Over a path of 12 cm.

Drying In a current of warm air, then heat at 105 °C for 5 min.

Detection Spray the hot plate with *strong sodium hypochlorite solution R* diluted to a concentration of 5 g/L of active chlorine. Place in a current of cold air until an area of coating below the points of application gives at most a faint blue colour with a drop of *potassium iodide and starch solution R*; avoid prolonged exposure to cold air. Spray with *potassium iodide and starch solution R* and examine the chromatograms within 5 min.

Limit Test solution (a):

— *impurity A*: any spot due to impurity A is not more intense than the corresponding spot in the chromatogram obtained with reference solution (b) (0.1 per cent).

Impurities B, C and D

Gas chromatography (*2.2.28*).

Internal standard solution Dissolve 2 µL of *tetradecane R* in *methylene chloride R* and dilute to 100 mL with the same solvent.

Test solution Dissolve 2.00 g of the substance to be examined in 20 mL of *water R*, add 0.5 mL of *strong sodium hydroxide solution R* and shake with 30 mL of *toluene R*. Shake 20 mL of the upper layer with 4 mL of a mixture of equal volumes of *dilute acetic acid R* and *water R*. Separate the lower layer, add 0.5 mL of *strong sodium hydroxide solution R* and 0.5 mL of the internal standard solution and shake. Use the lower layer immediately after separation.

Reference solution Dissolve 10.0 mg (about 12 µL) of *cyclohexylamine R* (impurity C), 1.0 mg (about 1.1 µL) of *dicyclohexylamine R* (impurity D) and 1.0 mg (about 1 µL) of *aniline R* (impurity B) in *water R*, then dilute to 1000 mL with the same solvent. Dilute 10.0 mL of this solution to 100.0 mL with *water R* (solution A). To 20.0 mL of solution A, add 0.5 mL of *strong sodium hydroxide solution R* and extract with 30 mL of *toluene R*. Shake 20 mL of the upper layer with 4 mL of a mixture of equal volumes of *dilute acetic acid R* and *water R*. Separate the lower layer, add 0.5 mL of *strong sodium hydroxide solution R* and 0.5 mL of the internal standard solution and shake. Use the lower layer immediately after separation.

Column:

— *material*: fused silica;

— *size*: *l* = 25 m, Ø = 0.32 mm;

— *stationary phase*: *phenyl(5)methyl(95)polysiloxane R* (film thickness 0.51 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.8 mL/min.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 1	85
	1 - 9	85 → 150
	9 - 13	150
Injection port		250
Detector		270

Detection Flame ionisation.

Injection 1.5 µL; use a split vent at a flow rate of 20 mL/min.

Relative retention With reference to impurity C (retention time = about 2.3 min): impurity B = about 1.4; tetradecane = about 4.3; impurity D = about 4.5.

Limits:

— *impurity C*: maximum 10 ppm;

— *impurities B, D*: for each impurity, maximum 1 ppm.

Sulfates (*2.4.13*)

Maximum 0.1 per cent.

Dilute 1.5 mL of solution S to 15 mL with *distilled water R*.

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

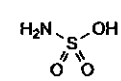
ASSAY

Dissolve without heating 0.150 g in 60 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

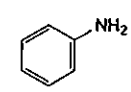
1 mL of *0.1 M perchloric acid* is equivalent to 20.12 mg of $C_6H_{12}NNaO_3S$.

IMPURITIES

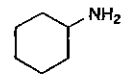
Specified impurities *A, B, C, D.*



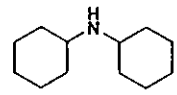
A. sulfamic acid,



B. aniline (phenylamine),



C. cyclohexanamine,



D. *N*-cyclohexylcyclohexanamine.

Ph Eur

Sodium Dihydrogen Phosphate

Anhydrous Sodium Dihydrogen Phosphate

NaH_2PO_4 120.0 *7558-80-7*

Action and use
Excipient.

Preparation
Phosphates Enema

DEFINITION
Sodium Dihydrogen Phosphate contains not less than 98.0% and not more than 100.5% of NaH_2PO_4, calculated with reference to the dried substance.

CHARACTERISTICS
White, slightly deliquescent crystals or granules.

Very soluble in *water*; very slightly soluble in *ethanol (96%)*.

Dissolve 10.0 g in sufficient *carbon dioxide-free water* prepared from *distilled water* to produce 100 mL (solution S).

IDENTIFICATION
A. Solution S is *slightly acid*, Appendix V K.

B. Solution S yields the reactions characteristic of *phosphates*, Appendix VI.

C. Solution S neutralised with a 10% w/v solution of *potassium hydroxide* yields reaction A characteristic of *sodium salts*, Appendix VI.

D. Complies with the test for Loss on drying.

TESTS
Acidity
pH of a mixture of 5 mL of solution S and 5 mL of *carbon dioxide-free water*, 4.2 to 4.5, Appendix V L.

Clarity and colour of solution
Solution S is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method II.

Arsenic
0.5 g complies with the *limit test for arsenic*, Appendix VII (2 ppm).

Iron
10 mL of solution S complies with the *limit test for iron*, Appendix VII (10 ppm).

Chloride
Dilute 2.5 mL of solution S to 15 mL with *water*.
The resulting solution complies with the *limit test for chlorides*, Appendix VII (200 ppm).

Sulfate
To 5 mL of solution S add 0.5 mL of *hydrochloric acid* and dilute to 15 mL with *distilled water*. The resulting solution complies with the *limit test for sulfates*, Appendix VII (300 ppm).

Reducing substances
To 5 mL of solution S add 0.25 mL of 0.02M *potassium permanganate* and 5 mL of 1M *sulfuric acid* and heat in a water bath for 5 minutes. The solution retains a slight red colour.

Loss on drying
When dried to constant weight at 130°, loses not more than 1.0% of its weight. Use 1 g.

ASSAY
Dissolve 2 g in 50 mL of *water* and titrate with carbonate-free *1M sodium hydroxide VS* determining the end point potentiometrically. Each mL of *1M sodium hydroxide VS* is equivalent to 0.120 g of NaH_2PO_4.

Sodium Dihydrogen Phosphate Dihydrate

Sodium Acid Phosphate

(Ph. Eur. monograph 0194)

$NaH_2PO_4,2H_2O$ 156.0 *13472-35-0*

Action and use
Excipient.

Preparation
Phosphates Enema

Ph Eur

DEFINITION
Content
98.0 per cent to 100.5 per cent (dried substance).

CHARACTERS
Appearance
White or almost white powder or colourless crystals.

Solubility
Very soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION
A. Solution S (see Tests) is slightly acid (*2.2.4*).

B. Solution S gives the reactions of phosphates (*2.3.1*).

C. Solution S previously neutralised using a 100 g/L solution of *potassium hydroxide R* gives reaction (a) of sodium (*2.3.1*).

TESTS
Solution S
Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)
4.2 to 4.5.

To 5 mL of solution S add 5 mL of *carbon dioxide-free water R*.

Reducing substances
To 5 mL of solution S add 0.25 mL of a 3.2 g/L solution of *potassium permanganate R* and 5 mL of *dilute sulfuric acid R* and heat in a water-bath for 5 min. The colour of the permanganate is not completely discharged.

Chlorides (*2.4.4*)
Maximum 200 ppm.
Dilute 2.5 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 300 ppm.
To 5 mL of solution S add 0.5 mL of *hydrochloric acid R* and dilute to 15 mL with *distilled water R*.

Iron (*2.4.9*)
Maximum 10 ppm, determined on solution S.

Loss on drying (*2.2.32*)
21.5 per cent to 24.0 per cent, determined on 0.500 g by drying in an oven at 130 °C.

ASSAY
Dissolve 2.500 g in 40 mL of *water R*. Titrate with carbonate-free *1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).
1 mL of *1 M sodium hydroxide* is equivalent to 0.120 g of NaH_2PO_4.

Ph Eur

Sodium Dihydrogen Phosphate Monohydrate

NaH_2PO_4,H_2O 138.0 *10049-21-5*

Action and use
Excipient.

Preparation
Phosphates Enema

DEFINITION
Sodium Dihydrogen Phosphate Monohydrate contains not less than 98.0% and not more than 100.5% of NaH_2PO_4, calculated with reference to the dried substance.

CHARACTERISTICS
Colourless crystals or a white powder.
Very soluble in *water*; very slightly soluble in *ethanol (96%)*.
Dissolve 10.0 g in sufficient *carbon dioxide-free water* prepared from *distilled water* to produce 100 mL (solution S).

IDENTIFICATION
A. Solution S is *slightly acid*, Appendix V K.
B. Solution S yields the reactions characteristic of *phosphates*, Appendix VI.
C. Solution S neutralised with a 10% w/v solution of *potassium hydroxide* yields reaction A characteristic of *sodium salts*, Appendix VI.
D. Complies with the test for Loss on drying.

TESTS
Acidity
pH of a mixture of 5 mL of solution S and 5 mL of *carbon dioxide-free water*, 4.2 to 4.5, Appendix V L.

Clarity and colour of solution
Solution S is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method II.

Arsenic
0.5 g complies with the *limit test for arsenic*, Appendix VII (2 ppm).

Iron
10 mL of solution S complies with the *limit test for iron*, Appendix VII (10 ppm).

Chloride
Dilute 2.5 mL of solution S to 15 mL with *water*.
The resulting solution complies with the *limit test for chlorides*, Appendix VII (200 ppm).

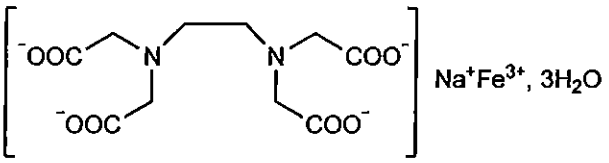
Sulfate
To 5 mL of solution S add 0.5 mL of *hydrochloric acid* and dilute to 15 mL with *distilled water*. The resulting solution complies with the *limit test for sulfates*, Appendix VII (300 ppm).

Reducing substances
To 5 mL of solution S add 0.25 mL of 0.02M *potassium permanganate* and 5 mL of 1M *sulfuric acid* and heat in a water bath for 5 minutes. The solution retains a slight red colour.

Loss on drying
When dried to constant weight at 130°, loses 11.5 to 14.5% of its weight. Use 0.5 g.

ASSAY
Dissolve 2.2 g in 50 mL of *water* and titrate with carbonate-free *1M sodium hydroxide VS* determining the end point potentiometrically. Each mL of *1M sodium hydroxide VS* is equivalent to 0.120 g of NaH_2PO_4.

Sodium Feredetate



$C_{10}H_{12}N_2O_8FeNa,3H_2O$ 421.1 *15708-41-5 (anhydrous)*

Action and use
Source of iron.

Preparation
Sodium Feredetate Oral Solution

DEFINITION
Sodium Feredetate is iron(III) sodium ethylenediaminetetra-acetate trihydrate. It contains not less than 98.0% and not more than 102.0% of $C_{10}H_{12}N_2O_8FeNa$, calculated with reference to the anhydrous substance.

CHARACTERISTICS
A yellow or yellowish brown, crystalline powder.

IDENTIFICATION
A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of sodium feredetate *(RS 378)*.
B. Ignite 0.5 g and allow to cool. Dissolve the residue in 2 mL of *hydrochloric acid*, add sufficient *water* to produce 20 mL and filter. The filtrate yields reaction C characteristic of *iron salts*, Appendix VI. Dilute 1 volume of the filtrate to 10 volumes with *water*; the solution yields reaction B characteristic of *iron salts*, Appendix VI.
C. Dissolve 2 g in 30 mL of *water*, slowly add 6.5 mL of a 20% w/v solution of *potassium hydroxide*, shake and filter the resulting suspension. Evaporate 8 mL of the filtrate to dryness, ignite and dissolve the residue in 4 mL of *water*. The solution yields reaction B characteristic of *sodium salts*, Appendix VI.

TESTS

Acidity
pH of a 1% w/v solution, 3.5 to 5.5, Appendix V L.

Free iron
Not more than 500 ppm, when determined by the following method. Dissolve 0.2 g in sufficient *water* to produce 20 mL and filter. Label three tubes A, B and C. Place 5 mL of the filtrate into each of tubes A and B and 4 mL of *water* and 1 mL of an iron standard solution prepared in the following manner in tube C. For the iron standard solution dilute 25 volumes of a 0.1726% w/v solution of *ammonium iron(III) sulfate* in 0.05M *sulfuric acid* to 200 volumes with *water* (25 ppm of Fe(III)). Add 1 mL of a 1.0% w/v solution of *disodium catechol-3,5-disulfonate* into each of tubes A and C and 1 mL of *water* to tube B. Measure the *absorbance* of solution A at 670 nm, Appendix II B, using solution B in the reference cell and of solution C using *water* in the reference cell. The absorbance of solution A is not greater than that of solution C.

Free sodium edetate
To 4 mL of a 1.0% w/v solution add 2 mL of an iron standard solution prepared in the following manner and 1 mL of a 1.0% w/v solution of *disodium catechol-3,5-disulfonate* and mix. For the iron standard solution dilute 50 volumes of a 0.1726% w/v solution of *ammonium iron(III) sulfate* in 0.05M *sulfuric acid* to 200 volumes with *water* (50 ppm of Fe(III)). Prepare a standard in the same manner using 4 mL of a 0.010% w/v solution of *disodium edetate* in place of the solution of the substance being examined. Measure the *absorbance* of the solutions at 670 nm, Appendix II B, using *water* in the reference cell. The absorbance of the solution of the substance being examined is not less than that of the standard solution (1%).

Nitrilotriacetic acid
Carry out the method for *liquid chromatography*, Appendix III D, protected from light, using the following solutions.

Solvent A To 10 volumes of a 50% w/v solution of *iron(III) sulfate pentahydrate* in 0.5M *sulfuric acid* add 390 volumes of *water*, adjust to a pH of 2.0 with 1M *sodium hydroxide* and dilute to 500 volumes with *water*.

(1) 0.4% w/v of the substance being examined in solvent A. Mix with the aid of ultrasound.

(2) 0.0004% w/v of *nitrilotriacetic acid* in solvent A.

(3) 0.0004% w/v of *nitrilotriacetic acid* in solution (1).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with spherical *graphitised carbon for chromatography R1* (5 µm) with a specific surface area of 120 m^2/g and a pore size of 25 nm (Hypercarb is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 273 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

To 50 volumes of a 0.01% w/v solution of *iron(III) sulfate pentahydrate* in 0.5M *sulfuric acid*, add 750 volumes of *water*. Adjust to a pH of 1.5 with 0.5M *sulfuric acid* or 1M *sodium hydroxide* and add 20 volumes of *ethylene glycol*. Dilute to 1000 volumes with *water*.

When the chromatograms are recorded under the prescribed conditions the retention of sodium feredetate relative to nitrilotriacetic acid (retention time, about 5 minutes) is about 2.0.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to nitrilotriacetic acid and sodium feredetate is at least 4.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to nitrilotriacetic acid is not greater than the area of the principal peak in the chromatogram obtained with solution 2 (0.1%).

Chlorides
Dissolve 1 g in 100 mL of *water* and filter. 15 mL of the filtrate complies with the *limit test for chlorides*, Appendix VII (350 ppm).

Sulfates
Dissolve 2 g in 40 mL of *water*, add 5 mL of a 20% w/v solution of *sodium hydroxide* and sufficient *water* to produce 50 mL, shake and filter. Evaporate 6.2 mL of this solution to dryness and ignite until no trace of carbon remains. Cool and dissolve the residue in 10 mL of *distilled water*. Neutralise the solution with 2M *hydrochloric acid* using *litmus paper* as external indicator and add 2 mL in excess. Boil the solution, cool, dilute to 15 mL with *distilled water* and filter. The filtrate complies with the *limit test for sulfates*, Appendix VII (600 ppm).

Water
12.0% to 13.5% w/w, Appendix IX C, Method I. Use 0.1 g.

ASSAY
Dissolve 1 g in 40 mL of *water* in an iodine flask, add 20 mL of *hydrochloric acid* and 3 g of *potassium iodide*, close the flask and allow to stand for 5 minutes. Titrate the liberated iodine with 0.1M *sodium thiosulfate VS* using *starch mucilage* as indicator. Repeat the procedure without the substance being examined. The difference between the titrations represents the amount of sodium thiosulfate required. Each mL of 0.1M *sodium thiosulfate VS* is equivalent to 36.71 mg of $C_{10}H_{12}N_2O_8FeNa$.

Sodium Fluoride

(Ph. Eur. monograph 0514)

NaF 41.99 *7681-49-4*

Action and use
Used in prevention of dental caries.

Preparations
Sodium Fluoride Mouthwash

Sodium Fluoride Oral Drops

Sodium Fluoride Oral Solution

Sodium Fluoride Tablets

Ph Eur

DEFINITION
Content
98.5 per cent to 100.5 per cent (dried substance).

CHARACTERS
Appearance
White or almost white powder or colourless crystals.

Solubility
Soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION
A. To 2 mL of solution S (see Tests) add 0.5 mL of *calcium chloride solution R*. A gelatinous white precipitate is formed that dissolves on adding 5 mL of *ferric chloride solution R1*.

B. To about 4 mg add a mixture of 0.1 mL of *alizarin S solution R* and 0.1 mL of *zirconyl nitrate solution R* and mix. The colour changes from red to yellow.

C. Solution S gives reaction (a) of sodium (*2.3.1*).

TESTS
Solution S
Dissolve 2.5 g in *carbon dioxide-free water R* without heating and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity
Dissolve 2.5 g of *potassium nitrate R* in 40 mL of solution S and dilute to 50 mL with *carbon dioxide-free water R*. Cool to 0 °C and add 0.2 mL of *phenolphthalein solution R*. If the solution is colourless, not more than 1.0 mL of *0.1 M sodium hydroxide* is required to produce a red colour that persists for at least 15 s. If the solution is red, not more than 0.25 mL of *0.1 M hydrochloric acid* is required to change the colour of the indicator.

Chlorides (*2.4.4*)
Maximum 200 ppm.

Dilute 10 mL of solution S to 15 mL with *water R*.

Fluorosilicates
Heat to boiling the neutralised solution obtained in the test for acidity or alkalinity and titrate whilst hot. Not more than 0.75 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator to red.

Sulfates (*2.4.13*)
Maximum 200 ppm.

Dissolve 0.25 g in 10 mL of a 223 g/L solution of *aluminium nitrate R*. Add 5 mL of *distilled water R* and 0.6 mL of *hydrochloric acid R1*. Prepare the standard by mixing 0.6 mL of *hydrochloric acid R1*, 5 mL of *sulfate standard solution (10 ppm SO_4) R* and 10 mL of a 223 g/L solution of *aluminium nitrate R*.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 130 °C for 3 h.

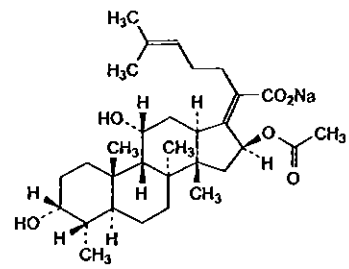
ASSAY
Dissolve 0.100 g in *water R* and dilute to 60 mL with the same solvent. Titrate with *0.1 M lanthanum nitrate*, determining the end-point potentiometrically (*2.2.20*) using a fluoride-selective indicator electrode and a silver-silver chloride reference electrode.

1 mL of *0.1 M lanthanum nitrate* is equivalent to 12.60 mg of NaF.

Ph Eur

Sodium Fusidate

(Ph. Eur. monograph 0848)



$C_{31}H_{47}NaO_6$ 538.7 *751-94-0*

Action and use
Antibacterial.

Preparation
Sodium Fusidate Ointment

Ph Eur

DEFINITION
Sodium *ent*-(17*Z*)-16α-(acetyloxy)-3β,11β-dihydroxy-4β,8,14-trimethyl-18-nor-5β,10α-cholesta-17(20),24-dien-21-oate.

Antimicrobial substance produced by fermentation of certain strains of *Fusidium coccineum* or by any other means.

Content
97.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white, crystalline powder, slightly hygroscopic.

Solubility
Freely soluble in water and in ethanol (96 per cent).

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *sodium fusidate CRS*.

B. Ignite 1 g. The residue gives reaction (a) of sodium (*2.3.1*).

TESTS
Appearance of solution
The solution is not more intensely coloured than reference solution B_6 (*2.2.2, Method II*).

Dissolve 1.5 g in 10 mL of *water R*.

pH (*2.2.3*)
7.5 to 9.0.

Dissolve 0.125 g in 10 mL of *carbon dioxide-free water R*.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Solvent mixture *methanol R*, 5 g/L solution of *phosphoric acid R*, *acetonitrile R* (10:40:50 *V/V/V*).

Test solution Dissolve 25 mg of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (a) Dissolve 2 mg of *fusidic acid for peak identification CRS* (containing impurities A, B, C, D, F, G, H and N) in the solvent mixture and dilute to 1.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture.

Reference solution (c) Dilute 1.0 mL of reference solution (b) to 10.0 mL with the solvent mixture.

Reference solution (d) Dissolve the contents of a vial of *fusidic acid impurity mixture CRS* (containing impurities I, K, L and M) in 1.0 mL of the solvent mixture.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3.5 µm);
- *temperature*: 30 °C.

Mobile phase:
- *mobile phase A*: *methanol R, acetonitrile R*, 5 g/L solution of *phosphoric acid R* (20:40:40 *V/V/V*);
- *mobile phase B*: 5 g/L solution of *phosphoric acid R, methanol R, acetonitrile R* (10:20:70 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	100	0
3 - 28	100 → 0	0 → 100
28 - 33	0	100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 235 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram supplied with *fusidic acid for peak identification CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D, F, G, H and N; use the chromatogram supplied with *fusidic acid impurity mixture CRS* and the chromatogram obtained with reference solution (d) to identify the peaks due to impurities I, K, L and M.

Relative retention With reference to fusidic acid (retention time = about 18 min): impurity A = about 0.4; impurity B = about 0.5; impurity C = about 0.6; impurity D = about 0.63; impurity N = about 0.65; impurity F = about 0.7; impurity G = about 0.82; impurity H = about 0.85; impurity I = about 0.96; impurity K = about 1.18; impurity L = about 1.23; impurity M = about 1.4.

System suitability Reference solution (a):
- *resolution*: minimum 1.5 between the peaks due to impurities G and H.

Limits:
- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity C = 0.7; impurity D = 0.7; impurity F = 0.3; impurity I = 0.6; impurity K = 0.6;
- *impurity M*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
- *impurity G*: not more than 0.7 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.7 per cent);
- *impurity L*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *impurity B*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.4 per cent);
- *impurity A*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent);
- *impurities C, D, F, I, K, N*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Water (*2.5.12*)
Maximum 2.0 per cent, determined on 0.500 g.

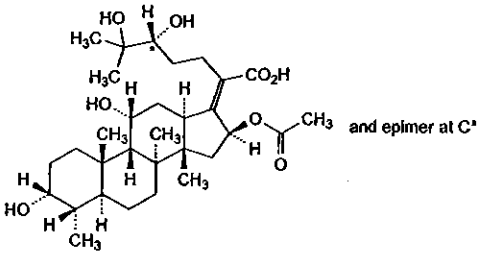
ASSAY
Dissolve 0.400 g in 30 mL of *water R* and add 40 mL of *ethanol (96 per cent) R*. Titrate with *0.1 M hydrochloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M hydrochloric acid* is equivalent to 53.87 mg of $C_{31}H_{47}NaO_6$.

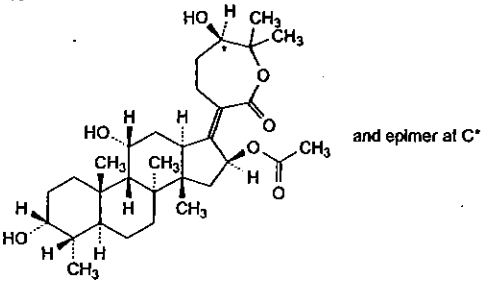
STORAGE
In an airtight container, protected from light, at a temperature of 2 °C to 8 °C.

IMPURITIES
Specified impurities *A, B, C, D, F, G, I, K, L, M, N.*

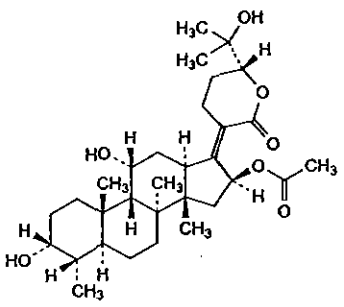
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *E, H, J, O.*



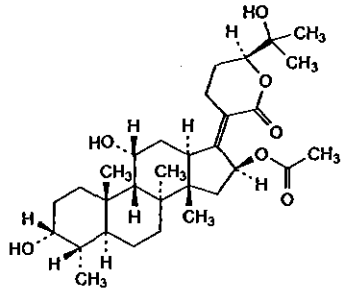
A. *ent*-(24*SR*,17*Z*)-16α-(acetyloxy)-3β,11β,24,25-tetrahydroxy-4β,8,14-trimethyl-18-nor-5β,10α-cholest-17(20)-en-21-oic acid (24,25-dihydro-24,25-dihydroxyfusidic acid),



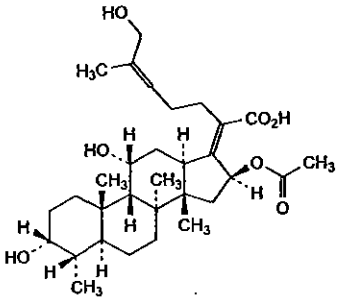
B. *ent*-(17*Z*)-3β,11β-dihydroxy-17-[(6*SR*)-6-hydroxy-7,7-dimethyl-2-oxooxepan-3-ylidene]-4β,8,14-trimethyl-18-nor-5β,10α-androstan-16α-yl acetate (24,25-dihydro-24,25-dihydroxyfusidic acid 21,25-lactone),



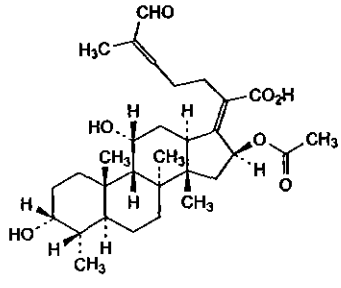
C. *ent*-(17*Z*)-3β,11β-dihydroxy-17-[(6*S*)-6-(1-hydroxy-1-methylethyl)-2-oxodihydro-2*H*-pyran-3(4*H*)-ylidene]-4β,8,14-trimethyl-18-nor-5β,10α-androstan-16α-yl acetate ((24*R*)-24,25-dihydro-24,25-dihydroxyfusidic acid 21,24-lactone),



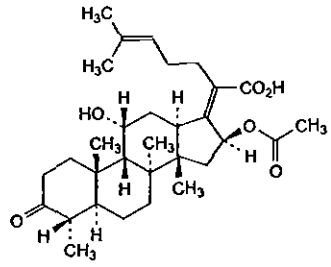
D. *ent*-(17*Z*)-3β,11β-dihydroxy-17-[(6*R*)-6-(1-hydroxy-1-methylethyl)-2-oxodihydro-2*H*-pyran-3(4*H*)-ylidene]-4β,8,14-trimethyl-18-nor-5β,10α-androstan-16α-yl acetate ((24*S*)-24,25-dihydro-24,25-dihydroxyfusidic acid 21,24-lactone),



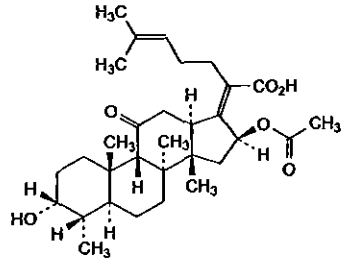
E. *ent*-(17*Z*,24*EZ*)-16α-(acetyloxy)-3β,11β,26-trihydroxy-4β,8,14-trimethyl-18-nor-5β,10α-cholesta-17(20),24-dien-21-oic acid (26-hydroxyfusidic acid),



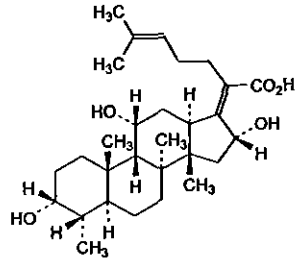
F. *ent*-(17*Z*,24*EZ*)-16α-(acetyloxy)-3β,11β-dihydroxy-4β,8,14-trimethyl-26-oxo-18-nor-5β,10α-cholesta-17 (20),24-dien-21-oic acid (26-oxofusidic acid),



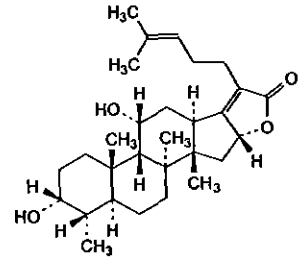
G. *ent*-(17*Z*)-16α-(acetyloxy)-11β-hydroxy-4β,8,14-trimethyl-3-oxo-18-nor-5β,10α-cholesta-17(20),24-dien-21-oic acid (3-didehydrofusidic acid),



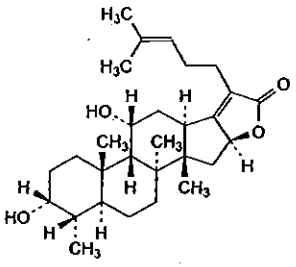
H. *ent*-(17*Z*)-16α-(acetyloxy)-3β-hydroxy-4β,8,14-trimethyl-11-oxo-18-nor-5β,10α-cholesta-17(20),24-dien-21-oic acid (11-didehydrofusidic acid),



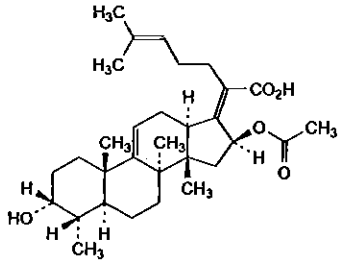
I. *ent*-(17*Z*)-3β,11β,16β-trihydroxy-4β,8,14-trimethyl-18-nor-5β,10α-cholesta-17(20),24-dien-21-oic acid (16-*epi*-deacetylfusidic acid),



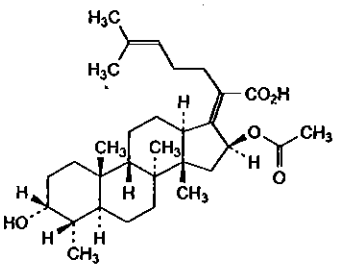
J. *ent*-(17*Z*)-3β,11β-dihydroxy-4β,8,14-trimethyl-18-nor-5β,10α-cholesta-17(20),24-dieno-21(16β)-lactone (16-*epi*-deacetylfusidic acid 21,16-lactone),



K. *ent*-(17*Z*)-3β,11β-dihydroxy-4β,8,14-trimethyl-18-nor-5β,10α-cholesta-17(20),24-dieno-21(16α)-lactone (deacetylfusidic acid 21,16-lactone),

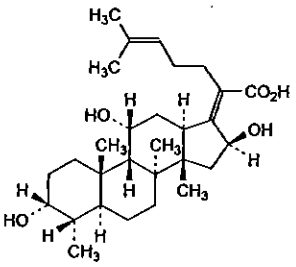


L. *ent*-(17*Z*)-16α-(acetyloxy)-3β-hydroxy-4β,8,14-trimethyl-18-nor-5β,10α-cholesta-9(11),17(20),24-trien-21-oic acid (9,11-anhydrofusidic acid),



M. *ent*-(17*Z*)-16α-(acetyloxy)-3β-hydroxy-4β,8,14-trimethyl-18-nor-5β,10α-cholesta-17(20),24-dien-21-oic acid (11-deoxyfusidic acid),

N. unknown structure,

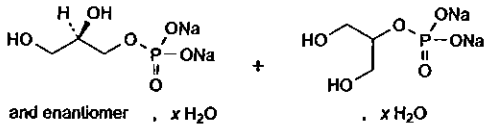


O. *ent*-(17*Z*)-3β,11β,16α-trihydroxy-4β,8,14-trimethyl-18-nor-5β,10α-cholesta-17(20),24-dien-21-oic acid (deacetylfusidic acid).

Ph Eur

Hydrated Sodium Glycerophosphate

(Ph. Eur. monograph 1995)



$C_3H_7Na_2O_6P,xH_2O$ 216.0
(anhydrous substance)

Ph Eur

DEFINITION

Mixture of variable proportions of hydrated disodium (2*RS*)-2,3-dihydroxypropyl phosphate and hydrated disodium 2-hydroxy-1-(hydroxymethyl)ethyl phosphate. The mixture may contain various amounts of other glycerophosphate esters.

Content

98.0 per cent to 105.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or crystals.

Solubility

Freely soluble in water, practically insoluble in acetone and in ethanol (96 per cent).

IDENTIFICATION

A. Solution S (see Tests) gives reaction (a) of sodium (*2.3.1*).

B. To 0.1 g add 5 mL of *dilute nitric acid R*. Heat to boiling and boil for 1 min. Cool. The solution gives reaction (b) of phosphates (*2.3.1*).

C. In a test-tube fitted with a glass tube, mix 0.1 g with 5 g of *potassium hydrogen sulfate R*. Heat strongly and direct the white vapour into 5 mL of *decolorised fuchsin solution R*. A violet-red colour develops which becomes violet upon heating for 30 min on a water-bath.

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 mL with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Alkalinity

To 10 mL of solution S add 0.2 mL of *phenolphthalein solution R*. Not more than 1.0 mL of *0.1 M hydrochloric acid* is required to change the colour of the indicator (n_2).

Glycerol and ethanol (96 per cent)-soluble substances

Maximum 1.0 per cent.

Shake 1.000 g with 25 mL of *ethanol (96 per cent) R* for 10 min. Filter. Evaporate the filtrate on a water-bath and dry the residue at 70 °C for 1 h. The residue weighs not more than 10 mg.

Chlorides (*2.4.4*)

Maximum 200 ppm.

Dilute 2.5 mL of solution S to 15 mL with *water R*.

Phosphates (*2.4.11*)

Maximum 0.1 per cent.

Dilute 1 mL of solution S to 10 mL with *water R*. Dilute 1 mL of this solution to 100 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 500 ppm.

Dilute 3 mL of solution S to 15 mL with *water R*.

Calcium
Maximum 100 ppm, if intended for use in the manufacture of parenteral preparations.

Inductively coupled plasma-atomic emission spectrometry (*2.2.57*).

Test solution Dissolve 0.50 g in a 1 per cent *V/V* solution of *nitric acid R* and dilute to 50.0 mL with the same solution.

Reference solutions Prepare the reference solutions using *calcium standard solution (100 ppm Ca) R*, diluting with a 1 per cent *V/V* solution of *nitric acid R*.

Wavelength 396.8 nm.

Iron (*2.4.9*)
Maximum 20 ppm.

Dilute 5 mL of solution S to 10 mL with *water R*.

Water (*2.5.12*)
25.0 per cent to 35.0 per cent, determined on 0.100 g.

ASSAY
Dissolve 0.250 g in 30 mL of *water R*. Titrate with *0.05 M sulfuric acid*, determining the end-point potentiometrically (*2.2.20*), (n_1).

Calculate the percentage content of sodium glycerophosphate (anhydrous substance) using the following expression:

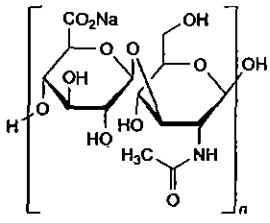
$$\frac{216.0\left(n_1 - \frac{n_2}{4}\right)}{m(100 - a)}$$

a = percentage content of water;
n_1 = volume of *0.05 M sulfuric acid* used in the assay, in millilitres;
n_2 = volume of *0.1 M hydrochloric acid* used in the test for alkalinity, in millilitres;
m = mass of the substance to be examined, in grams.

Ph Eur

Sodium Hyaluronate

(Ph. Eur. monograph 1472)



$(C_{14}H_{20}NNaO_{11})_n$ 9067-32-7

Action and use
High viscosity mucopolysaccharide.

Ph Eur

DEFINITION
Sodium salt of hyaluronic acid, a glycosaminoglycan consisting of D-glucuronic acid and *N*-acetyl-D-glucosamine disaccharide units. It is extracted from cocks' combs or obtained by fermentation from *Streptococci*, Lancefield Groups A and C.

Content
95.0 per cent to 105.0 per cent (dried substance).

Intrinsic viscosity: 90 per cent to 120 per cent of the value stated on the label.

PRODUCTION
Where applicable, the animals from which sodium hyaluronate is derived must fulfil the requirements for the health of animals suitable for human consumption.

When produced by fermentation of gram-positive bacteria, the process must be shown to reduce or eliminate pyrogenic or inflammatory components of the cell wall.

CHARACTERS

Appearance
White or almost white, very hygroscopic powder or fibrous aggregate.

Solubility
Sparingly soluble or soluble in water, practically insoluble in acetone and in anhydrous ethanol.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of sodium hyaluronate.*

B. It gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S
Weigh a quantity of the substance to be examined equivalent to 0.10 g of the dried substance and add 30.0 mL of a 9 g/L solution of *sodium chloride R*. Mix gently on a shaker until dissolved (about 12 h).

Appearance of solution
Solution S is clear (*2.2.1*) and its absorbance (*2.2.25*) at 600 nm is not greater than 0.01.

pH (*2.2.3*)
5.0 to 8.5.

Dissolve the substance to be examined in *carbon dioxide-free water R* to obtain a solution containing a quantity equivalent to 5 mg of the dried substance per millilitre.

Intrinsic viscosity
Sodium hyaluronate is very hygroscopic and must be protected from moisture during weighing.

Buffer solution *(0.15 M sodium chloride in 0.01 M phosphate buffer solution pH 7.0)*. Dissolve 0.78 g of *sodium dihydrogen phosphate R* and 4.50 g of *sodium chloride R* in *water R* and dilute to 500.0 mL with the same solvent (solution A). Dissolve 1.79 g of *disodium hydrogen phosphate dodecahydrate R* and 4.50 g of *sodium chloride R* in *water R* and dilute to 500.0 mL with the same solvent (solution B). Mix solutions A and B until a pH of 7.0 is reached. Filter through a sintered-glass filter (4) (*2.1.2*).

Test solution (a) Weigh 0.200 g (m_{0p}) *(NOTE: this value is only indicative and should be adjusted after an initial measurement of the viscosity of test solution (a))* of the substance to be examined and dilute with 50.0 g (m_{0s}) of buffer solution at 4 °C. Mix the solution by shaking at 4 °C during 24 h. Weigh 5.00 g (m_{1p}) of the solution and dilute with 100.0 g (m_{1s}) of buffer solution at 25 °C. Mix this solution by shaking for 20 min. Filter the solution through a sintered-glass filter (100) (*2.1.2*), and discard the first 10 mL.

Test solution (b) Weigh 30.0 g (m_{2p}) of test solution (a) and dilute with 10.0 g (m_{2s}) of buffer solution at 25 °C. Mix this

solution by shaking for 20 min. Filter the solution through a sintered-glass filter (100) (*2.1.2*) and discard the first 10 mL.

Test solution (c) Weigh 20.0 g (m_{3p}) of test solution (a) and dilute with 20.0 g (m_{3s}) of buffer solution at 25 °C. Mix this solution by shaking for 20 min. Filter the solution through a sintered-glass filter (100) (*2.1.2*) and discard the first 10 mL.

Test solution (d) Weigh 10.0 g (m_{4p}) of test solution (a) and dilute with 30.0 g (m_{4s}) of buffer solution at 25 °C. Mix this solution by shaking for 20 min. Filter the solution through a sintered-glass filter (100) (*2.1.2*) and discard the first 10 mL.

Determine the flow-times (*2.2.9*) for the buffer solution (t_0) and for the 4 test solutions (t_1, t_2, t_3 and t_4), at 25.00 ± 0.03 °C. Use an appropriate suspended level viscometer (specifications: viscometer constant about 0.005 mm^2/s^2, kinematic viscosity of 1-5 mm^2/s, internal diameter of tube *R* 0.53 mm, volume of bulb *C* 5.6 mL, internal diameter of tube *N* 2.8-3.2 mm) with a funnel-shaped lower capillary end. Use the same viscometer for all measurements; measure all outflow times in triplicate.

The test is not valid unless the results do not differ by more than 0.35 per cent from the mean and if the flow time t_1 is not less than 1.6 and not more than 1.8 times t_0. If this is not the case, adjust the value of m_{0p} and repeat the procedure.

Calculation of the relative viscosities

Since the densities of the sodium hyaluronate solutions and of the solvent are almost equal, the relative viscosities η_{ri} (being η_{r1}, η_{r2}, η_{r3} and η_{r4}) can be calculated from the ratio of the flow times for the respective solutions t_i (being t_1, t_2, $t_3$and t_4) to the flow time of the solvent t_0, but taking into account the kinetic energy correction factor for the capillary (B = 30 800 s^3), using the following expression:

$$\frac{t_i - \frac{B}{t_i^2}}{t_0 - \frac{B}{t_0^2}}$$

Calculation of the concentrations

Calculate the concentration c_1 (expressed in kg/m^3) of sodium hyaluronate in test solution (a) using the following expression:

$$\frac{m_{0p} \times x \times (100 - h) \times m_{1p} \times \rho_{25}}{100 \times 100 \times (m_{0p} + m_{0s}) \times (m_{1p} + m_{1s})}$$

x = percentage content of sodium hyaluronate as determined under Assay;
h = percentage loss on drying;
ρ_{25} = 1005 kg/m^3 (density of the test solution at 25 °C).

Calculate the concentration c_2 (expressed in kg/m^3) of sodium hyaluronate in test solution (b) using the following expression:

$$c_1 \times \frac{m_{2p}}{m_{2s} + m_{2p}}$$

Calculate the concentration c_3 (expressed in kg/m^3) of sodium hyaluronate in test solution (c) using the following expression:

$$c_1 \times \frac{m_{3p}}{m_{3s} + m_{3p}}$$

Calculate the concentration c_4 (expressed in kg/m^3) of sodium hyaluronate in test solution (d) using the following expression:

$$c_1 \times \frac{m_{4p}}{m_{4s} + m_{4p}}$$

Calculation of the intrinsic viscosity

Calculate the intrinsic viscosity [η] by linear least-squares regression analysis using the Martin equation:

$$\log_{10}\left(\frac{\eta_r - 1}{c}\right) = \log_{10}[\eta] + k[\eta]c$$

The decimal antilogarithm of the intercept is the intrinsic viscosity expressed in m^3/kg.

Sulfated glycosaminoglycans

Maximum 1 per cent, if the product is extracted from cocks' combs.

Appropriate safety precautions are to be taken when handling perchloric acid at elevated temperature.

Test solution Introduce a quantity of the substance to be examined equivalent to 50.0 mg of the dried substance into a test-tube 150 mm long and 16 mm in internal diameter and dissolve in 1.0 mL of *perchloric acid R*.

Reference solution Dissolve 0.149 g of *anhydrous sodium sulfate R* in *water R* and dilute to 100.0 mL with the same solvent. Dilute 10.0 mL of this solution to 100.0 mL with *water R*. Evaporate 1.0 mL in a test-tube 150 mm long and 16 mm in internal diameter in a heating block at 90-95 °C, and dissolve the residue in 1.0 mL of *perchloric acid R*.

Plug each test-tube with a piece of glass wool. Place the test-tubes in a heating block or a silicone oil bath maintained at 180 °C and heat until clear, colourless solutions are obtained (about 12 h). Remove the test-tubes and cool to room temperature. Add to each test-tube 3.0 mL of a 33.3 g/L solution of *barium chloride R*, cap and shake vigorously. Allow the test-tubes to stand for 30 min. Shake each test-tube once again, and determine the absorbance (*2.2.25*) at 660 nm, using *water R* as a blank.

The absorbance obtained with the test solution is not greater than the absorbance obtained with the reference solution.

Nucleic acids

The absorbance (*2.2.25*) of solution S at 260 nm is maximum 0.5.

Protein

Maximum 0.3 per cent; maximum 0.1 per cent, if intended for use in the manufacture of parenteral preparations.

Test solution (a) Dissolve the substance to be examined in *water R* to obtain a solution containing a quantity equivalent to about 10 mg of the dried substance per millilitre.

Test solution (b) Mix equal volumes of test solution (a) and *water R*.

Reference solutions Prepare a 0.5 mg/mL stock solution of *bovine albumin R* in *water R*. Prepare 5 dilutions of the stock solution containing between 5 µg/mL and 50 µg/mL of *bovine albumin R*.

Add 2.5 mL of freshly prepared *cupri-tartaric solution R3* to test-tubes containing 2.5 mL of *water R* (blank), 2.5 mL of the test solutions (a) or (b) or 2.5 mL of the reference solutions. Mix after each addition. After about 10 min, add to each test-tube 0.50 mL of a mixture of equal volumes of *phosphomolybdotungstic reagent R* and *water R* prepared immediately before use. Mix after each addition. After 30 min, measure the absorbance (*2.2.25*) of each solution at 750 nm against the blank. From the calibration curve

obtained with the 5 reference solutions determine the content of protein in the test solutions.

Chlorides (*2.4.4*)
Maximum 0.5 per cent.

Dissolve 67 mg in 100 mL of *water R*.

Iron
Maximum 80 ppm.

Atomic absorption spectrometry (*2.2.23, Method II*).

Test solution Dissolve a quantity of the substance to be examined equivalent to 0.25 g of the dried substance in 1 mL of *nitric acid R* by heating on a water-bath. Cool and dilute to 10.0 mL with *water R*.

Reference solutions Prepare 2 reference solutions in the same manner as the test solution, adding 1.0 mL and 2.0 mL respectively of *iron standard solution (10 ppm Fe) R* to the dissolved substance to be examined.

Source Iron hollow-cathode lamp using a transmission band of 0.2 nm.

Wavelength 248.3 nm.

Atomisation device Air-acetylene flame.

Loss on drying (*2.2.32*)
Maximum 20.0 per cent, determined on 0.500 g by drying at 100-110 °C over *diphosphorus pentoxide R* for 6 h.

Microbial contamination
TAMC: acceptance criterion 10^2 CFU/g (*2.6.12*). Use 1 g of the substance to be examined.

Bacterial endotoxins (*2.6.14*)
Less than 0.5 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins; less than 0.05 IU/mg, if intended for use in the manufacture of intra-ocular preparations or intra-articular preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Determine the glucuronic acid content by reaction with carbazole as described below.

Reagent A Dissolve 0.95 g of *disodium tetraborate R* in 100.0 mL of *sulfuric acid R*.

Reagent B Dissolve 0.125 g of *carbazole R* in 100.0 mL of *anhydrous ethanol R*.

Test solution Prepare this solution in triplicate. Dissolve 0.170 g of the substance to be examined in *water R* and dilute to 100.0 g with the same solvent. Dilute 10.0 g of this solution to 200.0 g with *water R*.

Reference stock solution Dissolve 0.100 g of *D-glucuronic acid R*, previously dried to constant mass in vacuum over *diphosphorus pentoxide R* (*2.2.32*), in *water R* and dilute to 100.0 g with the same solvent.

Reference solutions Prepare 5 dilutions of the reference stock solution containing between 6.5 µg/g and 65 µg/g of *D-glucuronic acid R*.

Place 25 test-tubes, numbered 1 to 25, in iced water. Add 1.0 mL of the 5 reference solutions in triplicate to the test-tubes 1 to 15 (reference tubes), 1.0 mL of the 3 test solutions in triplicate to the test-tubes 16 to 24 (sample tubes), and 1.0 mL of *water R* to test-tube 25 (blank). Add to each test-tube 5.0 mL of freshly prepared reagent A, previously cooled in iced water. Tightly close the test-tubes with plastic caps, shake the contents, and place on a water bath for exactly 15 min. Cool in iced water, and add to each test tube 0.20 mL of reagent B. Recap the tubes, shake, and put them again on a water-bath for exactly 15 min. Cool to room temperature and measure the absorbance (*2.2.25*) of the solutions at 530 nm, against the blank.

From the calibration curve obtained with the mean absorbances read for each reference solution, determine the mean concentrations of D-glucuronic acid in the test solutions.

Calculate the percentage content of sodium hyaluronate using the following expression:

$$\frac{c_g}{c_s} \times Z \times \frac{100}{100 - h} \times \frac{401.3}{194.1}$$

c_g	=	mean of concentrations of D-glucuronic acid in the test solutions, in milligrams per gram;
c_s	=	mean of concentrations of the substance to be examined in the test solutions, in milligrams per gram;
Z	=	determined percentage content of $C_6H_{10}O_7$ in *D-glucuronic acid R*;
h	=	percentage loss on drying;
401.3	=	relative molecular mass of the disaccharide fragment;
194.1	=	relative molecular mass of glucuronic acid.

STORAGE

In an airtight container, protected from light and humidity. If the substance is sterile, store in a sterile, airtight, tamper-evident container.

LABELLING

The label states:
- the intrinsic viscosity;
- the origin of the substance;
- the intended use of the substance;
- where applicable, that the substance is suitable for parenteral administration other than intra-articular administration;
- where applicable, that the substance is suitable for parenteral administration, including intra-articular administration;
- where applicable that the material is suitable for intra-ocular use.

Ph Eur

Sodium Hydroxide

Caustic Soda

(Ph. Eur. monograph 0677)

NaOH 40.00 *1310-73-2*

Ph Eur

DEFINITION

Content
97.0 per cent to 100.5 per cent.

CHARACTERS

Appearance
White or almost white, crystalline masses, supplied as pellets, sticks or slabs, deliquescent, readily absorbing carbon dioxide.

Solubility
Very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

A. pH (*2.2.3*): minimum 11.0.

Dissolve 0.1 g in 10 mL of *water R*. Dilute 1 mL of the solution to 100 mL with *water R*.

B. 2 mL of solution S (see Tests) gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S

Carry out the procedure described below with caution Dissolve 5.0 g in 12 mL of *water R*. Add 17 mL of *hydrochloric acid R1*, adjust to pH 7 with a 103 g/L solution of *hydrochloric acid R* and dilute to 50 mL with *water R*.

Appearance of solution

The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 1.0 g in 10 mL of *water R*.

Carbonates

Maximum 2.0 per cent, calculated as Na_2CO_3 as determined in the assay.

Chlorides (*2.4.4*)

Maximum 200 ppm.

Dissolve 0.25 g in 5 mL of *water R*, acidify the solution with about 4 mL of *nitric acid R* and dilute to 15 mL with *water R*. The solution, without addition of *dilute nitric acid R*, complies with the test.

Sulfates (*2.4.13*)

Maximum 200 ppm.

Dissolve 0.75 g in 6 mL of *distilled water R*, adjust to pH 7 with *hydrochloric acid R* and dilute to 15 mL with *distilled water R*.

Iron (*2.4.9*)

Maximum 10 ppm, determined on solution S.

ASSAY

Dissolve 2.000 g in about 80 mL of *carbon dioxide-free water R*. Add 0.3 mL of *phenolphthalein solution R* and titrate with *1 M hydrochloric acid*. Add 0.3 mL of *methyl orange solution R* and continue the titration with *1 M hydrochloric acid*.

1 mL of *1 M hydrochloric acid* used in the 2nd part of the titration is equivalent to 0.1060 g of Na_2CO_3.

1 mL of *1 M hydrochloric acid* used in the combined titrations is equivalent to 40.00 mg of total alkali, calculated as NaOH.

STORAGE

In an airtight, non-metallic container.

Ph Eur

Sodium Iodide

(Ph. Eur. monograph 0196)

NaI	149.9	*7681-82-5*

Preparation

Sodium Iodide Injection

Ph Eur

DEFINITION

Content

99.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals, hygroscopic.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

A. Solution S (see Tests) gives the reactions of iodides (*2.3.1*).

B. Solution S gives the reactions of sodium (*2.3.1*).

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Alkalinity

To 12.5 mL of solution S add 0.1 mL of *bromothymol blue solution R1*. Not more than 0.7 mL of *0.01 M hydrochloric acid* is required to change the colour of the indicator.

Iodates

To 10 mL of solution S add 0.25 mL of *iodide-free starch solution R* and 0.2 mL of *dilute sulfuric acid R* and allow to stand protected from light for 2 min. No blue colour develops.

Sulfates (*2.4.13*)

Maximum 150 ppm.

Dilute 10 mL of solution S to 15 mL with *distilled water R*.

Thiosulfates

To 10 mL of solution S add 0.1 mL of *starch solution R* and 0.1 mL of 0.005 M iodine. A blue colour is produced.

Iron (*2.4.9*)

Maximum 20 ppm.

Dilute 5 mL of solution S to 10 mL with *water R*.

Loss on drying (*2.2.32*)

Maximum 3.0 per cent, determined on 1.00 g by drying in an oven at 105 °C for 3 h.

ASSAY

Dissolve 1.300 g in *water R* and dilute to 100.0 mL with the same solvent. To 20.0 mL of the solution add 40 mL of *hydrochloric acid R* and titrate with *0.05 M potassium iodate* until the colour changes from red to yellow. Add 5 mL of *chloroform R* and continue the titration, shaking vigorously, until the chloroform layer is decolorised.

1 mL of *0.05 M potassium iodate* is equivalent to 14.99 mg of NaI.

STORAGE

Protected from light.

Ph Eur

Sodium Lactate Solution

(Ph. Eur. monograph 1151)

Action and use

Systemic alkalinising agent.

Ph Eur

DEFINITION

Solution of a mixture of the enantiomers of sodium (2*RS*)-2-hydroxypropanoate in approximately equal proportions.

Content

Minimum declared content 50 per cent *m/m* of sodium (2*RS*)-2-hydroxypropanoate ($C_3H_5NaO_3$; M_r 112.1); 96.0 per cent to 104.0 per cent of the content of sodium lactate stated on the label.

CHARACTERS

Appearance
Clear, colourless, slightly syrupy liquid.

Solubility
Miscible with water and with ethanol (96 per cent).

IDENTIFICATION

A. To 0.1 mL add 10 mL of *water R*. 5 mL of the solution gives the reaction of lactates (*2.3.1*).

B. It gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S
Dilute a quantity of the substance to be examined corresponding to 40.0 g of sodium lactate to 200 mL with *distilled water R*.

Appearance of solution
The substance to be examined is clear (*2.2.1*) and not more intensely coloured than reference solution BY_7 (*2.2.2, Method II*).

pH (*2.2.3*)
6.5 to 9.0 for the substance to be examined.

Reducing sugars and sucrose
To 5 mL of the substance to be examined add 0.2 mL of *copper sulfate solution R* and 2 mL of *dilute sodium hydroxide solution R*. The solution is clear and blue and remains so on boiling. Add to the hot solution 4 mL of *hydrochloric acid R*. Boil for 1 min. Add 6 mL of *strong sodium hydroxide solution R* and heat to boiling again. The solution is clear and blue.

Methanol
Gas chromatography (*2.4.24*).

Limit:
— *methanol*: maximum 50 ppm, calculated with reference to sodium lactate, if intended for use in the manufacture of parenteral preparations, dialysis, haemodialysis or haemofiltration solutions.

Chlorides (*2.4.4*)
Maximum 50 ppm calculated with reference to sodium lactate.

Dilute 5 mL of solution S to 15 mL with *water R*.

Oxalates and phosphates
To 1 mL of the substance to be examined add 15 mL of *ethanol (96 per cent) R* and 2 mL of *calcium chloride solution R*. Heat at 75 °C for 5 min. Any opalescence in the solution is not more intense than that of a standard prepared at the same time and in the same manner using a mixture of 1 mL of the substance to be examined, 15 mL of *ethanol (96 per cent) R* and 2 mL of *water R*.

Sulfates (*2.4.13*)
Maximum 100 ppm calculated with reference to sodium lactate.

To 7.5 mL of solution S, add 1.9 mL of *hydrochloric acid R1* and dilute to 15 mL with *distilled water R*. The solution complies with the test for sulfates without addition of 0.5 mL of *acetic acid R*. Acidify the standard solution with 0.05 mL of *hydrochloric acid R1* instead of 0.5 mL of *acetic acid R*.

Aluminium
Maximum 0.1 ppm, if intended for use in the manufacture of parenteral preparations, dialysis, haemodialysis or haemofiltration solutions.

Atomic absorption spectrometry (*2.2.23, Method I*). For the preparation of the solutions, use equipment that is aluminium-free or that will not release aluminium under the conditions of use (glass, polyethylene, etc).

Modifier solution Dissolve 100.0 g of *ammonium nitrate R* in a mixture of 4 mL of *nitric acid R* and 50 mL of *water R* and dilute to 200 mL with *water R*.

Blank solution Dilute 2.0 mL of the modifier solution to 25.0 mL with *water R*.

Test solution To 5.0 g add 2.0 mL of the modifier solution and dilute to 25.0 mL with *water R*.

Reference solutions Prepare the reference solutions immediately before use (0.010 ppm to 0.050 ppm of aluminium) using *aluminium standard solution (200 ppm Al) R*.

Source Aluminium hollow-cathode lamp.

Wavelength 309.3 nm.

Atomisation device Graphite furnace.

Carrier gas *argon R*.

Conditions The device is equipped with a non-specific absorption correction system. Heat the oven to 120 °C for as many seconds as there are microlitres of solution introduced into the apparatus, then heat at 1000 °C for 30 s and finally at 2700 °C for 6 s.

Iron (*2.4.9*)
Maximum 10 ppm calculated with reference to sodium lactate.

Dilute 5 mL of solution S to 10 mL with *water R*.

ASSAY

Dissolve a quantity of the substance to be examined corresponding to 75.0 mg of sodium lactate in a mixture of 10 mL of *glacial acetic acid R* and 20 mL of *acetic anhydride R*. Allow to stand for 15 min. Add 1 mL of *naphtholbenzein solution R* and titrate with *0.1 M perchloric acid*.

1 mL of *0.1 M perchloric acid* is equivalent to 11.21 mg of $C_3H_5NaO_3$.

LABELLING

The label states:
— where applicable, that the substance is suitable for use in the manufacture of dialysis, haemodialysis and haemofiltration solutions;
— where applicable, that the substance is suitable for use in the manufacture of parenteral preparations;
— the declared content of sodium lactate.

Ph Eur

Sodium (*S*)-Lactate Solution

(Ph. Eur. monograph 2033)

Ph Eur

DEFINITION

Content
Minimum 50.0 per cent *m/m* of sodium (2*S*)-2-hydroxypropanoate ($C_3H_5NaO_3$; M_r 112.1); 96.0 per cent to 104.0 per cent of the content of sodium lactate stated on the label, not less than 95.0 per cent of which is the (*S*)-enantiomer.

CHARACTERS

Appearance
Clear, colourless, slightly syrupy liquid.

Solubility
Miscible with water and with ethanol (96 per cent).

IDENTIFICATION
A. To 0.1 mL add 10 mL of *water R*. 5 mL of the solution gives the reaction of lactates (*2.3.1*).

B. It gives reaction (a) of sodium (*2.3.1*).

C. It complies with the limits of the assay.

TESTS

Solution S
Dilute a quantity of the substance to be examined corresponding to 40.0 g of sodium lactate to 200 mL with *distilled water R*.

Appearance of solution
The substance to be examined is clear (*2.2.1*) and not more intensely coloured than reference solution BY_7 (*2.2.2, Method II*).

pH (*2.2.3*)
6.5 to 9.0 for the substance to be examined.

Reducing sugars and sucrose
To 5 mL of the substance to be examined add 2 mL of *dilute sodium hydroxide solution R* and 0.2 mL of *copper sulfate solution R*. The solution is clear and blue and remains so on boiling. Add to the hot solution 4 mL of *hydrochloric acid R*. Boil for 1 min. Add 6 mL of *strong sodium hydroxide solution R* and heat to boiling again. The solution is clear and blue.

Methanol
Gas chromatography (*2.4.24*).

Limit:
— *methanol*: maximum 50 ppm, calculated with reference to sodium lactate, if intended for use in the manufacture of parenteral preparations, dialysis, haemodialysis or haemofiltration solutions.

Chlorides (*2.4.4*)
Maximum 50 ppm calculated with reference to sodium lactate.

Dilute 5 mL of solution S to 15 mL with *water R*.

Oxalates and phosphates
To 1 mL of the substance to be examined add 15 mL of *ethanol (96 per cent) R* and 2 mL of *calcium chloride solution R*. Heat at 75 °C for 5 min. Any opalescence in the solution is not more intense than that of a standard prepared at the same time and in the same manner using a mixture of 1 mL of the substance to be examined, 15 mL of *ethanol (96 per cent) R* and 2 mL of *water R*.

Sulfates (*2.4.13*)
Maximum 100 ppm calculated with reference to sodium lactate.

To 7.5 mL of solution S, add 1.9 mL of *hydrochloric acid R1* and dilute to 15 mL with *distilled water R*. The solution complies with the limit test for sulfates without addition of 0.5 mL of *acetic acid R*. Acidify the standard solution with 0.05 mL of *hydrochloric acid R1* instead of 0.5 mL of *acetic acid R*.

Aluminium
Maximum 0.1 ppm, if intended for use in the manufacture of parenteral preparations, dialysis, haemodialysis or haemofiltration solutions.

Atomic absorption spectrometry (*2.2.23, Method I*). For the preparation of the solutions, use equipment that is aluminium-free or that will not release aluminium under the conditions of use (glass, polyethylene, etc).

Modifier solution Dissolve 100.0 g of *ammonium nitrate R* in a mixture of 50 mL of *water R* and 4 mL of *nitric acid R* and dilute to 200 mL with *water R*.

Blank solution Dilute 2.0 mL of the modifier solution to 25.0 mL with *water R*.

Test solution To 5.0 g add 2.0 mL of the modifier solution and dilute to 25.0 mL with *water R*.

Reference solutions Prepare the reference solutions immediately before use (0.010 ppm to 0.050 ppm of aluminium) using *aluminium standard solution (200 ppm Al) R*.

Source Aluminium hollow-cathode lamp.

Wavelength 309.3 nm.

Atomisation device Graphite furnace.

Carrier gas *argon R*.

Conditions The device is equipped with a non-specific absorption correction system. Heat the oven to 120 °C for as many seconds as there are microlitres of solution introduced into the apparatus, then heat at 1000 °C for 30 s and finally at 2700 °C for 6 s.

Iron (*2.4.9*)
Maximum 10 ppm calculated with reference to sodium lactate.

Dilute 5 mL of solution S to 10 mL with *water R*.

ASSAY
Dissolve a quantity of the substance to be examined corresponding to 75.0 mg of sodium lactate in a mixture of 10 mL of *glacial acetic acid R* and 20 mL of *acetic anhydride R*. Allow to stand for 15 min. Add 1 mL of *naphtholbenzein solution R* and titrate with *0.1 M perchloric acid*.

1 mL of *0.1 M perchloric acid* is equivalent to 11.21 mg of $C_3H_5NaO_3$.

(*S*)-enantiomer
Transfer a quantity of the substance to be examined corresponding to 2.50 g of sodium lactate into a 50 mL volumetric flask, dilute with about 30 mL of *water R* and add 5.0 g of *ammonium molybdate R*. Dissolve and dilute with *water R* to 50.0 mL. Measure the angle of optical rotation (*2.2.7*). Calculate the percentage content of (*S*)-enantiomer using the expression:

$$50 + \left(24.04 \times \alpha \times \frac{5.0}{m} \times \frac{50}{c}\right)$$

α = angle of optical rotation (absolute value);
m = mass of the substance to be examined, in grams;
c = percentage content *m/m* of $C_3H_5NaO_3$ in the substance to be examined.

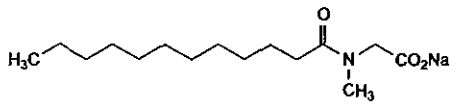
The complex of sodium (*S*)-lactate formed under these test conditions is laevorotatory.

LABELLING
The label states:
— where applicable, that the substance is suitable for use in the manufacture of dialysis, haemodialysis and haemofiltration solutions;
— where applicable, that the substance is suitable for use in the manufacture of parenteral preparations;
— the declared content of sodium lactate.

Ph Eur

Sodium Lauroylsarcosinate for External Use

(Ph. Eur. monograph 2542)



$C_{15}H_{28}NNaO_3$ 293.4 *137-16-6*

Action and use
Surfactant.

Ph Eur

DEFINITION

Mixture of sodium *N*-acyl-*N*-methylglycinates, mainly consisting of sodium *N*-lauroyl-*N*-methylglycinate (sodium [dodecanoyl(methyl)amino]acetate).

A suitable chelating agent (e.g. edetic acid (EDTA)) may be added.

PRODUCTION

Sodium lauroylsarcosinate is typically obtained using the reaction of lauroyl chloride with sodium sarcosinate (sodium *N*-methylglycinate) under aqueous alkaline conditions.

The production method is validated to ensure a limit of maximum 0.1 per cent of free sodium sarcosinate.

CHARACTERS

Appearance
White or almost white powder.

Solubility
Freely soluble in water giving a clear or slightly turbid solution, sparingly soluble in ethanol 96 per cent.

mp
About 145 °C.

IDENTIFICATION

A. Dissolve 0.1 g in 10 mL of *water R* and shake. Copious foam is formed.

B. Examine the chromatograms obtained in the test for composition of fatty acids.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (c).

C. Place 0.5 g in a silica crucible and ignite in a muffle furnace at 600 ± 25 °C for 1 h. Allow to cool to room temperature and dissolve the residue in 10 mL of *water R*. 0.5 mL of the solution obtained gives reaction (b) of sodium (*2.3.1*). Rub the inside of the tube to initiate the precipitation.

TESTS

pH (*2.2.3*)
7.5 to 8.5.

Dissolve 3.0 g in *carbon dioxide-free water R* and dilute to 100 mL with the same solvent.

Composition of fatty acids
Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution (a) To 2.5 g in a 150 mL round-bottomed flask add 30.0 mL of *boron trifluoride-methanol solution R*, fit a reflux condenser and boil for 20 min. Add 120 mL of hot *water R* so that the upper layer is brought into the neck of the flask in order to recover the methyl esters. Dissolve 0.50 g of the methyl esters in 10.0 mL of *heptane R*.

Test solution (b) Dilute 1.0 mL of test solution (a) to 10.0 mL with *heptane R*.

Reference solution (a) Prepare 0.50 g of the mixture of calibrating substances with the composition described in Table 2542.-1, dissolve in *heptane R* and dilute to 50.0 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of test solution (a) to 100.0 mL with *heptane R*. Dilute 1.0 mL of this solution to 10.0 mL with *heptane R*.

Reference solution (c) To 0.5 g of *sodium lauroylsarcosinate CRS* in a 25 mL round-bottomed flask, add 6.0 mL of *boron trifluoride-methanol solution R*, fit a reflux condenser and boil for 20 min. Add 20.0 mL of hot *water R* so that the upper layer is brought into the neck of the flask in order to recover the methyl esters. Dissolve 0.25 g of the methyl esters in 50.0 mL of *heptane R*.

Table 2542.-1. – *Mixture of calibrating substances*

Mixture of the following substances	Composition (per cent *m/m*)
Methyl caproate R	10
Methyl caprylate R	10
Methyl decanoate R	20
Methyl laurate R	20
Methyl myristate R	40

Column:
— *material*: fused silica;
— *size*: *l* = 30 m, Ø = 0.25 mm;
— *stationary phase*: *macrogol 20 000 R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R*.

Split ratio 1:5.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 3	100
	3 - 13	100 → 200
	13 - 19	200 → 230
	19 - 29	230
	29 - 31	230 → 250
	31 - 40	250
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 1 µL.

Identification of peaks Use the chromatogram obtained with reference solution (a) to identify the peaks due to methyl caprylate, methyl decanoate, methyl laurate and methyl myristate.

System suitability:
— *resolution*: minimum 4.0 between the peaks due to methyl caprylate and methyl decanoate in the chromatogram obtained with reference solution (a);
— *signal-to-noise ratio*: minimum 20 for the peak due to methyl laurate in the chromatogram obtained with reference solution (b).

Disregard any peak eluting after the peak due to methyl myristate, as well as any peak with an area less than

0.05 per cent of the total area of the peaks used for quantification.

Composition of the fatty-acid fraction of the substance Test solution (a):
— *capric acid*: maximum 0.5 per cent;
— *lauric acid*: minimum 98.0 per cent;
— *myristic acid*: maximum 1.5 per cent.

Sodium laurate
Proceed as described in the test for composition of fatty acids with the following modifications.

Internal standard *undecanoic acid R.*

Test solution To 2.5 g in a 150 mL round-bottomed flask add 0.100 g of the internal standard. Add 30.0 mL of *boron trifluoride-methanol solution R*, fit a reflux condenser and boil for 20 min. Add 120 mL of hot *water R* so that the upper layer is brought into the neck of the flask in order to recover the methyl esters. Dissolve 0.50 g of the methyl esters in 10.0 mL of *heptane R*.

Calculate the percentage content of sodium laurate using the following expression:

$$\frac{A_1 \times m_2 \times 1.110 \times 100}{A_2 \times m_1}$$

A_1 = area of the peak due to methyl laurate in the chromatogram obtained with the test solution;
A_2 = area of the peak due to methyl undecanoate in the chromatogram obtained with the test solution;
m_1 = mass of the substance to be examined in the test solution, in grams;
m_2 = mass of the internal standard in the test solution, in grams;
1.110 = ratio of the molecular mass of sodium laurate to that of lauric acid (222/200).

Limit:
— *sodium laurate*: maximum 5.0 per cent.

Sodium chloride
Maximum 0.5 per cent.

Dissolve 0.500 g in 50 mL of a mixture of equal volumes of *ethanol (96 per cent) R* and *water R*. Add 10 mL of *nitric acid R* and titrate with *0.005 M silver nitrate*, determining the end-point potentiometrically (*2.2.20*).

Calculate the percentage content of sodium chloride using the following expression:

$$\frac{M \times V \times 58.44}{m \times 10}$$

M = molarity of the silver nitrate solution, in moles per litre;
V = volume of *0.005 M silver nitrate* used, in mL;
m = mass of the substance to be examined, in grams.

Sodium sulfate
Maximum 1.5 per cent.

Test solution In a 150 mL borosilicate-glass beaker, dissolve 0.3 g in 20 mL of *deionised distilled water R*. Add 0.3 mL of *naphtharson solution R1*, 1.0 mL of *perchloric acid R* and 70.0 mL of *2-propanol R*.

Degas the solution obtained under vacuum for 30 s or using an ultrasonic bath for 2 min, then titrate with 0.005 M barium perchlorate.

Determine the end-point using a suitable autotitrator equipped with a photometric sensor at 523 nm and at 25 °C. Adjust the stirrer speed to avoid the formation of bubbles.

1 mL of 0.005 M barium perchlorate is equivalent to 0.7102 mg of Na_2SO_4.

Ph Eur

Sodium Lauryl Sulfate[1]

Sodium Dodecyl Sulfate
Sodium Lauryl Sulphate
(Sodium Laurilsulfate, Ph. Eur. monograph 0098)
Sodium dodecyl sulfate: 151-21-3

Action and use
Anionic emulsifying agent.

Ph Eur

DEFINITION
Mixture of sodium alkyl sulfates consisting mainly of sodium dodecyl sulfate ($C_{12}H_{25}NaO_4S$; M_r 288.4).

Content
— *sodium alkyl sulfates*: minimum 85.0 per cent, expressed as $C_{12}H_{25}NaO_4S$.

♦CHARACTERS
Appearance
White or pale yellow, powder or crystals.

Solubility
Freely soluble in water giving an opalescent solution, partly soluble in ethanol (96 per cent).♦

IDENTIFICATION
First identification: A, B, C.
◊Second identification: B, C, D.◊

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *sodium laurilsulfate CRS.*

B. Place 2.5 g in a silica or platinum crucible and add 2 mL of a 500 g/L solution of *sulfuric acid R*. Heat on a water-bath, then cautiously raise the temperature progressively over an open flame. Ignite, preferably in a muffle furnace, at 600 ± 25 °C. Continue heating until all black particles have disappeared. Cool, add a few drops of a 100 g/L solution of *sulfuric acid R*, and heat and ignite as above. Add a few drops of *ammonium carbonate solution R*, evaporate to dryness, and ignite as above. Cool, dissolve the residue in 50 mL of *water R* and mix. To 2 mL of this solution add 4 mL of *potassium pyroantimonate solution R1*. If necessary, rub the inside of the test-tube with a glass rod. A white, crystalline precipitate is formed.

C. Prepare a 100 g/L solution. After acidification with *hydrochloric acid R* and boiling for 20 min, no precipitate is formed. Add a 120 g/L solution of *barium chloride R*; a white precipitate is produced.

◊D. Dissolve 0.1 g in 10 mL of *water R* and shake. A copious foam is formed.◊

TESTS
Alkalinity
Dissolve 1.0 g in 100 mL of *carbon dioxide-free water R* and add 0.1 mL of *phenol red solution R*. Not more than 0.5 mL of *0.1 M hydrochloric acid* is required to change the colour of the indicator.

Non-esterified alcohols
Maximum 4.0 per cent.

Dissolve 10.0 g in 100 mL of *water R*, add 100 mL of *ethanol (96 per cent) R* and shake the solution with 3 quantities, each of 50 mL, of *light petroleum R*, adding *sodium chloride R*, if necessary, to promote separation of the 2 layers. Wash the

[1] *This monograph has undergone pharmacopoeial harmonisation. See chapter 5.8 Pharmacopoeial harmonisation.*

combined organic layers with 3 quantities, each of 50 mL, of *water R*, dry over *anhydrous sodium sulfate R*, filter and evaporate on a water-bath until the solvent has evaporated. Heat the residue at 105 °C for 30 min and cool. The residue weighs a maximum of 0.4 g.

Sodium chloride and sodium sulfate
Maximum 8.0 per cent for the total percentage content.

Sodium chloride Dissolve 5.00 g in 50 mL of *water R*, add *dilute nitric acid R* dropwise, if necessary, until the solution is neutral, and add 5.0 mL of a 5.84 g/L solution of *sodium chloride R*. Titrate with *0.1 M silver nitrate*, while stirring vigorously to disperse the silver chloride, using 0.1 mL of a 2 g/L solution of *sodium fluoresceinate R* as indicator, to the first appearance of turbidity with solution colour change from yellow-green to orange through yellow. Perform a blank determination.

1 mL of *0.1 M silver nitrate* is equivalent to 5.844 mg of NaCl.

Sodium sulfate Dissolve 1.00 g in 10 mL of *water R*, add 100 mL of *ethanol (96 per cent) R* and heat at a temperature just below the boiling point for 2 h. Filter while hot through a glass or porcelain filter with a porosity of 4-10 µm and wash with 100 mL of boiling *ethanol (96 per cent) R*. Dissolve the precipitate by washing with 150 mL of *water R*, collecting the washings in a beaker. Add 10 mL of *dilute hydrochloric acid R*, heat to boiling, add 25 mL of a 120 g/L solution of *barium chloride R* and allow to stand overnight. Collect the precipitate by filtration (maximum pore size 16 µm) and wash with *water R* until the last washing shows no opalescence with *0.1 M silver nitrate*. Dry the precipitate, ignite to constant mass at 500-600 °C by raising the temperature gradually, and weigh as barium sulfate.

1 mg of barium sulfate is equivalent to 0.609 mg of Na_2SO_4.

ASSAY
Dissolve 1.15 g in *water R*, warming if necessary, and dilute to 1000.0 mL with the same solvent. To 20.0 mL of the solution add 15 mL of *methylene chloride R* and 10 mL of *dimidium bromide-sulfan blue mixed solution R* and mix. Titrate with *0.004 M benzethonium chloride*, shaking vigorously and allowing the layers to separate before each addition, until the pink colour of the methylene chloride layer is completely discharged and a greyish-blue colour is obtained.

1 mL of *0.004 M benzethonium chloride* is equivalent to 1.154 mg of sodium alkyl sulfates, expressed as $C_{12}H_{25}NaO_4S$.

Ph Eur

Sodium Metabisulfite

Sodium Metabisulphite
Sodium Pyrosulphite
(Ph. Eur. monograph 0849)

$Na_2S_2O_5$ 190.1 *7681-57-4*

Action and use
Antioxidant.

Ph Eur

DEFINITION
Sodium metabisulfite also called sodium disulfite.

Content
95.0 per cent to 100.5 per cent.

CHARACTERS
Appearance
White or almost white, crystalline powder or colourless crystals.

Solubility
Freely soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION
A. pH (see Tests).

B. To 0.4 mL of *iodinated potassium iodide solution R* add 8 mL of *distilled water R* and 1 mL of solution S diluted 1 to 10 in *distilled water R*. The solution is colourless and gives reaction (a) of sulfates (*2.3.1*).

C. Solution S gives reaction (a) of sodium (*2.3.1*).

TESTS
Solution S
Dissolve 5.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)
3.5 to 5.0 for solution S.

Thiosulfates
To 5 mL of solution S add 5 mL of *dilute hydrochloric acid R*. The solution remains clear (*2.2.1*) for at least 15 min.

Iron (*2.4.9*)
Maximum 20 ppm, determined on solution S.

ASSAY
Dissolve 0.200 g in 50.0 mL of *0.05 M iodine* and add 5 mL of *dilute hydrochloric acid R*. Titrate the excess of iodine with *0.1 M sodium thiosulfate* using 1 mL of *starch solution R*, added towards the end of the titration, as indicator.

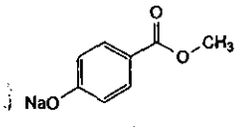
1 mL of *0.05 M iodine* is equivalent to 4.753 mg of $Na_2S_2O_5$.

STORAGE
Protected from light.

Ph Eur

Sodium Methyl Hydroxybenzoate

Sodium Methylparaben
(Sodium Methyl Parahydroxybenzoate, Ph. Eur. monograph 1262)



$C_8H_7NaO_3$ 174.1 *5026-62-0*

Action and use
Antimicrobial preservative.

Ph Eur

DEFINITION
Sodium 4-(methoxycarbonyl)phenolate.

Content
95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, hygroscopic, crystalline powder.

Solubility

Freely soluble in water, sparingly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Dissolve 0.5 g in 50 mL of *water R*. Immediately add 5 mL of *hydrochloric acid R1*. Filter and wash the precipitate with *water R*. Dry *in vacuo* at 80 °C for 2 h. The precipitate melts (*2.2.14*) at 125 °C to 128 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Precipitate obtained in identification test A.

Comparison *methyl parahydroxybenzoate CRS*.

C. Thin-layer chromatography (*2.2.27*).

Test solution (a) Dissolve 0.10 g of the substance to be examined in 10 mL of *water R*. Immediately add 2 mL of *hydrochloric acid R* and shake with 50 mL of *1,1-dimethylethyl methyl ether R*. Evaporate the upper layer to dryness and take up the residue with 10 mL of *acetone R*.

Test solution (b) Dilute 1 mL of test solution (a) to 10 mL with *acetone R*.

Reference solution (a) Dissolve 10 mg of *methyl parahydroxybenzoate CRS* in *acetone R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 10 mg of *ethyl parahydroxybenzoate CRS* in 1 mL of test solution (a) and dilute to 10 mL with *acetone R*.

Plate *TLC octadecylsilyl silica gel F_{254} plate R*.

Mobile phase *glacial acetic acid R, water R, methanol R* (1:30:70 *V/V/V*).

Application 5 µL of test solution (b) and reference solutions (a) and (b).

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):
- the chromatogram shows 2 clearly separated principal spots.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To 1 mL of solution S (see Tests) add 1 mL of *water R*. The solution gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50 mL with the same solvent.

Appearance of solution

Solution S examined immediately after preparation is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

pH (*2.2.3*)

Dilute 1 mL of solution S to 100 mL with *carbon dioxide-free water R*. The pH of the solution is 9.5 to 10.5.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in 2.5 mL of *methanol R* and dilute to 50.0 mL with the mobile phase. Dilute 10.0 mL of the solution to 100.0 mL with the mobile phase.

Reference solution (a) Dissolve 5 mg of *4-hydroxybenzoic acid R* (impurity A) and 5 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 50.0 mg of *methyl parahydroxybenzoate CRS* in 2.5 mL of *methanol R* and dilute to 50.0 mL with the mobile phase. Dilute 10.0 mL of the solution to 100.0 mL with the mobile phase.

Reference solution (c) Dilute 1.0 mL of the test solution to 20.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase 6.8 g/L solution of *potassium dihydrogen phosphate R, methanol R* (35:65 *V/V*).

Flow rate 1.3 mL/min.

Detection Spectrophotometer at 272 nm.

Injection 10 µL of the test solution and reference solutions (a) and (c).

Run time 5 times the retention time of methyl parahydroxybenzoate.

Relative retention With reference to methyl parahydroxybenzoate (retention time = about 2.3 min): impurity A = about 0.6.

System suitability Reference solution (a):
- *resolution*: minimum 2.0 between the peaks due to impurity A and methyl parahydroxybenzoate.

Limits:
- *correction factor*: for the calculation of content, multiply the peak area of impurity A by 1.4;
- *impurity A*: not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (c) (3.0 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);
- *sum of impurities other than A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent);
- *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Chlorides (*2.4.4*)

Maximum 350 ppm.

To 10 mL of solution S, add 30 mL of *water R* and 1 mL of *nitric acid R* and dilute to 50 mL with *water R*. Shake and filter. Dilute 10 mL of the filtrate to 15 mL with *water R*. Prepare the standard using 14 mL of *chloride standard solution (5 ppm Cl) R* to which 1 mL of *water R* has been added.

Sulfates (*2.4.13*)

Maximum 300 ppm.

To 25 mL of solution S, add 5 mL of *distilled water R* and 10 mL of *hydrochloric acid R* and dilute to 50 mL with *distilled water R*. Shake and filter. Dilute 10 mL of the filtrate to 15 mL with *distilled water R*.

Water (*2.5.12*)
Maximum 5.0 per cent, determined on 0.500 g.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

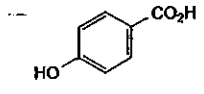
Injection Test solution and reference solution (b).

Calculate the percentage content of $C_8H_7NaO_3$ using the declared content of *methyl parahydroxybenzoate CRS* and multiplying by a correction factor of 1.145.

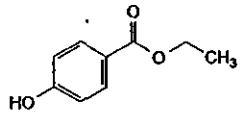
STORAGE
In an airtight container.

IMPURITIES
Specified impurities *A.*

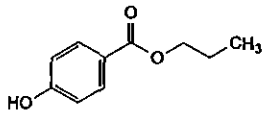
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D.*



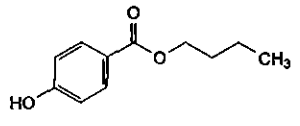
A. 4-hydroxybenzoic acid,



B. ethyl 4-hydroxybenzoate (ethyl parahydroxybenzoate),



C. propyl 4-hydroxybenzoate (propyl parahydroxybenzoate),



D. butyl 4-hydroxybenzoate (butyl parahydroxybenzoate).

Ph Eur

Sodium Molybdate Dihydrate

(Ph. Eur. monograph 1565)

$MoNa_2O_4,2H_2O$ 241.9 *10102-40-6*

Ph Eur

DEFINITION
Content
98.0 per cent to 100.5 per cent (dried substance).

CHARACTERS
Appearance
White or almost white powder or colourless crystals.

Solubility
Freely soluble in water.

IDENTIFICATION
A. Loss on drying (see Tests).

B. Dissolve 0.2 g in 5 mL of a mixture of equal volumes of *nitric acid R* and *water R* and add 0.1 g of *ammonium chloride R*. Add 0.3 mL of *disodium hydrogen phosphate solution R* and heat slowly at 50-60 °C. A yellow precipitate is formed.

C. Dissolve 0.15 g in 2 mL of *water R*. The solution gives reaction (a) of sodium (*2.3.1*).

TESTS
Solution S
Dissolve 5.0 g in *water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Chlorides
Maximum 50 ppm.

To 10 mL of a mixture of equal volumes of *nitric acid R* and *water R* add 10 mL of solution S with shaking. Add 1 mL of *0.1 M silver nitrate*. Any opalescence in the solution is not more intense after 5 min than that of a standard solution prepared at the same time in the same manner with 10 mL of *chloride standard solution (5 ppm Cl) R*.

Phosphates
Maximum 200 ppm.

Dissolve 2.0 g by heating in 13 mL of *water R*. In the still-hot solution, dissolve 8.0 g of *ammonium nitrate R1*. Add this solution to 27 mL of a mixture of equal volumes of *nitric acid R* and *water R*. Any yellow colour or opalescence in the solution is not more intense within 3 h than that in a standard solution prepared at the same time in the same manner as follows: dissolve 1.0 g in 12 mL of *water R* and add 1 mL of *phosphate standard solution (200 ppm PO_4) R*.

Ammonium (*2.4.1, Method B*)
Maximum 10 ppm, determined on 0.10 g.

Prepare the standard using 1 mL of *ammonium standard solution (1 ppm NH_4) R*.

Loss on drying (*2.2.32*)
14.0 per cent to 16.0 per cent, determined on 1.000 g by drying in an oven at 140 °C for 3 h.

ASSAY
Dissolve 0.100 g in 30 mL of *water R*, add 0.5 g of *hexamethylenetetramine R* and 0.1 mL of a 250 g/L solution of *nitric acid R*. Heat to 60 °C. Titrate with *0.05 M lead nitrate* using *4-(2-pyridylazo)resorcinol monosodium salt R* as indicator.

1 mL of *0.05 M lead nitrate* is equivalent to 10.30 mg of $MoNa_2O_4$.

Ph Eur

Sodium Nitrite

(Ph. Eur. monograph 1996)

$NaNO_2$ 69.0 *7632-00-0*

Action and use
Used in treatment of cyanide poisoning.

Ph Eur

DEFINITION
Content
98.5 per cent to 100.5 per cent (dried substance).

CHARACTERS
Appearance
Colourless crystals or mass or yellowish rods, hygroscopic.

Solubility
Freely soluble in water, soluble in ethanol (96 per cent).

IDENTIFICATION
A. Dilute 1 mL of solution S1 (see Tests) to 25 mL with *water R*. To 0.1 mL of the solution add 1 mL of *sulfanilic acid solution R1*. Allow to stand for 2-3 min. Add 1 mL of *β-naphthol solution R* and 1 mL of *dilute sodium hydroxide solution R*. An intense red colour develops.

B. To 1 mL of the solution prepared for identification test A add 3 mL of a 20 g/L solution of *phenazone R* and 0.4 mL of *dilute sulfuric acid R*. An intense green colour develops.

C. To 0.15 mL of solution S1, add 0.35 mL of *water R*. The solution gives reaction (b) of sodium (*2.3.1*).

TESTS
Solution S1
Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Solution S2
Dissolve 3 g in *distilled water R*. Cautiously add 10 mL of *nitric acid R* and evaporate to dryness. Dissolve the residue in 10 mL of *distilled water R*, neutralise with *dilute sodium hydroxide solution R* and dilute to 30 mL with *distilled water R*.

Appearance of solution
Solution S1 is clear (*2.2.1*) and not more intensely coloured than reference solution B_6 (*2.2.2, Method II*).

Acidity or alkalinity
To 10 mL of solution S1, add 0.05 mL of *phenol red solution R*. Add 0.1 mL of *0.01 M sodium hydroxide*. The solution is red. Add 0.3 mL of *0.01 M hydrochloric acid*. The solution is yellow.

Chlorides (*2.4.4*)
Maximum 50 ppm.

Dilute 10 mL of solution S2 to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 200 ppm.

Dilute 7.5 mL of solution S2 to 15 mL with *distilled water R*.

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying *in vacuo*.

ASSAY
Dissolve 0.400 g in 100.0 mL of *water R*. Introduce 20.0 mL of the solution, while stirring continuously and keeping the tip of the pipette below the surface of the liquid, into a conical flask containing 30.0 mL of *0.1 M cerium sulfate*. Immediately stopper the flask and allow to stand for 2 min. Add 10 mL of a 200 g/L solution of *potassium iodide R* and 2 mL of *starch solution R*.

While stirring continuously, titrate with *0.1 M sodium thiosulfate* until the blue colour disappears. Carry out a blank titration.

1 mL of *0.1 M cerium sulfate* is equivalent to 3.45 mg of $NaNO_2$.

STORAGE
In an airtight container.

Ph Eur

Sodium Nitroprusside

(Ph. Eur. monograph 0565)

$Na_2[Fe(CN)_5(NO)],2H_2O$ 298.0 *13755-38-9*

Action and use
Vasodilator.

Preparation
Sodium Nitroprusside Infusion

Ph Eur

DEFINITION
Sodium pentacyanonitrosylferrate (III) dihydrate.

Content
99.0 per cent to 100.5 per cent (anhydrous substance).

CHARACTERS
Appearance
Reddish-brown powder or crystals.

Solubility
Freely soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION
A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 0.700 g in *water R* and dilute to 100.0 mL with the same solvent. Examine the solution immediately after preparation.

Spectral range 350-600 nm.

Absorption maximum At 395 nm.

Shoulder At about 510 nm.

Absorption minimum At 370 nm.

Specific absorbance at the absorption maximum 0.65 to 0.80.

B. Dissolve about 20 mg in 2 mL of *water R* and add 0.1 mL of *sodium sulfide solution R*. A deep violet-red colour is produced.

C. Dissolve 50 mg in 1 mL of *water R* and acidify the solution by the addition of *hydrochloric acid R*. Place a drop of the solution in an oxidising flame. A persistent yellow colour is produced.

TESTS
Insoluble matter
Maximum 100 ppm.

Dissolve 10 g without heating in 50 mL of *water R*. Allow to stand for 30 min and filter through a sintered-glass filter (16) (*2.1.2*). Wash the filter with cold *water R* until the filtrate is colourless. Dry the residue on the filter at 105 °C. The residue weighs a maximum of 1 mg.

Chlorides (*2.4.4*)
Maximum 200 ppm.

In a metallic crucible (nickel) mix 1.0 g with 8 mL of a 200 g/L solution of *sodium hydroxide R*. Heat slowly and evaporate carefully to dryness over a small flame, then heat to a dull red colour for 30 min. Allow to cool and transfer the solid residue with 3 quantities, each of 8 mL, of *dilute sulfuric acid R*. Filter the sulfuric acid extracts on a filter paper and collect the filtrates. Render the filtrate acid to *blue litmus paper R* by adding, if necessary, a few drops of *dilute sulfuric acid R*. Wash the crucible and the filter paper with 3 quantities, each of 10 mL, of *water R*, add the washings to the main sulfuric acid solution and dilute to 60 mL with *water R*. Mix.

Ferricyanides
Maximum 200 ppm.

Dissolve 1.25 g in *acetate buffer solution pH 4.6 R* and dilute to 50.0 mL with the same buffer solution. Use three 50 mL volumetric flasks (*A*, *B*, *C*). To flask *B* add 1.0 mL of *ferricyanide standard solution (50 ppm $Fe(CN)_6$) R*. To flasks *A* and *B* add 1 mL of a 5 g/L solution of *ferrous ammonium sulfate R*. To the 3 flasks add 10.0 mL of the solution of the substance to be examined. Dilute the contents of each flask to 50.0 mL with *water R*. Allow to stand for 30 min.
The absorbance (*2.2.25*) of the solution in flask *A* measured at 720 nm using the solution in flask *C* as the compensation liquid is not greater than the absorbance of the solution in flask *B* measured at 720 nm using the solution in flask *A* as the compensation liquid.

Ferrocyanides
Maximum 200 ppm.

Dissolve 4.0 g in *water R* and dilute to 100.0 mL with the same solvent. Use three 50 mL volumetric flasks (*A*, *B*, *C*). To flask *B* add 2.0 mL of *ferrocyanide standard solution (100 ppm $Fe(CN)_6$) R*. To flasks *A* and *B* add 1 mL of *ferric chloride solution R2*. To the 3 flasks add 25.0 mL of the solution of the substance to be examined. Dilute the contents of each flask to 50.0 mL with *water R*. Allow to stand for 30 min. The absorbance (*2.2.25*) of the solution in flask *A* measured at 695 nm using the solution in flask *C* as the compensation liquid is not greater than the absorbance of the solution in flask *B* measured at 695 nm using the solution in flask *A* as the compensation liquid.

Sulfates
Maximum 100 ppm.

Test solution Dissolve 3.6 g in 120 mL of *distilled water R*, add with mixing 4 mL of *sulfate standard solution (10 ppm SO_4) R* and 20 mL of a 250 g/L solution of *cupric chloride R* and dilute to 150.0 mL with *distilled water R*. Allow to stand for 16 h and filter or centrifuge until a clear light-blue solution is obtained.

Reference solution To 40 mL of *sulfate standard solution (10 ppm SO_4) R* add 80 mL of *distilled water R* and 12-13 mL of a 250 g/L solution of *cupric chloride R*. Dilute to 150.0 mL with *distilled water R*. The volume of cupric chloride solution added is such that the colour of the final solution matches that of the test solution.

Allow the solutions to stand. Filter both solutions, discarding the first 25 mL of filtrate. To 100 mL of each filtrate, add 0.5 mL of *acetic acid R*. Mix and add 2 mL of a 250 g/L solution of *barium chloride R* and mix again. The test solution is not more opalescent than the reference solution.

Water (*2.5.12*)
9.0 per cent to 15.0 per cent, determined on 0.250 g.

ASSAY
Dissolve 0.250 g in 100 mL of *water R* and add 0.1 mL of *dilute sulfuric acid R*. Titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (*2.2.20*).

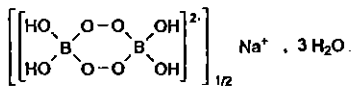
1 mL of *0.1 M silver nitrate* is equivalent to 13.10 mg of $Na_2[Fe(CN)_5(NO)]$.

STORAGE
Protected from light.

Ph Eur

Sodium Perborate

(Hydrated Sodium Perborate, Ph. Eur. monograph 1997)



$NaBO_3,4H_2O$ or $NaBO_2,H_2O_2,3H_2O$ 153.9

Action and use
Antiseptic.

Ph Eur

DEFINITION
Content
96.0 per cent to 103.0 per cent.

CHARACTERS
Appearance
Colourless, prismatic crystals or white or almost white powder, stable in the crystalline form.

Solubility
Sparingly soluble in water, with slow decomposition. It dissolves in dilute mineral acids.

IDENTIFICATION
A. Dissolve 20 mg in a mixture of 1 mL of *dilute sulfuric acid R* and 1 mL of *water R*. Add 1 mL of *potassium iodide solution R*. A reddish-brown colour appears.

B. The mixture obtained by treating about 100 mg with 0.1 mL of *sulfuric acid R* and 5 mL of *methanol R* burns with a greenish flame when ignited.

C. It gives reaction (a) of sodium (*2.3.1*).

TESTS
Chlorides (*2.4.4*)
Maximum 330 ppm.

Dissolve 0.15 g in 15 mL of *water R*.

Sulfates (*2.4.13*)
Maximum 1.2 per cent.

Dissolve 0.13 g in 150 mL of *distilled water R*.

Iron (*2.4.9*)
Maximum 20 ppm.

Dissolve 2.5 g in 10 mL of *dilute hydrochloric acid R* with heating, evaporate to dryness, with stirring, and dissolve the residue in 25 mL of hot *water R*. Dilute 5 mL of the obtained solution to 10 mL with *water R*.

ASSAY
Dissolve 0.300 g in 50.0 mL of *water R*. Dilute 10.0 mL of the solution to 50 mL with *water R* and add 10 mL of *dilute sulfuric acid R*. Titrate with *0.02 M potassium permanganate*.

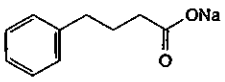
1 mL of *0.02 M potassium permanganate* is equivalent to 7.693 mg of NaH_8BO_7.

STORAGE
In an airtight container.

Ph Eur

Sodium Phenylbutyrate

(Ph. Eur. monograph 2183)



$C_{10}H_{11}NaO_2$ 186.2 *1716-12-7*

Action and use
Glutamine conjugate; treatment of hyperammonaemia.

Ph Eur

DEFINITION
Sodium 4-phenylbutanoate.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or yellowish-white powder.

Solubility
Freely soluble in water and in methanol, practically insoluble in methylene chloride.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *sodium phenylbutyrate CRS.*

B. Dissolve 0.15 g in 2 mL of *water R*. The solution gives reaction (a) of sodium (*2.3.1*).

TESTS

pH (*2.2.3*)
6.5 to 7.5.

Dissolve 0.20 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Impurity C
Gas chromatography (*2.2.28*).

Silylation solution To 2 mL of *N,O-bis(trimethylsilyl) trifluoroacetamide R* add 0.04 mL of *chlorotrimethylsilane R* and mix.

Test solution Dissolve 50.0 mg of the substance to be examined in 3 mL of *water R* and add 0.5 mL of *hydrochloric acid R*. Extract with 2 quantities, each of 5 mL, of *methylene chloride R*. Evaporate the combined methylene chloride extracts to dryness in a vial with a screw cap and add 0.5 mL of the silylation solution. Seal the vial and heat at 70 ± 5 °C for 20 min.

Reference solution (a) Dissolve 5.0 mg of *sodium phenylbutyrate impurity C CRS* in *methylene chloride R* and dilute to 10.0 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 10.0 mL with *methylene chloride R*. Place 1.0 mL of this solution in a vial with a screw cap, evaporate to dryness and add 0.5 mL of the silylation solution. Seal the vial and heat at 70 ± 5 °C for 20 min.

Reference solution (c) Dissolve 10 mg of the substance to be examined in 25 mL of *water R*. To 3 mL of this solution add 0.1 mL of *hydrochloric acid R*. Extract with 2 quantities, each of 5 mL, of *methylene chloride R*. Combine the methylene chloride extracts in a vial with a screw cap and add 2 mL of reference solution (a). Evaporate to dryness and add 0.5 mL of the silylation solution. Seal the vial and heat at 70 ± 5 °C for 20 min.

Column:
— *material*: fused silica;
— *size*: *l* = 25 m, Ø = 0.25 mm;
— *stationary phase*: *phenyl(5)methyl(95)polysiloxane R* (film thickness 1.0 µm).

Carrier gas *helium for chromatography R.*

Flow rate 0.9 mL/min.

Split ratio 1:100.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 5	50
	5 - 27	50 → 270
	27 - 32	270
Injection port		270
Detector		270

Detection Flame ionisation.

Injection 1 µL.

Relative retention With reference to phenylbutyrate (retention time = about 20 min): impurity C = about 0.98.

System suitability Reference solution (c):
— *resolution*: minimum 3.0 between the peaks due to impurity C and phenylbutyrate.

Limit:
— *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.20 g of the substance to be examined in 10 mL of *methanol R* and dilute to 50.0 mL with *water R*.

Reference solution (a) Dissolve 4.0 mg of *α-tetralone R* (impurity B) in 10 mL of *methanol R* and dilute to 200.0 mL with the same solvent.

Reference solution (b) Dissolve 0.20 g of the substance to be examined in 10 mL of *methanol R*, add 1 mL of reference solution (a) and dilute to 50 mL with *water R*.

Reference solution (c) Dilute 1.0 mL of reference solution (a) to 50.0 mL with *water R*.

Reference solution (d) Dissolve 5.0 mg of *3-benzoylpropionic acid R* (impurity A) in 2.5 mL of *methanol R* and dilute to 50.0 mL with the same solvent. Dilute 1.0 mL of this solution to 50.0 mL with *water R*.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *glacial acetic acid R, methanol R, water R* (1:49:50 *V/V/V*).

Flow rate 1.3 mL/min.

Detection Spectrophotometer at 245 nm.

Injection 20 µL of the test solution and reference solutions (b), (c) and (d).

Run time Twice the retention time of phenylbutyrate.

Relative retention With reference to phenylbutyrate (retention time = about 17 min): impurity A = about 0.3; impurity B = about 0.7.

System suitability Reference solution (b):
— *resolution*: minimum 6 between the peaks due to impurity B and phenylbutyrate.

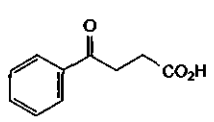
Limits:
— *impurity A*: not more than twice the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.1 per cent);
— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.01 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.05 per cent);
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (d) (0.1 per cent);
— *disregard limit of impurities other than B*: 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.03 per cent).

Water (*2.5.12*)
Maximum 0.5 per cent, determined on 2.00 g.

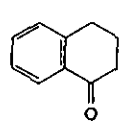
ASSAY
Disperse 0.150 g in 50 mL of *anhydrous acetic acid R*. The opalescence of the solution disappears during the titration. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 18.62 mg of $C_{10}H_{11}NaO_2$.

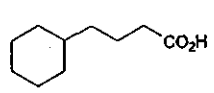
IMPURITIES
Specified impurities *A, B, C.*



A. 4-oxo-4-phenylbutanoic acid (3-benzoylpropionic acid),



B. 3,4-dihydronaphthalen-1(2*H*)-one (α-tetralone),

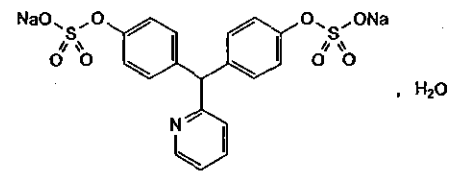


C. 4-cyclohexylbutanoic acid.

Ph Eur

Sodium Picosulfate

(Ph. Eur. monograph 1031)



$C_{18}H_{13}NNa_2O_8S_2,H_2O$ 499.4

Action and use
Stimulant laxative.

Preparations
Compound Sodium Picosulfate Powder for Oral Solution
Sodium Picosulfate Oral Solution

Ph Eur

DEFINITION
4,4′-[(Pyridin-2-yl)methylene]diphenyl bis(sodium sulfate) monohydrate.

Content
98.5 per cent to 100.5 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION
First identification: A, D.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *sodium picosulfate CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 5 mL with the same solvent.

Reference solution Dissolve 20 mg of *sodium picosulfate CRS* in *methanol R* and dilute to 5 mL with the same solvent.

Plate *TLC silica gel GF_{254} plate R.*

Mobile phase *anhydrous formic acid R, water R, methanol R, ethyl acetate R* (2.5:12.5:25:60 *V/V/V/V*).

Application 5 µL.

Development Over 1/2 of the plate.

Drying In a current of warm air for 15 min.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. To 5 mL of solution S (see Tests) add 1 mL of *dilute hydrochloric acid R* and heat to boiling. Add 1 mL of *barium chloride solution R1*. A white precipitate is formed.

D. Solution S gives reaction (a) of sodium (*2.3.1*).

TESTS
Solution S
Dissolve 2.5 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution GY_7 (*2.2.2, Method II*).

Acidity or alkalinity
To 10 mL of solution S add 0.05 mL of *phenolphthalein solution R*. The solution is colourless. Not more than 0.25 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 50 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 10.0 mL of this solution to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve the contents of a vial of *picosulfate for system suitability CRS* (containing impurities A and B) in 1.0 mL of the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.0 mm;
— *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R1* (5 µm);
— *temperature*: 40 °C.

Mobile phase Dissolve 2.3 g of *disodium hydrogen phosphate dihydrate R* in 800 mL of *water for chromatography R*, add 0.2 g of *cetyltrimethylammonium bromide R*, adjust to pH 7.5 with *phosphoric acid R* and dilute to 1000 mL with *water for chromatography R*; mix 550 mL of this solution and 450 mL of *acetonitrile R* (if necessary vary the buffer/acetonitrile proportion in 10 mL increments in order to fulfil the resolution requirement).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 263 nm.

Injection 40 µL.

Run time Twice the retention time of picosulfate.

Identification of impurities Use the chromatogram supplied with *picosulfate for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and B.

Relative retention With reference to picosulfate (retention time = about 7.4 min): impurity B = about 0.5; impurity A = about 0.7.

System suitability Reference solution (b):
— *resolution*: minimum 4.0 between the peaks due to impurities B and A.

Limits:
— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.7; impurity B = 0.5;
— *impurities A, B*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Chlorides (*2.4.4*)
Maximum 200 ppm.

Dilute 5 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 400 ppm.

Dilute 7.5 mL of solution S to 15 mL with *distilled water R*.

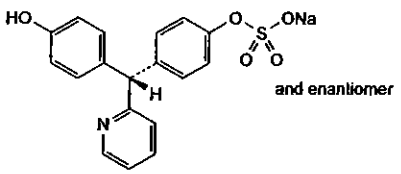
Water (*2.5.12*)
3.0 per cent to 5.0 per cent, determined on 0.500 g.

ASSAY
Dissolve 0.400 g in 80 mL of *methanol R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

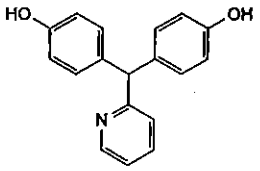
1 mL of *0.1 M perchloric acid* is equivalent to 48.14 mg of $C_{18}H_{13}NNa_2O_8S_2$.

IMPURITIES
Specified impurities *A, B.*

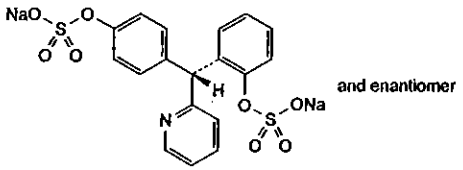
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C.*



A. 4-[(*RS*)-(4-hydroxyphenyl)(pyridin-2-yl)methyl]phenyl sodium sulfate,



B. 4,4′-[(pyridin-2-yl)methylene]diphenol,



C. 2-[(*RS*)-(pyridin-2-yl)[4-(sulfonatooxy)phenyl] methyl]phenyl disodium sulfate.

Sodium Polystyrene Sulfonate

Sodium Polystyrene Sulphonate

(Ph. Eur. monograph 1909)

Action and use
Used in the treatment of hyperkalaemia.

Ph Eur

DEFINITION

Polystyrene sulfonate resin prepared in the sodium form.

Exchange capacity 2.8 mmol to 3.4 mmol of potassium per gram (dried substance).

Content
9.4 per cent to 11.0 per cent of Na (dried substance).

CHARACTERS

Appearance
Almost white or light brown powder.

Solubility
Practically insoluble in water, in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs using finely ground substance.

Comparison *Ph. Eur. reference spectrum of sodium polystyrene sulfonate.*

B. Suspend 0.1 g in *water R*, add 2 mL of a 150 g/L solution of *potassium carbonate R*, and heat to boiling. Allow to cool and filter. To the filtrate add 4 mL of *potassium pyroantimonate solution R* and heat to boiling. Allow to cool in iced water and if necessary rub the inside of the test-tube with a glass rod. A dense white precipitate is formed.

TESTS

Styrene
Liquid chromatography (*2.2.29*).

Test solution Shake 10.0 g of the substance to be examined with 10 mL of *acetone R* for 30 min, centrifuge and use the supernatant.

Reference solution Dissolve 10 mg of *styrene R* in *acetone R* and dilute to 100 mL with the same solvent. Dilute 1 mL of this solution to 100 mL with *acetone R*.

Column:
— *size*: *l* = 0.25 m, Ø = 4 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R, water R* (1:1 *V/V*).

Flow rate 2 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL.

Limit:
— *styrene*: not more than the area of the principal peak in the chromatogram obtained with the reference solution (1 ppm).

Calcium
Maximum 0.10 per cent.

Atomic emission spectrometry (*2.2.22, Method I*).

Test solution To 1.10 g add 5 mL of *hydrochloric acid R*, heat to boiling, cool and add 10 mL of *water R*. Filter, wash the filter and residue with *water R* and dilute the filtrate and washing to 25.0 mL with *water R*.

Reference solutions Prepare the reference solutions using *calcium standard solution (400 ppm Ca) R*, diluted as necessary with *water R*.

Wavelength 422.7 nm.

Potassium
Maximum 0.10 per cent.

Atomic emission spectrometry (*2.2.22, Method I*).

Test solution To 1.10 g add 5 mL of *hydrochloric acid R*, heat to boiling, cool and add 10 mL of *water R*. Filter, wash the filter and residue with *water R* and dilute the filtrate and washings to 25.0 mL with *water R*.

Reference solutions Prepare the reference solutions using *potassium standard solution (100 ppm K) R*, diluted as necessary with *water R*.

Wavelength 766.5 nm.

Loss on drying (*2.2.32*)
Maximum 7.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Microbial contamination (*2.6.13*)
Bile-tolerant gram-negative bacteria: acceptance criterion less than 10^2 CFU/g.

ASSAY

Sodium
Atomic emission spectrometry (*2.2.22, Method I*).

Test solution In a platinum crucible moisten 0.90 g with a few drops of *sulfuric acid R*, ignite very gently and allow to cool. Moisten with a few drops of *sulfuric acid R* again, ignite at 800 ± 50 °C until a carbon-free ash is obtained and allow to cool.

Add 20 mL of *water R* to the crucible, warm gently on a water-bath until dissolution, cool, transfer quantitatively to a 100 mL graduated flask and dilute to 100.0 mL with *water R*. Dilute 5 mL of this solution to 1000.0 mL with *water R*.

Reference solutions Prepare the reference solutions using *sodium standard solution (200 ppm Na) R*, diluted as necessary with *water R*.

Wavelength 589 nm.

Exchange capacity
Atomic emission spectrometry (*2.2.22, Method I*).

Solution A 9.533 g/L solution of *potassium chloride R*.

Test solution To 1.6 g of the substance to be examined in a dry 250 mL ground-glass-stoppered flask add 100 mL of solution A, stopper and shake for 15 min. Filter, discard the first 20 mL of the filtrate and dilute 4 mL of the filtrate to 1000 mL with *water R*.

Reference solutions Prepare the reference solutions by diluting 0, 1, 2, 3 and 4 mL of solution A respectively and 4, 3, 2, 1 and 0 mL of a 7.63 g/L solution of *sodium chloride R* to 1000 mL with *water R*.

Wavelength 766.5 nm.

Prepare a calibration curve using the reference solutions and calculate the potassium exchange capacity of the substance to be examined in millimoles per gram taking the concentration of potassium in solution A as 128 mmoles of K per litre.

STORAGE

In an airtight container.

IMPURITIES

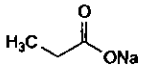
Specified impurities *A*.

A. styrene.

Ph Eur

Sodium Propionate

(Ph. Eur. monograph 2041)



$C_3H_5NaO_2$ 96.1 *137-40-6*

Action and use
Antifungal.

Ph Eur

DEFINITION
Sodium propanoate.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
Colourless crystals or, white or almost white powder, slightly hygroscopic.

Solubility
Freely soluble in water, sparingly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION
First identification: A, D.
Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *Ph. Eur. reference spectrum of sodium propionate.*

B. Dissolve 0.1 g in a mixture of 2 mL of *copper sulfate solution R* and 2 mL of *methylene chloride R*. Shake vigorously and allow to stand. Both the upper and the lower layer show a blue colour.

C. To 5 mL of solution S (see Tests) add 2 mL of *0.1 M silver nitrate*. A white precipitate is formed.

D. Solution S gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S
Dissolve 10 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)
7.8 to 9.2.
Dilute 1 mL of solution S to 5 mL with *water R*.

Related substances
Liquid chromatography (*2.2.29*).
Test solution Dissolve 0.250 g of the substance to be examined in *water R* and dilute to 100 mL with the same solvent.
Reference solution (a) Dissolve 10 mg of the substance to be examined and 10 mg of *sodium acetate R* in *water R* and dilute to 100 mL with the same solvent.
Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *water R*.
Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm,
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).
Mobile phase Dilute 1 mL of *phosphoric acid R* to 1000 mL with *water for chromatography R*.
Flow rate 1 mL/min.
Detection Spectrophotometer at 210 nm.
Injection 20 µL.
System suitability Reference solution (a):
— *resolution*: minimum 5.0 between the peaks due to sodium acetate and sodium propionate.
Limits:
— *any impurity*: not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),
— *total*: not more than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),
— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Readily oxidisable substances
In a ground-glass-stoppered conical flask introduce 10 g of the substance to be examined. Add 100 mL of *water R* and stir to dissolve. Add 25 mL of *sodium hypobromite solution R* and 10 mL of a 200 g/L solution of *sodium acetate R*, stopper the flask and allow to stand for 15 min. Add 10 mL of *potassium iodide solution R* and 20 mL of *hydrochloric acid R* while cooling. Titrate with *0.1 M sodium thiosulfate*, adding 2 mL of *starch solution R* towards the end of the titration. Carry out a blank titration. The difference between the volumes used in the 2 titrations is not greater than 2.2 mL.

Iron (*2.4.9*)
Maximum 10 ppm, determined on solution S.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by heating in an oven at 105 °C for 3 h.

ASSAY
Dissolve 80.0 mg in 30 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).
1 mL of *0.1 M perchloric acid* is equivalent to 9.61 mg of $C_3H_5NaO_2$.

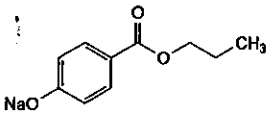
STORAGE
In an airtight container.

Ph Eur

Sodium Propyl Hydroxybenzoate

Sodium Propylparaben

(Sodium Propyl Parahydroxybenzoate, Ph. Eur. monograph 1263)



$C_{10}H_{11}NaO_3$ 202.2 *35285-69-9*

Action and use
Antimicrobial preservative.

Ph Eur

DEFINITION
Sodium 4-(propoxycarbonyl)phenolate.

Content
94.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, hygroscopic, crystalline powder.

Solubility

Freely soluble in water, sparingly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Dissolve 0.5 g in 50 mL of *water R*. Immediately add 5 mL of *hydrochloric acid R1*. Filter and wash the precipitate with *water R*. Dry *in vacuo* at 80 °C for 2 h. The precipitate melts (*2.2.14*) at 96 °C to 99 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Precipitate obtained in identification test A.

Comparison *propyl parahydroxybenzoate CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution (a) Dissolve 0.10 g of the substance to be examined in 10 mL of *water R*. Immediately add 2 mL of *hydrochloric acid R* and shake with 50 mL of *1,1-dimethylethyl methyl ether R*. Evaporate the upper layer to dryness and take up the residue with 10 mL of *acetone R*.

Test solution (b) Dilute 1 mL of test solution (a) to 10 mL with *acetone R*.

Reference solution (a) Dissolve 10 mg of *propyl parahydroxybenzoate CRS* in *acetone R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 10 mg of *ethyl parahydroxybenzoate CRS* in 1 mL of test solution (a) and dilute to 10 mL with *acetone R*.

Plate *TLC octadecylsilyl silica gel F_{254} plate R.*

Mobile phase *glacial acetic acid R, water R, methanol R* (1:30:70 *V/V/V*).

Application 5 µL of test solution (b) and reference solutions (a) and (b).

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated principal spots.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To 1 mL of solution S (see Tests) add 1 mL of *water R*. The solution gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* prepared from *distilled water R*, and dilute to 50 mL with the same solvent.

Appearance of solution

Solution S, examined immediately after preparation, is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

pH (*2.2.3*)

9.5 to 10.5.

Dilute 1 mL of solution S to 100 mL with *carbon dioxide-free water R*.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in 2.5 mL of *methanol R* and dilute to 50.0 mL with the mobile phase. Dilute 10.0 mL of the solution to 100.0 mL with the mobile phase.

Reference solution (a) Dissolve 5 mg of *ethyl parahydroxybenzoate CRS* (impurity C), 5 mg of *4-hydroxybenzoic acid R* (impurity A) and 5 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 50.0 mg of *propyl parahydroxybenzoate CRS* in 2.5 mL of *methanol R* and dilute to 50.0 mL with the mobile phase. Dilute 10.0 mL of the solution to 100.0 mL with the mobile phase.

Reference solution (c) Dilute 1.0 mL of the test solution to 20.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:

— *size: l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase 6.8 g/L solution of *potassium dihydrogen phosphate R, methanol R* (35:65 *V/V*).

Flow rate 1.3 mL/min.

Detection Spectrophotometer at 272 nm.

Injection 10 µL of the test solution and reference solutions (a) and (c).

Run time 2.5 times the retention time of propyl parahydroxybenzoate.

Relative retention With reference to propyl parahydroxybenzoate (retention time = about 4.5 min): impurity A = about 0.3; impurity C = about 0.7.

System suitability Reference solution (a):

— *resolution*: minimum 5.0 between the peaks due to impurity C and propyl parahydroxybenzoate.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity A by 1.4;

— *impurity A*: not more than 8 times the area of the principal peak in the chromatogram obtained with reference solution (c) (4.0 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *sum of impurities other than A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent);

— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Chlorides (*2.4.4*)

Maximum 350 ppm.

To 10 mL of solution S, add 1 mL of *nitric acid R* and 30 mL of *water R* and dilute to 50 mL with *water R*. Shake and filter. Dilute 10 mL of the filtrate to 15 mL with *water R*. Prepare the standard using 14 mL of *chloride standard solution (5 ppm Cl) R* to which 1 mL of *water R* has been added.

Sulfates (*2.4.13*)

Maximum 300 ppm.

To 25 mL of solution S, add 5 mL of *distilled water R* and 10 mL of *hydrochloric acid R* and dilute to 50 mL with *distilled water R*. Shake and filter. Dilute 10 mL of the filtrate to 15 mL with *distilled water R*.

Water (*2.5.12*)
Maximum 5.0 per cent, determined on 0.500 g.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

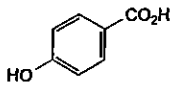
Injection Test solution and reference solution (b).

Calculate the percentage content of $C_{10}H_{11}NaO_3$ from the declared content of *propyl parahydroxybenzoate CRS*, multiplied by a correction factor of 1.122.

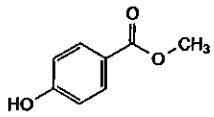
STORAGE
In an airtight container.

IMPURITIES
Specified impurities *A.*

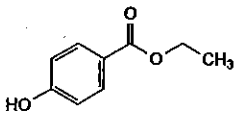
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D.*



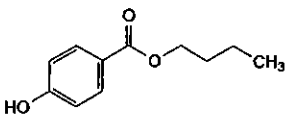
A. 4-hydroxybenzoic acid,



B. methyl 4-hydroxybenzoate (methyl parahydroxybenzoate),



C. ethyl 4-hydroxybenzoate (ethyl parahydroxybenzoate),

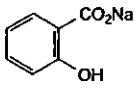


D. butyl 4-hydroxybenzoate (butyl parahydroxybenzoate).

Ph Eur

Sodium Salicylate

(Ph. Eur. monograph 0413)



$C_7H_5NaO_3$ 160.1 *54-21-7*

Action and use
Anti-inflammatory; analgesic.

Ph Eur

DEFINITION
Sodium 2-hydroxybenzenecarboxylate.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder or small, colourless crystals or shiny flakes.

Solubility
Freely soluble in water, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION
First identification: A, C.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *sodium salicylate CRS.*

B. Solution S (see Tests) gives the reactions of salicylates (*2.3.1*).

C. It gives reaction (b) of sodium (*2.3.1*).

TESTS
Solution S
Dissolve 5.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Acidity
To 20 mL of solution S add 0.1 mL of *phenol red solution R*. The solution is yellow. Not more than 2.0 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to violet-red.

Chlorides (*2.4.4*)
Maximum 200 ppm.

To 5 mL of solution S add 5 mL of *water R* and 10 mL of *dilute nitric acid R* and filter. Dilute 10 mL of the filtrate to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 600 ppm.

Dilute 2.5 mL of solution S to 15 mL with *distilled water R*.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.00 g by drying in an oven at 105 °C.

ASSAY
Dissolve 0.130 g in 30 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 16.01 mg of $C_7H_5NaO_3$.

STORAGE
In an airtight container, protected from light.

Ph Eur

Sodium Selenite

(Ph. Eur. monograph 2740)

Na_2SeO_3 172.9 *10102-18-8*

Action and use
Used in treatment of selenium deficiency.

Ph Eur

DEFINITION
Content
98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline deliquescent powder.

Solubility
Freely soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION
A. Dissolve 50 mg in 5 mL of a mixture of equal volumes of *dilute hydrochloric acid R* and *water R* and heat to boiling. Add 50 mg of *ascorbic acid R*; a red precipitate is formed which may become black.

B. Dissolve 50 mg in a mixture of 1 mL of *dilute hydrochloric acid R* and 5 mL of *water R*. Add 1 mL of *barium chloride solution R1*; the solution remains clear.

C. It gives reaction (a) of sodium (*2.3.1*).

D. It complies with the limits of the assay.

TESTS
Solution S
Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)
9.8 to 10.8 for solution S.

Chlorides (*2.4.4*)
Maximum 50 ppm.

Dissolve 1.0 g in 5 mL of *water R*, add 9 mL of *nitric acid R* and dilute to 15 mL with *water R*.

Sulfates and selenates (*2.4.13*)
Maximum 300 ppm (determined as sulfates).

Dissolve 0.5 g in 10 mL of *distilled water R*, add 0.5 mL of *hydrochloric acid R1* and dilute to 15 mL with *distilled water R*.

Iron
Maximum 50 ppm.

To 2 mL of solution S add 2 mL of a 200 g/L solution of *sulfosalicylic acid R* and 5 mL of *concentrated ammonia R*, and dilute to 10 mL with *water R*. The solution is not more intensely coloured than a reference solution prepared at the same time and in the same manner using 1 mL of *iron standard solution (10 ppm Fe) R*.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 130 °C for 2 h.

ASSAY
Dissolve 80.0 mg in 50 mL of *water R*. Add 1.0 mL of *anhydrous formic acid R*, 25.0 mL of *0.1 M sodium thiosulfate* and 0.5 g of *potassium iodide R*. Titrate immediately with *0.05 M iodine* using *starch solution R* as indicator. Carry out a blank titration.

1 mL of *0.1 M sodium thiosulfate* is equivalent to 4.323 mg of Na_2SeO_3.

STORAGE
In an airtight container.

Ph Eur

Sodium Selenite Pentahydrate

(Ph. Eur. monograph 1677)

$Na_2SeO_3,5H_2O$ 263.0 *26970-82-1*

Action and use
Used in treatment of selenium deficiency.

Ph Eur

DEFINITION
Content
98.5 per cent to 101.5 per cent.

CHARACTERS
Appearance
White or almost white, crystalline powder, hygroscopic.

Solubility
Freely soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION
A. Dissolve 50 mg in 5 mL of a mixture of equal volumes of *dilute hydrochloric acid R* and *water R* and heat to boiling. Add 50 mg of *ascorbic acid R*; a red precipitate is formed which may become black.

B. Dissolve 50 mg in a mixture of 1 mL of *dilute hydrochloric acid R* and 5 mL of *water R*. Add 1 mL of *barium chloride solution R1*; the solution remains clear.

C. It gives reaction (a) of sodium (*2.3.1*).

D. It complies with the limits of the assay.

TESTS
Solution S
Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)
9.8 to 10.8 for solution S.

Chlorides (*2.4.4*)
Maximum 50 ppm.

Dissolve 1.0 g in 5 mL of *water R*, add 9 mL of *nitric acid R* and dilute to 15 mL with *water R*.

Sulfates and selenates (*2.4.13*)
Maximum 300 ppm (determined as sulfates).

Dissolve 0.5 g in 10 mL of *distilled water R*, add 0.5 mL of *hydrochloric acid R1* and dilute to 15 mL with *distilled water R*.

Iron
Maximum 50 ppm.

To 2 mL of solution S add 2 mL of a 200 g/L solution of *sulfosalicylic acid R*, 5 mL of *concentrated ammonia R* and dilute to 10 mL with *water R*. The solution is not more intensely coloured than a reference solution prepared at the same time and in the same manner using 1 mL of *iron standard solution (10 ppm Fe) R*.

ASSAY
Dissolve 0.120 g in 50 mL of *water R*. Add 1.0 mL of *anhydrous formic acid R*, 25.0 mL of *0.1 M sodium thiosulfate* and 0.5 g of *potassium iodide R*. Titrate immediately with *0.05 M iodine* using *starch solution R* as indicator. Carry out a blank titration.

1 mL of *0.1 M sodium thiosulfate* is equivalent to 6.575 mg of $Na_2SeO_3,5H_2O$.

STORAGE
In an airtight container.

Ph Eur

Sodium Starch Glycolate (Type A)[1]

(Ph. Eur. monograph 0983)

Action and use
Excipient.

Ph Eur

DEFINITION
Sodium salt of a cross-linked partly *O*-carboxymethylated starch.

Content
2.8 per cent to 4.2 per cent of Na (A_r 22.99) (substance washed with ethanol (80 per cent *V/V*) and dried).

♦ CHARACTERS
Appearance
White or almost white, fine, free-flowing powder, very hygroscopic.

Solubility
Practically insoluble in methylene chloride. It gives a translucent suspension in water. ♦

IDENTIFICATION
◊ A. pH (see Tests).

B. Prepare with shaking and without heating a mixture of 4.0 g of the substance to be examined and 20 mL of *carbon dioxide-free water R*. The mixture has the appearance of a gel. Add 100 mL of *carbon dioxide-free water R* and shake. A suspension forms that settles after standing. ◊

C. To an acidified solution, add *iodinated potassium iodide solution R1*. The solution becomes blue or violet.

D. Solution S2 (see Tests) gives reaction (a) of sodium (*2.3.1*).

TESTS
Solution S1
Centrifuge the suspension obtained in identification test B at 2500 *g* for 10 min. Collect carefully the supernatant.

Solution S2
Place 2.5 g in a silica or platinum crucible and add 2 mL of a 500 g/L solution of *sulfuric acid R*. Heat on a water-bath, then cautiously over a naked flame, raising the temperature progressively, then incinerate in a muffle furnace at 600 ± 25 °C. Continue heating until all black particles have disappeared. Allow to cool, add a few drops of *dilute sulfuric acid R*, heat and incinerate as above. Allow to cool, add a few drops of *ammonium carbonate solution R*, evaporate to dryness and incinerate cautiously. Allow to cool and dissolve the residue in 50 mL of *water R*.

◊ Appearance of solution S1
Solution S1 is clear (*2.2.1*) and colourless (*2.2.2, Method II*). ◊

pH (*2.2.3*)
5.5 to 7.5.

Disperse 1.0 g in 30 mL of *carbon dioxide-free water R*.

Sodium glycolate
Maximum 2.0 per cent. *Carry out the test protected from light.*

Test solution Place 0.20 g in a beaker. Add 5 mL of *acetic acid R* and 5 mL of *water R*. Stir until dissolution is complete (about 10 min). Add 50 mL of *acetone R* and 1 g of *sodium chloride R*. Filter through a fast filter paper impregnated with *acetone R*, rinse the beaker and filter with *acetone R*. Combine the filtrate and washings and dilute to 100.0 mL with *acetone R*. Allow to stand for 24 h without shaking. Use the clear supernatant.

Reference solution Dissolve 0.310 g of *glycolic acid R*, previously dried in a desiccator (*2.2.32*) at room temperature overnight, in *water R* and dilute to 500.0 mL with the same solvent. To 5.0 mL of this solution add 5 mL of *acetic acid R* and allow to stand for about 30 min. Add 50 mL of *acetone R* and 1 g of *sodium chloride R*. Filter through a fast filter paper impregnated with *acetone R*, rinse the beaker and filter with *acetone R*. Combine the filtrate and washings and dilute to 100.0 mL with *acetone R*. Allow to stand for 24 h without shaking. Use the clear supernatant.

Heat 2.0 mL of the test solution on a water-bath for 20 min. Cool to room temperature and add 20.0 mL of *2,7-dihydroxynaphthalene solution R*. Shake and heat in a water-bath for 20 min. Cool under running water, transfer to a volumetric flask and dilute to 25.0 mL with *sulfuric acid R*, maintaining the flask under running water. Within 10 min, measure the absorbance at 540 nm (*2.2.25*) using *water R* as the compensation liquid. The absorbance of the solution prepared with the test solution is not greater than that of a solution prepared at the same time and in the same manner with 2.0 mL of the reference solution.

Sodium chloride
Maximum 7.0 per cent.

Place 0.500 g in a beaker and suspend in 100 mL of *water R*. Add 1 mL of *nitric acid R*. Titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (*2.2.20*), using a silver-based indicator electrode and a double-junction reference electrode containing a 100 g/L solution of *potassium nitrate R* in the outer jacket and a standard filling solution in the inner jacket.

1 mL of *0.1 M silver nitrate* is equivalent to 5.844 mg of NaCl.

Iron (*2.4.9*)
Maximum 20 ppm, determined on 10 mL of solution S2.

[1] *This monograph has undergone pharmacopoeial harmonisation. See chapter 5.8 Pharmacopoeial harmonisation.*

Loss on drying (*2.2.32*)
Maximum 10.0 per cent, determined on 1.000 g by drying in an oven at 130 °C for 1.5 h.

Microbial contamination
It complies with the test for *Escherichia coli* and *Salmonella* (*2.6.13*).

ASSAY
Shake about 1 g with 20 mL of *ethanol (80 per cent V/V) R*, stir for 10 min and filter. Repeat the operation until chloride has been completely extracted and verify the absence of chloride using *silver nitrate solution R2*. Dry the residue at 105 °C to constant mass. To 0.700 g of the dried residue, add 80 mL of *glacial acetic acid R* and heat under a reflux condenser for 2 h. Cool the solution to room temperature. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 mL of *0.1 M perchloric acid* is equivalent to 2.299 mg of Na.

♦ STORAGE
In an airtight container, protected from light. ♦

FUNCTIONALITY-RELATED CHARACTERISTICS
This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for sodium starch glycolate (type A) used as disintegrant in tablets.

Settling volume
Introduce 70 mL of *water R* in a flask, add 1.0 g of the substance to be examined (dried substance) and stir with a glass rod so that no lumps remain. Transfer the suspension to a 100 mL graduated cylinder, rinse the walls of the flask with *water R* and dilute to 100 mL with the same solvent. Stir again until the substance is evenly distributed. Allow to stand for 2 h. The volume of the sediment is typically less than 45 mL.

Degree of substitution
Place 1.000 g (dried substance) in a 500 mL conical flask, add 300 mL of a 100 g/L solution of *sodium chloride R* and 25.0 mL of *0.1 M sodium hydroxide*, stopper the flask and allow to stand for 5 min, shaking occasionally. Add 0.05 mL of *m-cresol purple solution R* and about 15 mL of *0.1 M hydrochloric acid* from a burette. Insert the stopper and shake. If the solution is violet, add *0.1 M hydrochloric acid* in 1 mL portions until the solution becomes yellow, shaking after each addition. Titrate with *0.1 M sodium hydroxide* until the colour turns to violet. Calculate the number of milliequivalents (*M*) of base required to neutralise the equivalent of 1 g of dried substance.

Calculate the degree of acid carboxymethyl substitution (*A*) using the following expression:

$$A = \frac{1150M}{(7102 - 412M - 80C)}$$

C = sulfated ash as a percentage, determined as follows.

Introduce 1.3 g of the substance to be examined in a flask and add 20 mL of *ethanol (80 per cent V/V) R*. Shake for 10 min and filter on a sintered-glass filter (*2.1.2*). Repeat this operation 4 times. Add to the residue 10 mL of *ethanol (96 per cent) R* and filter. Collect the residue and place it in an oven at 105 °C for 4 h. Place the dried residue in a mortar and crush. Determine the sulfated ash content (*C*) (*2.4.14*) on 1.000 g of the powder obtained.

Calculate the degree of sodium carboxymethyl substitution (*S*) using the following expression:

$$S = \frac{(162 + 58A)C}{(7102 - 80C)}$$

The degree of substitution is the sum of *A* and *S*.

Particle-size distribution (*2.9.31* or *2.9.38*)

Ph Eur

Sodium Starch Glycolate (Type B)[1]

(Ph. Eur. monograph 0984)

Action and use
Excipient.

Ph Eur

DEFINITION
Sodium salt of a cross-linked partly *O*-carboxymethylated starch.

Content
2.0 per cent to 3.4 per cent of Na (A_r 22.99) (substance washed with ethanol (80 per cent *V/V*) and dried).

♦ CHARACTERS
Appearance
White or almost white, fine, free-flowing powder, very hygroscopic.

Solubility
Practically insoluble in methylene chloride. It gives a translucent suspension in water. ♦

IDENTIFICATION
◊ A. pH (see Tests).

B. Prepare with shaking and without heating a mixture of 4.0 g of the substance to be examined and 20 mL of *carbon dioxide-free water R*. The mixture has the appearance of a gel. Add 100 mL of *carbon dioxide-free water R* and shake. A suspension forms that settles after standing. ◊

C. To an acidified solution, add *iodinated potassium iodide solution R1*. The solution becomes blue or violet.

D. Solution S2 (see Tests) gives reaction (a) of sodium (*2.3.1*).

TESTS
Solution S1
Centrifuge the suspension obtained in identification test B at 2500 *g* for 10 min. Collect carefully the supernatant.

[1] *This monograph has undergone pharmacopoeial harmonisation. See chapter 5.8 Pharmacopoeial harmonisation.*

Solution S2
Place 2.5 g in a silica or platinum crucible and add 2 mL of a 500 g/L solution of *sulfuric acid R*. Heat on a water-bath, then cautiously over a naked flame, raising the temperature progressively, and then incinerate in a muffle furnace at 600 ± 25 °C. Continue heating until all black particles have disappeared. Allow to cool, add a few drops of *dilute sulfuric acid R* and heat and incinerate as above. Allow to cool, add a few drops of *ammonium carbonate solution R*, evaporate to dryness and incinerate cautiously. Allow to cool and dissolve the residue in 50 mL of *water R*.

◊ **Appearance of solution S1**
Solution S1 is clear (*2.2.1*) and colourless (*2.2.2, Method II*). ◊

pH (*2.2.3*)
3.0 to 5.0.

Disperse 1.0 g in 30 mL of *carbon dioxide-free water R*.

Sodium glycolate
Maximum 2.0 per cent. *Carry out the test protected from light.*

Test solution Place 0.20 g in a beaker. Add 5 mL of *acetic acid R* and 5 mL of *water R*. Stir until dissolution is complete (about 10 min). Add 50 mL of *acetone R* and 1 g of *sodium chloride R*. Filter through a fast filter paper impregnated with *acetone R*, rinse the beaker and filter with *acetone R*. Combine the filtrate and washings and dilute to 100.0 mL with *acetone R*. Allow to stand for 24 h without shaking. Use the clear supernatant.

Reference solution Dissolve 0.310 g of *glycolic acid R*, previously dried in a desiccator (*2.2.32*) at room temperature overnight, in *water R* and dilute to 500.0 mL with the same solvent. To 5.0 mL of this solution add 5 mL of *acetic acid R* and allow to stand for about 30 min. Add 50 mL of *acetone R* and 1 g of *sodium chloride R*. Filter through a fast filter paper impregnated with *acetone R*, rinse the beaker and filter with *acetone R*. Combine the filtrate and washings and dilute to 100.0 mL with *acetone R*. Allow to stand for 24 h without shaking. Use the clear supernatant.

Heat 2.0 mL of the test solution on a water-bath for 20 min. Cool to room temperature and add 20.0 mL of *2,7-dihydroxynaphthalene solution R*. Shake and heat in a water-bath for 20 min. Cool under running water, transfer quantitatively to a volumetric flask and dilute to 25.0 mL with *sulfuric acid R*, maintaining the flasks under running water. Within 10 min, measure the absorbance at 540 nm (*2.2.25*) using *water R* as the compensation liquid.
The absorbance of the solution prepared with the test solution is not greater than that of a solution prepared at the same time and in the same manner with 2.0 mL of the reference solution.

Sodium chloride
Maximum 7.0 per cent.

Place 0.500 g in a beaker and suspend in 100 mL of *water R*. Add 1 mL of *nitric acid R*. Titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (*2.2.20*), using a silver-based indicator electrode and a double-junction reference electrode containing a 100 g/L solution of *potassium nitrate R* in the outerjacket and a standard filling solution in the inner jacket.

1 mL of *0.1 M silver nitrate* is equivalent to 5.844 mg of NaCl.

Iron (*2.4.9*)
Maximum 20 ppm, determined on 10 mL of solution S2.

Loss on drying (*2.2.32*)
Maximum 10.0 per cent, determined on 1.000 g by drying in an oven at 130 °C for 1.5 h.

Microbial contamination
It complies with the test for *Escherichia coli* and *Salmonella* (*2.6.13*).

ASSAY
Shake about 1 g with 20 mL of *ethanol (80 per cent V/V) R*, stir for 10 min and filter. Repeat the operation until chloride has been completely extracted and verify the absence of chloride using *silver nitrate solution R2*. Dry the residue at 105 °C to constant mass. To 0.700 g of the dried residue, add 80 mL of *glacial acetic acid R* and heat under a reflux condenser for 2 h. Cool the solution to room temperature. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 mL of *0.1 M perchloric acid* is equivalent to 2.299 mg of Na.

♦ **STORAGE**
In an airtight container, protected from light. ♦

FUNCTIONALITY-RELATED CHARACTERISTICS
This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for sodium starch glycolate (type B) used as disintegrant in tablets.

Settling volume
Introduce 70 mL of *water R* in a flask, add 1.0 g of the substance to be examined (dried substance) and stir with a glass rod so that no lumps remain. Transfer the suspension to a 100 mL graduated cylinder, rinse the walls of the flask with *water R* and dilute to 100 mL with the same solvent. Stir again until the substance is evenly distributed. Allow to stand for 2 h. The volume of the sediment is typically less than 45 mL.

Degree of substitution
Place 1.000 g (dried substance) in a 500 mL conical flask, add 300 mL of a 100 g/L solution of *sodium chloride R* and 25.0 mL of *0.1 M sodium hydroxide*, stopper the flask and allow to stand for 5 min, shaking occasionally. Add 0.05 mL of *m-cresol purple solution R* and about 15 mL of *0.1 M hydrochloric acid* from a burette. Insert the stopper and shake. If the solution is violet, add *0.1 M hydrochloric acid* in 1 mL portions until the solution becomes yellow, shaking after each addition. Titrate with *0.1 M sodium hydroxide* until the colour turns to violet. Calculate the number of milliequivalents (*M*) of base required to neutralise the equivalent of 1 g of dried substance.

Calculate the degree of acid carboxymethyl substitution (*A*) using the following expression:

$$A = \frac{1150M}{(7102 - 412M - 80C)}$$

C = sulfated ash as a percentage, determined as follows.

Introduce 1.3 g of the substance to be examined in a flask and add 20 mL of *ethanol (80 per cent V/V) R*. Shake for 10 min and filter on a sintered-glass filter (*2.1.2*). Repeat this operation 4 times. Add to the residue 10 mL of *ethanol (96 per cent) R* and filter. Collect the residue and place it in an oven at 105 °C for 4 h. Place the dried residue in a mortar and crush. Determine the sulfated ash content (*C*) (*2.4.14*) on 1.000 g of the powder obtained.

Calculate the degree of sodium carboxymethyl substitution (*S*) using the following expression:

$$S = \frac{(162 + 58A)C}{(7102 - 80C)}$$

The degree of substitution is the sum of *A* and *S*.

Particle-size distribution (*2.9.31* or *2.9.38*)

Ph Eur

Sodium Starch Glycolate (Type C)[1]

(Ph. Eur. monograph 1566)

Action and use
Excipient.

Ph Eur

DEFINITION

Sodium salt of a partly *O*-carboxymethylated starch, cross-linked by physical dehydration.

Content
2.8 per cent to 5.0 per cent of Na (A_r 22.99) (substance washed with ethanol (80 per cent *V/V*) and dried).

CHARACTERS

Appearance
White or almost white, fine, free-flowing powder, very hygroscopic.

Microscopic examination: it is seen to consist of granules, irregularly shaped, ovoid or pear-shaped, 30-100 µm in size, or rounded, 10-35 µm in size; compound granules consisting of 2-4 components occur occasionally; the granules have an eccentric hilum and clearly visible concentric striations; between crossed nicol prisms, the granules show a distinct black cross intersecting at the hilum; small crystals are visible at the surface of the granules. The granules show considerable swelling in contact with water.

Solubility
Soluble in water, practically insoluble in methylene chloride. It gives a translucent gel-like product in water.

IDENTIFICATION

A. pH (see Tests).

B. Prepare with shaking and without heating a mixture of 4.0 g of the substance to be examined and 20 mL of *carbon dioxide-free water R*. The mixture has the appearance of a gel. Add 100 mL of *carbon dioxide-free water R* and shake: the gel remains stable (difference from types A and B). Keep the gel for the tests for appearance of gel and pH.

C. To 5 mL of the gel obtained in identification test B add 0.05 mL of *iodine solution R1*. A dark blue colour is produced.

D. Solution S (see Tests) gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S
Place 2.5 g in a silica or platinum crucible and add 2 mL of a 500 g/L solution of *sulfuric acid R*. Heat on a water-bath, then cautiously over a naked flame, raising the temperature progressively, and then incinerate in a muffle furnace at 600 ± 25 °C. Continue heating until all black particles have disappeared. Allow to cool, add a few drops of *sulfuric acid R*, heat and incinerate as above. Allow to cool, add a few drops of *ammonium carbonate solution R*, evaporate to dryness and incinerate cautiously. Allow to cool and dissolve the residue in 50 mL of *water R*.

Appearance of gel
The gel obtained in identification test B is colourless (*2.2.2, Method II*).

pH (*2.2.3*)
5.5 to 7.5 for the gel obtained in identification test B.

Sodium glycolate
Maximum 2.0 per cent. *Carry out the test protected from light.*

Test solution Place 0.20 g in a beaker. Add 5 mL of *acetic acid R* and 5 mL of *water R*. Stir until dissolution is complete (about 10 min). Add 50 mL of *acetone R* and 1 g of *sodium chloride R*. Filter through a fast filter paper impregnated with *acetone R*, rinse the beaker and filter with *acetone R*. Combine the filtrate and washings and dilute to 100.0 mL with *acetone R*. Allow to stand for 24 h without shaking. Use the clear supernatant.

Reference solution Dissolve 0.310 g of *glycolic acid R*, previously dried in a desiccator (*2.2.32*), in *water R* and dilute to 500.0 mL with the same solvent. To 5.0 mL of this solution, add 5 mL of *acetic acid R* and allow to stand for about 30 min. Add 50 mL of *acetone R* and 1 g of *sodium chloride R* and dilute to 100.0 mL with *acetone R*.

Heat 2.0 mL of the test solution on a water-bath for 20 min. Cool to room temperature and add 20.0 mL of *2,7-dihydroxynaphthalene solution R*. Shake and heat on a water-bath for 20 min. Cool under running water, transfer to a volumetric flask and dilute to 25.0 mL with *sulfuric acid R*, maintaining the flask under running water. Within 10 min, measure the absorbance (*2.2.25*) at 540 nm using *water R* as the compensation liquid. The absorbance of the solution prepared with the test solution is not greater than that of a solution prepared at the same time and in the same manner with 2.0 mL of the reference solution.

Sodium chloride
Maximum 1 per cent.

Shake 1.00 g with 20 mL of *ethanol (80 per cent V/V) R* for 10 min and filter. Repeat the operation 4 times. Dry the residue to constant mass at 100 °C and set aside for the assay. Combine the filtrates. Evaporate to dryness, take up the residue with *water R* and dilute to 25.0 mL with the same solvent. To 10.0 mL of the solution add 30 mL of *water R* and 5 mL of *dilute nitric acid R*. Titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (*2.2.20*), using a silver-based indicator electrode and a double-junction reference electrode containing a 100 g/L

[1] *In contrast to sodium starch glycolate type A and type B, this monograph has NOT undergone pharmacopoeial harmonisation.*

solution of *potassium nitrate R* in the outer jacket and a standard filling solution in the inner jacket.

1 mL of *0.1 M silver nitrate* is equivalent to 5.844 mg of NaCl.

Iron (*2.4.9*)
Maximum 20 ppm, determined on solution S.

Loss on drying (*2.2.32*)
Maximum 7.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Microbial contamination
It complies with the test for *Escherichia coli* and *Salmonella* (*2.6.13*).

ASSAY
To 0.500 g of the dried and crushed residue obtained in the test for sodium chloride add 80 mL of *anhydrous acetic acid R* and heat under a reflux condenser for 2 h. Cool the solution to room temperature. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank test.

1 mL of *0.1 M perchloric acid* is equivalent to 2.299 mg of Na.

STORAGE
In an airtight container, protected from light.

Ph Eur

Sodium Stearate

(Ph. Eur. monograph 2058)

Action and use
Excipient.

Ph Eur

DEFINITION
Mixture of sodium salts of different fatty acids consisting mainly of stearic (octadecanoic) acid [$C_{17}H_{35}COONa$; M_r 306.5] and palmitic (hexadecanoic) acid [$C_{15}H_{31}COONa$; M_r 278.4].

Content
— *sodium*: 7.4 per cent to 8.5 per cent (A_r 22.99) (dried substance);
— *stearic acid in the fatty acid fraction*: minimum 40 per cent;
— *sum of stearic acid and palmitic acid in the fatty acid fraction*: minimum 90 per cent.

CHARACTERS
Appearance
White or yellowish, fine powder, greasy to the touch.

Solubility
Slightly soluble in water and in ethanol (96 per cent).

IDENTIFICATION
First identification: C, D.

Second identification: A, B, D.

A. Freezing point (*2.2.18*): minimum 53 °C for the residue obtained in the preparation of solution S (see Tests).

B. Acid value (*2.5.1*): 195 to 210, determined on 0.200 g of the residue obtained in the preparation of solution S dissolved in 25 mL of the prescribed mixture of solvents.

C. Examine the chromatograms obtained in the assay of stearic acid and palmitic acid.

Results The 2 principal peaks in the chromatogram obtained with the test solution are similar in retention time and size to the 2 principal peaks in the chromatogram obtained with the reference solution.

D. Solution S gives reaction (b) of sodium (*2.3.1*).

TESTS
Solution S
To 10.0 g add 100 mL of *peroxide-free ether R* and 80 mL of *acetic acid R*. Boil under a reflux condenser until dissolution is complete. Allow to cool. In a separating funnel, separate the aqueous layer and shake the ether layer with 2 quantities, each of 8 mL, of *acetic acid R*. Combine the aqueous layers, wash with 30 mL of *peroxide-free ether R* and dilute to 100 mL with *distilled water R* (solution S). Evaporate the ether layers to dryness on a water-bath and dry the residue at 100-105 °C.

Acidity or alkalinity
Suspend 2.0 g in 50 mL of previously neutralised *ethanol (96 per cent) R*. Heat under reflux to dissolve and add 3 drops of *phenolphthalein solution R*; the solution is colourless. Not less than 0.60 mL and not more than 0.85 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Chlorides (*2.4.4*)
Maximum 0.2 per cent.

Dilute 0.25 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 0.3 per cent.

Dilute 0.5 mL of solution S to 15 mL with *distilled water R*.

Nickel
Maximum 5 ppm.

Atomic absorption spectrometry (*2.2.23, Method II*).

Test solution Place 50.0 mg of the substance to be examined in a polytetrafluoroethylene digestion flask and add 0.5 mL of a mixture of 1 volume of *heavy metal-free hydrochloric acid R* and 5 volumes of *heavy metal-free nitric acid R*. Allow to digest at 170 °C for 5 h. Allow to cool. Dissolve the residue in *water R* and dilute to 5.0 mL with the same solvent.

Reference solutions Prepare the reference solutions using *nickel standard solution (10 ppm Ni) R*, diluting as necessary with *water R*.

Source Nickel hollow-cathode lamp.

Wavelength 232.0 nm.

Atomisation device Furnace.

Loss on drying (*2.2.32*)
Maximum 5.0 per cent.

In a weighing glass introduce 1.0 g of previously washed *sand R*, dry at 105 °C and weigh. Add 0.500 g of the substance to be examined and 10 mL of *ethanol (96 per cent) R*. Evaporate at 80 °C and dry the residue at 105 °C for 4 h.

Microbial contamination
TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*).

TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

Absence of *Escherichia coli* (*2.6.13*).

Absence of *Salmonella* (*2.6.13*).

ASSAY
Sodium
Dissolve 0.250 g with gentle heating in a mixture of 5 mL of *acetic anhydride R* and 20 mL of *anhydrous acetic acid R*. Cool

and add 20 mL of *dioxan R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 2.299 mg of Na.

Stearic acid and palmitic acid

Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution In a conical flask fitted with a reflux condenser, dissolve 0.10 g of the substance to be examined in 5 mL of *boron trifluoride-methanol solution R*. Boil under a reflux condenser for 10 min. Add 4 mL of *heptane R* through the condenser and boil again under a reflux condenser for 10 min. Allow to cool. Add 20 mL of *saturated sodium chloride solution R*. Shake and allow the layers to separate. Remove about 2 mL of the organic layer and dry over 0.2 g of *anhydrous sodium sulfate R*. Dilute 1.0 mL of the solution to 100.0 mL with *heptane R*.

Reference solution Prepare the reference solution in the same manner as the test solution using 50.0 mg of *palmitic acid CRS* and 50.0 mg of *stearic acid CRS* instead of the substance to be examined.

Column:
- *material*: fused silica;
- *size*: *l* = 30 m, Ø = 0.32 mm;
- *stationary phase*: *macrogol 20 000 R* (film thickness 0.5 µm).

Carrier gas *helium for chromatography R*.

Flow rate 2.4 mL/min.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 2	70
	2 - 36	70 → 240
	36 - 41	240
Injection port		220
Detector		260

Detection Flame ionisation.

Injection 1 µL.

Relative retention With reference to methyl stearate (retention time = about 40 min): methyl palmitate = about 0.88.

System suitability Reference solution:
- *resolution*: minimum 5.0 between the peaks due to methyl palmitate and methyl stearate.

Calculate the content of stearic acid and palmitic acid.

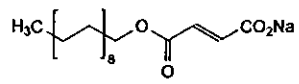
STORAGE

In an airtight container, protected from light.

Ph Eur

Sodium Stearyl Fumarate

(Ph. Eur. monograph 1567)



$C_{22}H_{39}NaO_4$ 390.5 *4070-80-8*

Ph Eur

DEFINITION

Sodium octadecyl (*E*)-butenedioate.

Content

99.0 per cent to 101.5 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, fine powder with agglomerates of flat, circular particles.

Solubility

Practically insoluble in water, slightly soluble in methanol, practically insoluble in acetone and in anhydrous ethanol.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *sodium stearyl fumarate CRS*.

TESTS

Related substances

Gas chromatography (*2.2.28*): use the normalisation procedure.

Silylation solutio n. To 2 mL of *N,O-bis(trimethylsilyl) trifluoroacetamide R* add 0.02 mL of *chlorotrimethylsilane R* and mix.

Test solution Introduce 15.0 mg of the substance to be examined in a vial with a screw cap and add 1 mL of the silylation solution. Seal the vial and heat at about 70 °C for 1 h. After the reaction a precipitate remains in the vial; filter the solution through a nylon filter (pore size 0.45 µm).

Reference solution Introduce 1.0 mg of *sodium stearyl maleate CRS* and 1.0 mg of *sodium stearyl fumarate CRS* into a vial with a screw cap and add 1 mL of the silylation solution. Seal the vial and heat at about 70 °C for 1 h.

Column:
- *material*: fused silica;
- *size*: *l* = 15 m, Ø = 0.53 mm;
- *stationary phase*: *methylpolysiloxane R* (film thickness 0.15 µm).

Carrier gas *helium for chromatography R*.

Flow rate 2 mL/min.

Split ratio 1:25.

Temperature:

	Time (min)	Temperature (°C)
	0 - 1	180
Column	1 - 21	180 → 320
	21 - 26	320
Injection port		250
Detector		320

Detection Flame ionisation.

Injection 2 µL.

Relative retention With reference to stearyl trimethylsilyl fumarate (retention time = about 9 min): stearyl

alcohol = 0.30; stearyl trimethylsilyl ether = 0.35; palmityl trimethylsilyl fumarate = 0.80; heptadecyl trimethylsilyl fumarate = 0.85; stearyl trimethylsilyl maleate = 0.90; nonadecyl trimethylsilyl fumarate = 1.05; eicos-11-enyl trimethylsilyl fumarate = 1.15; distearyl fumarate = 2.25.

System suitability:

— *resolution:* minimum 1.5 between the peaks in the chromatogram obtained with the reference solution.

Limits:

— *any impurity:* maximum 0.5 per cent;

— *total:* maximum 5.0 per cent.

Water (*2.5.12*)

Maximum 5.0 per cent, determined on 0.250 g.

ASSAY

Dissolve 0.250 g, accurately weighed, in 10 mL of *methylene chloride R* and add 30 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 39.05 mg of $C_{22}H_{39}NaO_4$.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

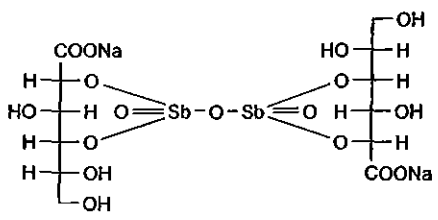
The following characteristics may be relevant for sodium stearyl fumarate used as a lubricant in tablets and capsules.

Particle-size distribution (*2.9.31*)

Specific surface area (*2.9.26, Method I*)

Ph Eur

Sodium Stibogluconate



16037-91-5

Action and use

Pentavalent antimony compound; antiprotozoal.

Preparation

Sodium Stibogluconate Injection

DEFINITION

Sodium Stibogluconate is mainly the disodium salt of μ-oxy-bis[gluconato(3-)-O^2, O^3,O^4-hydroxoantimony]. It contains not less than 30.0% and not more than 34.0% of antimony (V), calculated with reference to the dried and methanol-free substance.

PRODUCTION

The method of manufacture is such as to ensure consistently controlled reaction stoichiometry in order to yield sodium stibogluconate that is satisfactory with regard to intrinsic toxicity.

CHARACTERISTICS

A colourless, mostly amorphous powder.

Very soluble in *water*; practically insoluble in *ethanol (96%)* and in *ether*.

IDENTIFICATION

A. A solution is dextrorotatory.

B. Pass *hydrogen sulfide* into a 5% w/v solution for several minutes. An orange precipitate is produced.

C. When heated it chars, without melting, leaving a residue which yields the reactions characteristic of *antimony compounds* and the reactions characteristic of *sodium salts*, Appendix VI.

TESTS

Stability and acidity of solution

Heat a solution containing the equivalent of 10% w/v of antimony(V) in an autoclave at 115.5° and at a pressure of 70 kPa for 30 minutes. The resulting solution is colourless or almost colourless and has a pH of 5.0 to 5.6, Appendix V L.

Antimony(III)

Dissolve 2 g in 30 mL of *water*, add 15 mL of *hydrochloric acid* and titrate with *0.00833M potassium bromate VS* using *methyl orange solution* as indicator. Not more than 1.3 mL of *0.00833M potassium bromate VS* is required.

Chloride

Dissolve 2.5 g in 50 mL of *water* and add 2 mL of 2M *nitric acid* and 75 mL of *acetate buffer pH 5.0*. Titrate with *0.1M silver nitrate VS* determining the end point potentiometrically. Not more than 3.0 mL of *0.1M silver nitrate VS* is required.

Methanol

Not more than 2.0% w/w when determined by the following method. Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. For solution (1) add 1 mL of a 1.0% v/v solution of *methanol* to 5 mL of a 0.2% v/v solution of *absolute ethanol* (internal standard). For solution (2) add 5 mL of *water* to 0.5 g of the substance being examined and mix with the aid of ultrasound until solution is complete. For solution (3) add 5 mL of a 0.2% v/v solution of the internal standard to 0.5 g of the substance being examined and mix with the aid of ultrasound until solution is complete.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with porous polymer beads (80 to 100 mesh) (Porapak Q and Chromosorb 101 are suitable) and maintained at 130°.

Calculate the percentage w/w of methanol taking 0.792 g as its weight per mL at 20°.

Loss on drying

When dried to constant weight at 130° at a pressure not exceeding 0.7 kPa, loses not more than 15.0% of its weight. Use 1 g.

ASSAY

Dissolve 0.16 g in 30 mL of *hydrochloric acid*, add 70 mL of *orthophosphoric acid* and stir carefully until completely mixed. Titrate with 0.05M *ammonium iron(II) sulfate VS* prepared using *sulfuric acid (1%)* and determining the end point

potentiometrically using a platinum electrode and a silver-silver chloride reference electrode. Each mL of 0.05M *ammonium iron(II) sulfate VS* is equivalent to 3.044 mg of antimony(V).

Anhydrous Sodium Sulfate

Anhydrous Sodium Sulphate

(Ph. Eur. monograph 0099)

Na_2SO_4 142.0 *7757-82-6*

Action and use
Laxative.

Ph Eur

DEFINITION
Anhydrous disodium sulfate.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white powder, hygroscopic.

Solubility
Freely soluble in water.

IDENTIFICATION
A. It gives reaction (a) of sulfates (*2.3.1*).

B. It gives reaction (a) of sodium (*2.3.1*).

C. Loss on drying (see Tests).

TESTS

Solution S
Dissolve 2.2 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity
To 10 mL of solution S add 0.1 mL of *bromothymol blue solution R1*. Not more than 0.5 mL of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Chlorides (*2.4.4*)
Maximum 450 ppm.

Dilute 5 mL of solution S to 15 mL with *water R*.

Calcium (*2.4.3*)
Maximum 450 ppm, if intended for use in the manufacture of parenteral preparations.

Dilute 10 mL of solution S to 15 mL with *distilled water R*.

Iron (*2.4.9*)
Maximum 90 ppm, if intended for use in the manufacture of parenteral preparations.

Dilute 5 mL of solution S to 10 mL with *water R*.

Magnesium
Maximum 200 ppm, if intended for use in the manufacture of parenteral preparations.

To 10 mL of solution S add 1 mL of *glycerol (85 per cent) R*, 0.15 mL of *titan yellow solution R*, 0.25 mL of *ammonium oxalate solution R* and 5 mL of *dilute sodium hydroxide solution R* and shake. Any pink colour in the test solution is not more intense than that in a standard prepared at the same time in the same manner using a mixture of 5 mL of *magnesium standard solution (10 ppm Mg) R* and 5 mL of *water R*.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 130 °C.

ASSAY
Pack 20 g of *strongly acidic ion-exchange resin R* into a glass column, at least 20 cm long and 20 mm in internal diameter, and cover it with *water R*. After 5 min, wash the resin with *water R* until the pH of the eluate is about 6 to 7 using a *pH indicator strip R*. Keep the resin covered with *water R*. Dissolve 0.500 g of the substance to be examined in 10 mL of *water R* in a beaker. Load the solution onto the column and pass through the resin at a flow rate of about 4 mL/min until the resin is just covered with the solution. Using about 200 mL of *water R*, rinse the beaker and pass the rinsings through the column at the same flow rate. Check that the pH of the last eluate is about 6 to 7 using a *pH indicator strip R*.

Titrate the eluate with *1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *1 M sodium hydroxide* is equivalent to 71.00 mg of Na_2SO_4.

STORAGE
Store in an airtight container.

LABELLING
The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

Ph Eur

Sodium Sulfate

Glauber's Salt

(Sodium Sulfate Decahydrate, Ph. Eur. monograph 0100)

$Na_2SO_4,10H_2O$ 322.2 *7727-73-3*

Action and use
Laxative.

Ph Eur

DEFINITION
Disodium sulfate decahydrate.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder or colourless, transparent crystals.

Solubility
Freely soluble in water, practically insoluble in ethanol (96 per cent). It partly dissolves in its own water of crystallisation at about 33 °C.

IDENTIFICATION
A. It gives reaction (a) of sulfates (*2.3.1*).

B. It gives reaction (a) of sodium (*2.3.1*).

C. Loss on drying (see Tests).

TESTS

Solution S
Dissolve 5.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity
To 10 mL of solution S add 0.1 mL of *bromothymol blue solution R1*. Not more than 0.5 mL of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Chlorides (*2.4.4*)
Maximum 200 ppm.

Dilute 5 mL of solution S to 15 mL with *water R*.

Calcium (*2.4.3*)
Maximum 200 ppm, if intended for use in the manufacture of parenteral preparations.

Dilute 10 mL of solution S to 15 mL with *distilled water R*.

Iron (*2.4.9*)
Maximum 40 ppm, if intended for use in the manufacture of parenteral preparations.

Dilute 5 mL of solution S to 10 mL with *water R*.

Magnesium
Maximum 100 ppm, if intended for use in the manufacture of parenteral preparations.

To 10 mL of solution S add 1 mL of *glycerol (85 per cent) R*, 0.15 mL of *titan yellow solution R*, 0.25 mL of *ammonium oxalate solution R* and 5 mL of *dilute sodium hydroxide solution R* and shake. Any pink colour in the test solution is not more intense than that in a standard prepared at the same time in the same manner using a mixture of 5 mL of *magnesium standard solution (10 ppm Mg) R* and 5 mL of *water R*.

Loss on drying (*2.2.32*)
52.0 per cent to 57.0 per cent, determined on 1.000 g by drying at 30 °C for 1 h, then at 130 °C.

ASSAY

Pack 20 g of *strongly acidic ion-exchange resin R* into a glass column, at least 20 cm long and 20 mm in internal diameter, and cover it with *water R*. After 5 min, wash the resin with *water R* until the pH of the eluate is about 6 to 7 using a *pH indicator strip R*. Keep the resin covered with *water R*. Dissolve 1.100 g of the substance to be examined in 10 mL of *water R* in a beaker. Load the solution onto the column and pass through the resin at a flow rate of about 4 mL/min until the resin is just covered with the solution. Using about 200 mL of *water R*, rinse the beaker and pass the rinsings through the column at the same flow rate. Check that the pH of the last eluate is about 6 to 7 using a *pH indicator strip R*.

Titrate the eluate with *1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *1 M sodium hydroxide* is equivalent to 71.00 mg of Na_2SO_4.

LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

Ph Eur

Sodium Sulfite

Anhydrous Sodium Sulfite
Annydrous Sodium Sulphite
(Ph. Eur. monograph 0775)

Na_2SO_3 126.0 *7757-83-7*

Action and use
Antioxidant.

Ph Eur

DEFINITION

Content
95.0 per cent to 100.5 per cent of Na_2SO_3.

CHARACTERS

Appearance
White or almost white powder.

Solubility
Freely soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Solution S (see Tests) is slightly alkaline (*2.2.4*).

B. To 5 mL of solution S add 0.5 mL of *0.05 M iodine*. The solution is colourless and gives reaction (a) of sulfates (*2.3.1*).

C. Solution S gives reaction (a) of sodium (*2.3.1*).

D. It complies with the limits of the assay.

TESTS

Solution S
Dissolve 5 g in *water R* and dilute to 100 mL with the same solvent.

Solution S1
To 10.0 g add 25 mL of *water R*. Shake until mostly dissolved, carefully and progressively add 15 mL of *hydrochloric acid R*. Heat to boiling. Cool and dilute to 100.0 mL with *water R*.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method I*).

Thiosulfates
Maximum 0.1 per cent.

To 2.00 g add 100 mL of *water R*. Shake, add 10 mL of *formaldehyde solution R* and 10 mL of *acetic acid R*. Allow to stand for 5 min. Add 0.5 mL of *starch solution R* and titrate with *0.05 M iodine*. Carry out a blank titration.
The difference between the volumes used in the titrations is not more than 0.15 mL.

Iron (*2.4.9*)
Maximum 10 ppm, determined on solution S1.

Selenium
Maximum 10 ppm.

To 3.0 g add 10 mL of *formaldehyde solution R*, carefully and progressively add 2 mL of *hydrochloric acid R*. Heat on a water-bath for 20 min. Any pink colour in the solution is not more intense than that of a standard prepared at the same time and in the same manner using 1.0 g of the substance to be examined to which 0.2 mL of *selenium standard solution (100 ppm Se) R* has been added.

Zinc
Maximum 25 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Dilute 2.0 mL of solution S1 to 10.0 mL with *water R*.

Reference solutions Prepare the reference solutions using *zinc standard solution (100 ppm Zn) R*, diluting with *water R*.

Source Zinc hollow-cathode lamp.

Wavelength 213.9 nm.

Atomisation device Air-acetylene flame.

ASSAY

Introduce 0.250 g into a 500 mL conical flask containing 50.0 mL of *0.05 M iodine*. Shake until completely dissolved. Add 1 mL of *starch solution R* and titrate the excess of iodine with *0.1 M sodium thiosulfate*. Carry out a blank titration.

1 mL of *0.05 M iodine* is equivalent to 6.30 mg of Na_2SO_3.

Ph Eur

Sodium Sulfite Heptahydrate

Sodium Sulphite Heptahydrate

(Ph. Eur. monograph 0776)

$Na_2SO_3,7H_2O$ 252.2 *10102-15-5*

Action and use

Antioxidant.

Ph Eur

DEFINITION

Content

48.0 per cent to 52.5 per cent of Na_2SO_3.

CHARACTERS

Appearance

Colourless crystals.

Solubility

Freely soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Solution S (see Tests) is slightly alkaline (*2.2.4*).

B. To 5 mL of solution S add 0.5 mL of *0.05 M iodine*. The solution is colourless and gives reaction (a) of sulfates (*2.3.1*).

C. Solution S gives reaction (a) of sodium (*2.3.1*).

D. It complies with the limits of the assay.

TESTS

Solution S

Dissolve 10 g in *water R* and dilute to 100 mL with the same solvent.

Solution S1

To 20.0 g add 25 mL of *water R*. Shake until mostly dissolved, and carefully and progressively, add 15 mL of *hydrochloric acid R*. Heat to boiling. Cool and dilute to 100.0 mL with *water R*.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method I*).

Thiosulfates

Maximum 0.05 per cent.

To 4.00 g add 100 mL of *water R*. Shake to dissolve, add 10 mL of *formaldehyde solution R* and 10 mL of *acetic acid R*. Allow to stand for 5 min, then add 0.5 mL of *starch solution R* and titrate with *0.05 M iodine*. Carry out a blank titration. The difference between the volumes used in the titrations is not more than 0.15 mL.

Iron (*2.4.9*)

Maximum 5 ppm, determined on solution S1.

Selenium

Maximum 5 ppm.

To 6.0 g add 10 mL of *formaldehyde solution R*, carefully and progressively add 2 mL of *hydrochloric acid R*. Heat on a water-bath for 20 min. Any pink colour in the solution is not more intense than that of a standard prepared at the same time and in the same manner using 2.0 g of the substance to be examined to which 0.2 mL of *selenium standard solution (100 ppm Se) R* has been added.

Zinc

Maximum 12 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Dilute 2.0 mL of solution S1 to 10.0 mL with *water R*.

Reference solutions Prepare the reference solutions using *zinc standard solution (100 ppm Zn) R*, diluting with *water R*.

Source Zinc hollow-cathode lamp.

Wavelength 213.9 nm.

Atomisation device Air-acetylene flame.

ASSAY

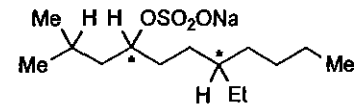
Introduce 0.500 g into a 500 mL conical flask containing 50.0 mL of *0.05 M iodine*. Shake until completely dissolved. Add 1 mL of *starch solution R* and titrate the excess of iodine with *0.1 M sodium thiosulfate*. Carry out a blank titration.

1 mL of *0.05 M iodine* is equivalent to 6.30 mg of Na_2SO_3.

Ph Eur

Sodium Tetradecyl Sulfate Concentrate

Sodium Tetradecyl Sulphate Concentrate



mixture of 4 stereoisomers

$C_{14}H_{29}NaO_4S$ 316.4 *139-88-8*

Action and use

Sclerosant.

Preparation

Sodium Tetradecyl Sulfate Injection

DEFINITION

Sodium Tetradecyl Sulfate Concentrate is an aqueous gel containing sodium *all-rac*-4-ethyl-1-isobutyloctyl sulfate. It contains not less than 46.0% w/w and not more than 52.0% w/w of $C_{14}H_{29}NaO_4S$.

CHARACTERISTICS

A clear, colourless gel.

IDENTIFICATION

A. Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. For solution (1) boil 0.2 g under a reflux condenser with 20 mL of 2M *hydrochloric acid* for 15 minutes, allow to cool, add 20 mL

of *ethanol (96%)* and extract the mixture with two 10 mL quantities of n-*pentane*. Wash the combined pentane extracts with 20 mL of *water* and dry over *anhydrous sodium sulfate*. Solution (2) contains 0.35% w/v of *decan-1-ol* and 0.7% w/v of *dodecan-1-ol* in n-*pentane*.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (80 to 100 mesh) coated with 3% w/w of polyethylene glycol (Carbowax 20M is suitable) and maintained at 120°.

The retention time of the principal peak in the chromatogram obtained with solution (1) is less than the retention time of the peak due to dodecan-1-ol and more than that of the peak due to decan-1-ol in the chromatogram obtained with solution (2).

B. Mix 0.1 mL of a 2% w/v solution with 0.1 mL of a 0.1% w/v solution of *methylene blue* and 2 mL of 1M *sulfuric acid*, add 2 mL of *chloroform* and shake. The chloroform layer is intensely blue.

C. Mix 20 mg with 10 mL of *ethanol (96%)* and heat to boiling on a water bath, shaking frequently. Filter immediately and evaporate the ethanol. Dissolve the residue in 8 mL of *water*, add 3 mL of 2M *hydrochloric acid*, evaporate the solution to half its volume and cool. Filter to remove the congealed fatty alcohols and add 1 mL of 0.25M *barium chloride* to the filtrate. A white, crystalline precipitate is produced.

D. Yields reaction B characteristic of *sodium salts*, Appendix VI.

TESTS

Alkalinity

Dissolve 1 g in 100 mL of *water* and add 0.1 mL of *methyl red solution*; the solution is yellow. Not more than 0.5 mL of *0.1M hydrochloric acid VS* is required to change the colour of the solution.

Non-ionic impurities

Not more than 3.0% w/w, with respect to the nominal content of Sodium Tetradecyl Sulfate, when determined by the method for *gas chromatography*, Appendix III B, using the following solutions. Dissolve 75 mg of *dodecan-1-ol* (internal standard) in sufficient n-*hexane* to produce 100 mL (solution A). For solution (1) extract 1 g of the concentrate being examined with 10 mL of solution A followed by two 10 mL quantities of n-*hexane*. Pass 10 mL of the combined extracts at a rate of about 1.5 mL per minute through a column, 1 cm in diameter, packed with 5 g of *basic aluminium oxide* (Brockmann grade II is suitable) and pre-washed with 25 mL of n-*hexane*. Elute with 20 mL of a mixture of equal volumes of n-*hexane* and *ether*, evaporate to dryness using a rotary evaporator and dissolve the residue in 2 mL of n-*hexane*. Prepare solution (2) in the same manner but using 10 mL of n-*hexane* in place of 10 mL of solution A.

The chromatographic procedure may be carried out using a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (80 to 100 mesh) coated with 3% w/w of polyethylene glycol (Carbowax 20M is suitable) and maintained at 120°.

Allow the chromatography to proceed for the retention time of the peak due to the internal standard.

In the chromatogram obtained with solution (1) the sum of the areas of any *secondary peaks* is not greater than twice the area of the peak due to the internal standard.

Chloride

Dissolve 33 mg in sufficient *water* to produce 100 mL. 15 mL of the resulting solution complies with the *limit test for chlorides*, Appendix VII (1%).

Sulfated ash

10.3 to 13.3%, Appendix IX A.

ASSAY

Dissolve 2.4 g in sufficient *water* to produce 1000 mL. To 20 mL add 15 mL of *chloroform* and 10 mL of *dimidium bromide-sulfan blue mixed solution* and titrate with *0.004M benzethonium chloride VS*, shaking vigorously and allowing the layers to separate after each addition, until the pink colour of the chloroform layer is completely discharged and a greyish blue colour is produced. Each mL of *0.004M benzethonium chloride VS* is equivalent to 1.266 mg of $C_{14}H_{29}NaO_4S$.

STORAGE

Sodium Tetradecyl Sulfate Concentrate should be protected from light.

Sodium Thiosulfate

Sodium Thiosulphate

(Ph. Eur. monograph 0414)

$Na_2S_2O_3,5H_2O$ 248.2 *10102-17-7*

Action and use

Used in treatment of cyanide poisoning.

Preparation

Sodium Thiosulfate Injection

Ph Eur

DEFINITION

Content

99.0 per cent to 101.0 per cent of $Na_2S_2O_3,5H_2O$.

CHARACTERS

Appearance

Transparent, colourless crystals, efflorescent in dry air.

Solubility

Very soluble in water, practically insoluble in ethanol 96 per cent. It dissolves in its water of crystallisation at about 49 °C.

IDENTIFICATION

A. It decolourises *iodinated potassium iodide solution R*.

B. To 0.5 mL of solution S (see Tests) add 0.5 mL of *water R* and 2 mL of *silver nitrate solution R2*. A white precipitate is formed which rapidly becomes yellowish and then black.

C. To 2,5 mL of solution S add 2.5 mL of *water R* and 1 mL of *hydrochloric acid R*. A precipitate of sulfur is formed and gas is evolved which gives a blue colour to *starch iodate paper R*.

D. 1 mL of solution S gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S

Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 mL with the same solvent.

Appearance of solution

The freshly prepared solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 10.0 g in 50 mL of *distilled water R*, add 1 mL of *0.1 M sodium hydroxide* and dilute to 100 mL with the same solvent.

pH (*2.2.3*)
6.0 to 8.4 for the freshly prepared solution S.

Sulfates and sulfites (*2.4.13*)
Maximum 0.2 per cent.

Dilute 2.5 mL of freshly prepared solution S to 10 mL with *distilled water R*. To 3 mL of this solution first add 2 mL of *iodinated potassium iodide solution R* and continue the addition dropwise until a very faint persistent yellow colour appears. Dilute to 15 mL with *distilled water R*.

Sulfides
To 10 mL of solution S add 0.05 mL of a freshly prepared 50 g/L solution of *sodium nitroprusside R*. The solution does not become violet.

ASSAY
Dissolve 0.500 g in 20 mL of *water R* and titrate with *0.05 M iodine*, using 1 mL of *starch solution R*, added towards the end of the titration, as indicator.

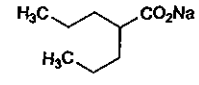
1 mL of *0.05 M iodine* is equivalent to 24.82 mg of $Na_2S_2O_3,5H_2O$.

STORAGE
In an airtight container.

Ph Eur

Sodium Valproate

(Ph. Eur. monograph 0678)



$C_8H_{15}NaO_2$ 166.2 *1069-66-5*

Action and use
Antiepileptic.

Preparations
Sodium Valproate Gastro-resistant Tablets
Sodium Valproate Oral Solution
Sodium Valproate Tablets
Sodium Valproate Prolonged-release Capsules
Sodium Valproate Prolonged-release Tablets

Ph Eur

DEFINITION
Sodium 2-propylpentanoate.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline, hygroscopic powder.

Solubility
Very soluble in water, freely soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *sodium valproate CRS*.

If the spectra obtained in the solid state show differences, record new spectra using discs prepared by placing 50 µL of a 100 g/L solution in *methanol R* on a disc of *potassium bromide R* and evaporating the solvent *in vacuo*. Examine immediately.

B. 2 mL of solution S (see Tests) gives reaction (a) of sodium (*2.3.1*).

TESTS
Solution S
Dissolve 1.25 g in 20 mL of *distilled water R* in a separating funnel, add 5 mL of *dilute nitric acid R* and shake. Allow the mixture to stand for 12 h. Use the aqueous lower layer.

Appearance of solution
The solution is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Dissolve 2.0 g in *water R* and dilute to 10 mL with the same solvent.

Acidity or alkalinity
Dissolve 1.0 g in 10 mL of *water R*. Add 0.1 mL of *phenolphthalein solution R*. Not more than 0.75 mL of *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Related substances
Gas chromatography (*2.2.28*).

Test solution Dissolve 0.500 g of the substance to be examined in 10 mL of *water R*. Add 5 mL of *dilute sulfuric acid R* and shake with 3 quantities, each of 20 mL, of *heptane R*. Dilute the combined upper layers to 100.0 mL with *heptane R*.

Reference solution (a) Dissolve 5 mg of *valproic acid for system suitability CRS* (containing impurity K) in 1.0 mL of *heptane R*.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *heptane R*.

Column:
— *material*: wide-bore fused silica;
— *size*: *l* = 30 m, Ø = 0.53 mm;
— *stationary phase*: *macrogol 20 000 2-nitroterephthalate R* (film thickness 0.5 µm).

Carrier gas *helium for chromatography R*.

Flow rate 8 mL/min.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 5	80
	5 - 15	80 → 150
	15 - 28.3	150 → 190
	28.3 - 30	190
Injection port		220
Detector		220

Detection Flame ionisation.

Injection 1 µL.

Relative retention With reference to valproic acid (retention time = about 17 min): impurity K = about 0.97.

System suitability Reference solution (a):
— *resolution*: minimum 2.0 between the peaks due to impurity K and valproic acid.

Limits:
- — *impurity K*: not more than 0.15 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
- — *unspecified impurities*: for each impurity, not more than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent);
- — *total*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- — *disregard limit*: 0.03 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.03 per cent).

Chlorides (*2.4.4*)
Maximum 200 ppm.
To 5 mL of solution S add 10 mL of *water R*.

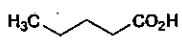
Sulfates (*2.4.13*)
Maximum 200 ppm, determined on solution S.

Loss on drying (*2.2.32*)
Maximum 2.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

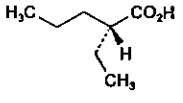
ASSAY
Dissolve 0.150 g in 25 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).
1 mL of *0.1 M perchloric acid* is equivalent to 16.62 mg of $C_8H_{15}NaO_2$.

STORAGE
In an airtight container.

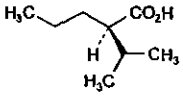
IMPURITIES
Specified impurities *K*.
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, F, G, I, J, L*.



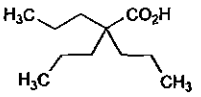
A. pentanoic acid (valeric acid),



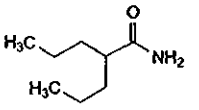
B. (2*RS*)-2-ethylpentanoic acid,



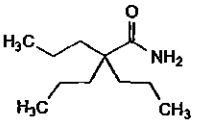
C. (2*RS*)-2-(1-methylethyl)pentanoic acid,



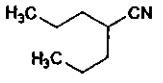
D. 2,2-dipropylpentanoic acid,



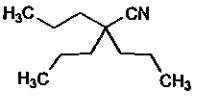
F. 2-propylpentanamide,



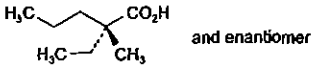
G. 2,2-dipropylpentanamide,



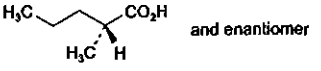
I. 2-propylpentanenitrile,



J. 2,2-dipropylpentanenitrile,



K. (2*RS*)-2-ethyl-2-methylpentanoic acid,

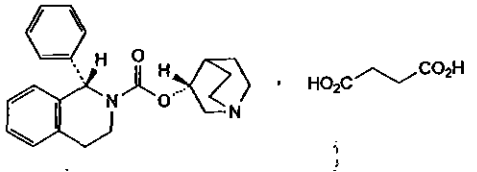


L. (2*RS*)-2-methylpentanoic acid.

Ph Eur

Solifenacin Succinate

(Ph. Eur. monograph 2779)



$C_{27}H_{32}N_2O_6$ 480.6 *242478-38-2*

Action and use
Muscarinic M_3 receptor antagonist; anticholinergic.

Ph Eur

DEFINITION
(3*R*)-1-Azabicyclo[2.2.2]octan-3-yl (1*S*)-1-phenyl-3,4-dihydroisoquinoline-2(1*H*)-carboxylate hydrogen butanedioate.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or light yellow powder.

Solubility

Very soluble or freely soluble in water, soluble in ethanol (96 per cent), practically insoluble in heptane.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *solifenacin succinate CRS.*

B. Isomeric purity (see Tests).

TESTS

Isomeric purity

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50 mg of the substance to be examined in the mobile phase and dilute to 20.0 mL with the mobile phase.

Reference solution (a) Dissolve the contents of a vial of *solifenacin for system suitability CRS* (containing impurities F, G and H) in 1.0 mL of the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:
- *size*: l = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *amylose derivative of silica gel for chiral separation R* (5 µm);
- *temperature*: 35 °C.

Mobile phase *diethylamine R, anhydrous ethanol R, heptane R* (0.1:200:800 *V/V/V*).

Flow rate 0.8 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µL.

Run time 1.5 times the retention time of solifenacin.

Identification of impurities Use the chromatogram supplied with *solifenacin for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities F, G and H.

Relative retention With reference to solifenacin (retention time = about 17 min): impurity F = about 0.7; impurity H = about 0.76; impurity G = about 0.84.

System suitability Reference solution (a):
- *resolution*: minimum 1.5 between the peaks due to impurities F and H;
- *peak-to-valley ratio*: minimum 10, where H_p = height above the baseline of the peak due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity H.

Calculation of percentage contents:
- for each impurity, use the concentration of solifenacin succinate in reference solution (b).

Limits:
- *impurities F, G, H*: for each impurity, maximum 0.15 per cent.

Impurity E

Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.500 g of the substance to be examined in *methanol R* and dilute to 5.0 mL with the same solvent.

Reference solution Dissolve 5.0 mg of *3-quinuclidinol R* (impurity E) in *methanol R* and dilute to 50.0 mL with the same solvent.

Plate *TLC silica gel plate R* (2-10 µm).

Mobile phase *concentrated ammonia R, anhydrous ethanol R, toluene R* (6:31:63 *V/V/V*).

Application 10 µL.

Development Over 2/3 of the plate.

Drying At 100-105 °C for 1 h.

Detection Expose to iodine vapour for at least 2 h.

Relative retention With reference to solifenacin (R_F = about 0.6): impurity E = about 0.2.

Limit:
- *impurity E*: any spot due to impurity E is not more intense than the corresponding spot in the chromatogram obtained with the reference solution (0.1 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture Dissolve 0.6 g of *ammonium phosphate R* in 1 L of *water R*, add 1.0 mL of *diethylamine R* and adjust to pH 11 with *concentrated ammonia R*.

Test solution Dissolve 0.100 g of the substance to be examined in mobile phase A and dilute to 100.0 mL with mobile phase A.

Reference solution (a) Dissolve 5 mg of *1-phenyl-1,2,3,4-tetrahydroisoquinoline R* (impurity A) in the test solution and dilute to 25.0 mL with the test solution.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

Column:
- *size*: l = 0.10 m, Ø = 2.1 mm;
- *stationary phase*: *end-capped ethylene-bridged polar-embedded octadecylsilyl silica gel for chromatography (hybrid material) R* (1.7 µm);
- *temperature*: 40 °C.

Mobile phase:
- *mobile phase A*: *acetonitrile R1*, solvent mixture (45:55 *V/V*);
- *mobile phase B*: *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 0.5	90	10
0.5 - 10.5	90 → 68	10 → 32
10.5 - 12.5	68	32

Flow rate 0.4 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 2.5 µL.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity A.

Relative retention With reference to solifenacin (retention time = about 2 min): succinate = about 0.2; impurity A = about 0.7.

System suitability Reference solution (a):
- *resolution*: minimum 4.0 between the peaks due to impurity A and solifenacin.

Calculation of percentage contents:
- for each impurity, use the concentration of solifenacin succinate in reference solution (b).

Limits:
- — *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- — *total*: maximum 0.3 per cent;
- — *reporting threshold*: 0.05 per cent; disregard the peak due to succinate.

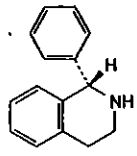
Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

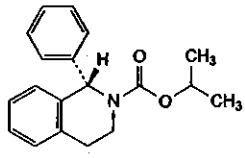
ASSAY
Dissolve 0.400 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 48.06 mg of $C_{27}H_{32}N_2O_6$.

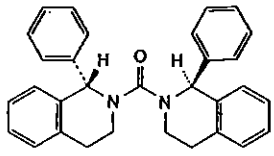
IMPURITIES
Specified impurities *E, F, G, H.*
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, I.*



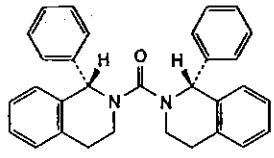
A. (1*S*)-1-phenyl-1,2,3,4-tetrahydroisoquinoline,



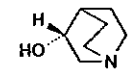
B. propan-2-yl (1*S*)-1-phenyl-3,4-dihydroisoquinoline-2(1*H*)-carboxylate,



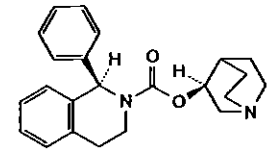
C. bis[(1*S*)-1-phenyl-3,4-dihydroisoquinolin-2(1*H*)-yl]methanone,



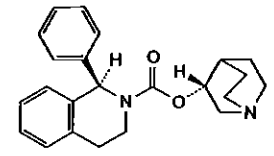
D. [(1*R*)-1-phenyl-3,4-dihydroisoquinolin-2(1*H*)-yl][(1*S*)-1-phenyl-3,4-dihydroisoquinolin-2(1*H*)-yl]methanone,



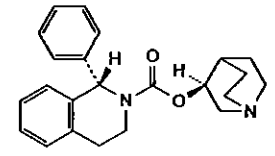
E. (3*R*)-1-azabicyclo[2.2.2]octan-3-ol (3-quinuclidinol),



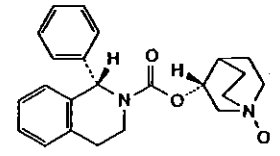
F. (3*S*)-1-azabicyclo[2.2.2]octan-3-yl (1*R*)-1-phenyl-3,4-dihydroisoquinoline-2(1*H*)-carboxylate,



G. (3*R*)-1-azabicyclo[2.2.2]octan-3-yl (1*R*)-1-phenyl-3,4-dihydroisoquinoline-2(1*H*)-carboxylate,



H. (3*S*)-1-azabicyclo[2.2.2]octan-3-yl (1*S*)-1-phenyl-3,4-dihydroisoquinoline-2(1*H*)-carboxylate,

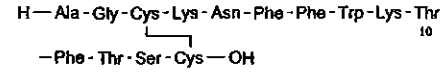


I. (3*R*)-3-[[(1*S*)-1-phenyl-3,4-dihydroisoquinoline-2(1*H*)-carbonyl]oxy]-1-azabicyclo[2.2.2]octane 1-oxide (solifenacin *N*-oxide).

Ph Eur

Somatostatin

(Ph. Eur. monograph 0949)



$C_{76}H_{104}N_{18}O_{19}S_2$ 1638 *38916-34-6*

Action and use
Growth hormone release inhibiting hormone.

Ph Eur

DEFINITION
S^3,S^{14}-Cyclo(L-alanylglycyl-L-cysteinyl-L-lysyl-L-asparaginyl-L-phenylalanyl-L-phenylalanyl-L-tryptophyl-L-lysyl-L-threonyl-L-phenylalanyl-L-threonyl-L-seryl-L-cysteine).

Synthetic cyclic tetradecapeptide having the structure of the hypothalamic hormone that inhibits the release of human growth hormone. It contains a variable quantity of acetic acid. It is available in the freeze-dried form.

Content
95.0 per cent to 104.0 per cent (anhydrous and acetic acid-free substance).

CHARACTERS

Appearance
White or almost white, hygroscopic, amorphous powder.

Solubility
Freely soluble in water and in acetic acid, practically insoluble in methylene chloride.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

B. Amino acid analysis (*2.2.56*). Method 1 for hydrolysis and method 1 for analysis are suitable.

Express the content of each amino acid in moles. Calculate the relative proportions of the amino acids taking 1/8 of the sum of the number of moles of aspartic acid, alanine, lysine, glycine and phenylalanine as equal to 1. The values fall within the following limits: aspartic acid: 0.90 to 1.10; glycine: 0.90 to 1.10; alanine: 0.90 to 1.10; phenylalanine: 2.7 to 3.3; serine: 0.7 to 1.05; threonine: 1.4 to 2.1; half-cystine: 1.4 to 2.1; lysine: 1.8 to 2.2. Not more than traces of other amino acids are present.

TESTS

Specific optical rotation (*2.2.7*)
−47 to −37 (anhydrous and acetic acid-free substance).

Dissolve 2.0 mg in 1.0 mL of a 1 per cent *V/V* solution of *glacial acetic acid R*.

Related substances
Liquid chromatography (*2.2.29*): use the normalisation procedure. *Prepare the solutions immediately before use.*

Test solution Dissolve the substance to be examined in *water R* to obtain a concentration of 1.0 mg/mL.

Reference solution (a) Dissolve the contents of a vial of *somatostatin CRS* in *water R* to obtain a concentration of 1.0 mg/mL.

Reference solution (b) Dissolve the contents of a vial of *somatostatin CRS* in a 1 mg/mL solution of *dithioerythritol R* to obtain a concentration of 1.0 mg/mL. Allow to stand at room temperature for about 1 h. Mix 1 volume of this solution and 1 volume of reference solution (a).

Reference solution (c) Dissolve the contents of a vial of *somatostatin impurity B CRS* in 1.0 mL of *water R*.

Reference solution (d) Dissolve the contents of a vial of *somatostatin impurity F CRS* in 1.0 mL of *water R*.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: *trifluoroacetic acid R, acetonitrile for chromatography R, water for chromatography R* (0.1:20:80 *V/V/V*);
— *mobile phase B*: *trifluoroacetic acid R, acetonitrile for chromatography R, water for chromatography R* (0.1:45:55 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 25	100 → 0	0 → 100
25 - 30	0	100
30 - 32	0 → 100	100 → 0

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 15 µL of the test solution and reference solutions (b), (c) and (d).

Identification of peaks Use the chromatogram obtained with reference solution (b) to identify the peak due to reduced somatostatin; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity B; use the chromatogram obtained with reference solution (d) to identify the peak due to impurity F.

Relative retention With reference to somatostatin (retention time = about 12 min): impurities B and F = about 1.03; reduced somatostatin = about 1.09; impurities C and D = about 1.17; impurity E = about 1.22.

System suitability Reference solution (b):
— the ratio of the area of the peak due to reduced somatostatin to the area of the peak due to somatostatin is between 1:9 and 9:1;
— *resolution*: minimum 3.5 between the peaks due to somatostatin and reduced somatostatin;
— *number of theoretical plates*: minimum 15 000, calculated for the peak due to somatostatin;
— *symmetry factor*: maximum 2.7 for the peak due to somatostatin.

Limits:
— *impurity B or F*: maximum 1.0 per cent;
— *unspecified impurities*: for each impurity, maximum 0.5 per cent;
— *total*: maximum 2.0 per cent;
— *reporting threshold*: 0.1 per cent.

Acetic acid (*2.5.34*)
3.0 per cent to 15.0 per cent.

Test solution Dissolve 7.0 mg of the substance to be examined in a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A, then dilute to 10.0 mL with the same mixture of mobile phases.

Water (*2.5.32*)
Maximum 8.0 per cent, determined on 10.0 mg.

Bacterial endotoxins (*2.6.14*)
Less than 10 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection 10 µL of the test solution and reference solution (a).

Calculate the percentage content of somatostatin ($C_{76}H_{104}N_{18}O_{19}S_2$) taking into account the assigned content of $C_{76}H_{104}N_{18}O_{19}S_2$ in *somatostatin CRS*.

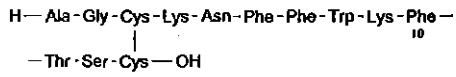
STORAGE

In an airtight container, protected from light, at a temperature of 2 °C to 8 °C. If the substance is sterile, the container is also sterile and tamper-evident.

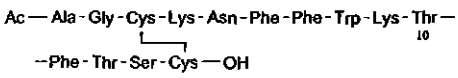
IMPURITIES

Specified impurities *B, F.*

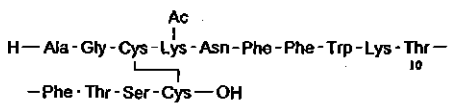
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) C, D, E.



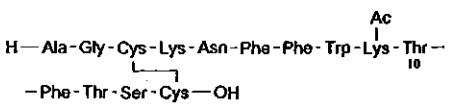
B. des-10-threonine-somatostatin (des-Thr^{10}-somatostatin),



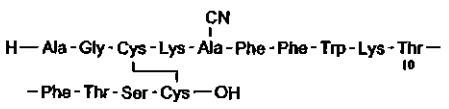
C. acetylsomatostatin ([Ac-Ala^1]somatostatin),



D. $N^{6.4}$-acetylsomatostatin ([Lys(Ac)^4]somatostatin),



E. $N^{6.9}$-acetylsomatostatin ([Lys(Ac)^9]somatostatin),



F. [5-(3-cyanoalanine)]somatostatin ([Ala(CN)^5] somatostatin).

Ph Eur

Somatropin

(Ph. Eur. monograph 0951)

```
FPTIPLSRLF   DNAMLRAHRL   HQLAFDTYQE   FEEAYIPKEQ
KYSFLQNPQT   SLCFSESIPT   PSNREETQQK   SNLELLRISL
LLIQSWLEPV   QFLRSVFANS   LVYGASDSNV   YDLLKDLEEG
IQTLMGRLED   GSPRTGQIFK   QTYSKFDTNS   HNDDALLKNY
GLLYCFRKDM   DKVETFLRIV   QCRSVEGSCG   F
```

$C_{990}H_{1528}N_{262}O_{300}S_7$ 22 125

Action and use
Growth hormone.

Preparation
Somatropin Injection

Ph Eur

DEFINITION

Protein having the structure (191 amino-acid residues) of the major component of growth hormone produced by the human pituitary.

Content
91.0 per cent to 105.0 per cent (anhydrous substance).

By convention, for the purpose of labelling somatropin preparations, 1 mg of anhydrous somatropin ($C_{990}H_{1528}N_{262}O_{300}S_7$) is equivalent to 3.0 IU of biological activity.

PRODUCTION

Somatropin is produced by a method based on recombinant DNA (rDNA) technology. During the course of product development, it must be demonstrated that the manufacturing process produces a product having a biological activity of not less than 2.5 IU/mg, using a validated bioassay based on growth promotion and approved by the competent authority.

Somatropin complies with the following additional requirements.

Host-cell-derived proteins
The limit is approved by the competent authority.

Host-cell- and vector-derived DNA
The limit is approved by the competent authority.

CHARACTERS

Appearance
White or almost white powder.

IDENTIFICATION

A. Capillary electrophoresis (*2.2.47*) as described in the test for charged variants with the following modifications.

Injection Test solution (b); under pressure or vacuum, using the following sequence: sample injection for at least 3 s then CZE buffer injection for 1 s.

Results In the electropherogram obtained, only 1 principal peak, corresponding to somatropin, is detected: no doubling of this peak is observed.

B. Examine the chromatograms obtained in the test for related proteins.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with the reference solution.

C. Peptide mapping (*2.2.55*).

SELECTIVE CLEAVAGE OF THE PEPTIDE BONDS

Test solution Prepare a solution of the substance to be examined in *0.05 M tris-hydrochloride buffer solution pH 7.5 R* to obtain a solution containing 2.0 mg/mL of somatropin and transfer about 1.0 mL to a tube made from a suitable material such as polypropylene. Prepare a 1 mg/mL solution of *trypsin for peptide mapping R* in *0.05 M tris-hydrochloride buffer solution pH 7.5 R* and add 30 µL to the solution of the substance to be examined. Cap the tube and place in a water-bath at 37 °C for 4 h. Remove from the water-bath and stop the reaction immediately, for example by freezing. If analysed immediately using an automatic injector, maintain at 2-8 °C.

Reference solution Prepare at the same time and in the same manner as for the test solution, but using *somatropin CRS* instead of the substance to be examined.

CHROMATOGRAPHIC SEPARATION Liquid chromatography (*2.2.29*).

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octylsilyl silica gel for chromatography R* (5-10 µm) with a pore size of 30 nm;
- *temperature*: 30 °C.

Mobile phase:
— *mobile phase A*: dilute 1 mL of *trifluoroacetic acid R* to 1000 mL with *water for chromatography R*;
— *mobile phase B*: to 100 mL of *water for chromatography R*, add 1 mL of *trifluoroacetic acid R* and dilute to 1000 mL with *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	100 → 80	0 → 20
20 - 40	80 → 75	20 → 25
40 - 65	75 → 50	25 → 50
65 - 70	50 → 20	50 → 80

Flow rate 1 mL/min.

Detection Spectrophotometer at 214 nm.

Injection 100 µL.

System suitability The chromatogram obtained with the reference solution is qualitatively similar to the chromatogram of somatropin digest supplied with *somatropin CRS*.

Results The profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.

D. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with the reference solution.

TESTS

Related proteins

Liquid chromatography (*2.2.29*): use the normalisation procedure. *Maintain the solutions at 2-8 °C and use within 24 h. If an automatic injector is used, maintain it at 2-8 °C.*

Test solution Prepare a solution of the substance to be examined in *0.05 M tris-hydrochloride buffer solution pH 7.5 R*, containing 2.0 mg/mL of somatropin.

Reference solution Prepare a solution of *somatropin CRS* in *0.05 M tris-hydrochloride buffer solution pH 7.5 R*, containing 2.0 mg/mL of somatropin.

Resolution solution Dissolve the contents of a vial of *somatropin/desamidosomatropin resolution mixture CRS* in *0.05 M tris-hydrochloride buffer solution pH 7.5 R* to obtain a concentration of 2 mg/mL of somatropin.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped butylsilyl silica gel for chromatography R* (5 µm) with a pore size of 30 nm; a silica saturation column is placed between the pump and the injector valve;
— *temperature*: 45 °C.

Mobile phase *propanol R, 0.05 M tris-hydrochloride buffer solution pH 7.5 R* (29:71 *V/V*).

Flow rate 0.5 mL/min.

Detection Spectrophotometer at 220 nm.

Preconditioning of the column Rinse with 200-500 mL of a 0.1 per cent *V/V* solution of *trifluoroacetic acid R* in a 50 per cent *V/V* solution of *acetonitrile R*; repeat as necessary, to improve column performance.

Injection 20 µL.

Relative retention With reference to somatropin (retention time = about 33 min; if necessary adjust the concentration of *propanol R* in the mobile phase): desamidosomatropin = about 0.85.

System suitability Resolution solution:
— *resolution*: minimum 1.0 between the peaks due to desamidosomatropin and somatropin;
— *symmetry factor*: 0.9 to 1.8 for the peak due to somatropin.

Limit:
— *total*: maximum 6.0 per cent.

Dimer and related substances of higher molecular mass

Size-exclusion chromatography (*2.2.30*): use the normalisation procedure.

Test solution Prepare a solution of the substance to be examined in *0.025 M phosphate buffer solution pH 7.0 R*, containing 1.0 mg/mL of somatropin.

Reference solution Dissolve the contents of a vial of *somatropin CRS* in *0.025 M phosphate buffer solution pH 7.0 R* and dilute with the same solution to obtain a concentration of 1.0 mg/mL.

Resolution solution Place 1 vial of *somatropin CRS* in an oven at 50 °C for a period sufficient to generate 1-2 per cent of dimer (typically 12-24 h). Dissolve its contents in *0.025 M phosphate buffer solution pH 7.0 R* and dilute with the same solution to obtain a concentration of 1.0 mg/mL.

Column:
— *size*: *l* = 0.30 m, Ø = 7.8 mm;
— *stationary phase*: *hydrophilic silica gel for chromatography R* of a grade suitable for fractionation of globular proteins in the relative molecular mass range of 5000 to 150 000.

Mobile phase *2-propanol R, 0.063 M phosphate buffer solution pH 7.0 R* (3:97 *V/V*); filter and degas.

Flow rate 0.6 mL/min.

Detection Spectrophotometer at 214 nm.

Injection 20 µL.

Relative retention With reference to somatropin monomer (retention time = 12 min to 17 min): related substances of higher molecular mass = about 0.65; somatropin dimer = about 0.9.

System suitability Resolution solution:
— *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to the dimer and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to the monomer.

Limit:
— *sum of the peaks with retention times less than that of the principal peak*: maximum 4.0 per cent.

Charged variants

Capillary electrophoresis (*2.2.47*).

Test solution (a) Prepare a solution of the substance to be examined containing 1 mg/mL of somatropin.

Test solution (b) Mix equal volumes of test solution (a) and the reference solution.

Reference solution Dissolve the contents of a vial of *somatropin CRS* in *water R* and dilute with the same solvent to obtain a concentration of 1 mg/mL.

Capillary:
— *material*: uncoated fused silica;
— *size*: effective length = at least 70 cm, Ø = 50 µm.

Temperature 30 °C.

CZE buffer 13.2 g/L solution of *ammonium phosphate R* adjusted to pH 6.0 with *phosphoric acid R* and filtered.

Detection Spectrophotometer at 200 nm.

Set the autosampler to store the samples at 4 °C during analysis.

Preconditioning of the capillary Rinse with *1 M sodium hydroxide* for 20 min, with *water R* for 10 min and with the CZE buffer for 20 min.

Between-run rinsing Rinse with *0.1 M sodium hydroxide* for 2 min and with the CZE buffer for 6 min.

Note: rinsing times may be adapted according to the length of the capillary and the equipment used.

Injection Test solution (a) and the reference solution; under pressure or vacuum, using the following sequence: sample injection for at least 3 s then CZE buffer injection for 1 s.

The injection time and pressure may be adapted in order to meet the system suitability criteria.

Migration Apply a field strength of 217 V/cm (20 kV for capillaries of 92 cm total length) for 80 min, using the CZE buffer as the electrolyte in both buffer reservoirs.

Relative migration With reference to somatropin: deamidated forms = 1.02 to 1.11.

System suitability Reference solution:

— the electropherogram obtained is similar to the electropherogram of somatropin supplied with *somatropin CRS*; 2 peaks (I_1, I_2) eluting prior to the principal peak and at least 2 peaks (I_3, I_4) eluting after the principal peak are clearly visible.

Note: peak I_2 corresponds to the cleaved form and peak I_4 corresponds to the deamidated forms, eluting as a doublet.

Limits:

— *deamidated forms*: maximum 5.0 per cent;

— *any other impurity*: for each impurity, maximum 2.0 per cent;

— *total*: maximum 10.0 per cent.

Water (*2.5.32*)
Maximum 10.0 per cent.

Bacterial endotoxins (*2.6.14*)
Less than 5 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for removal of bacterial endotoxins.

ASSAY

Size-exclusion chromatography (*2.2.30*) as described in the test for dimer and related substances of higher molecular mass.

Calculate the content of somatropin ($C_{990}H_{1528}N_{262}O_{300}S_7$) from the declared content of $C_{990}H_{1528}N_{262}O_{300}S_7$ in *somatropin CRS*.

STORAGE

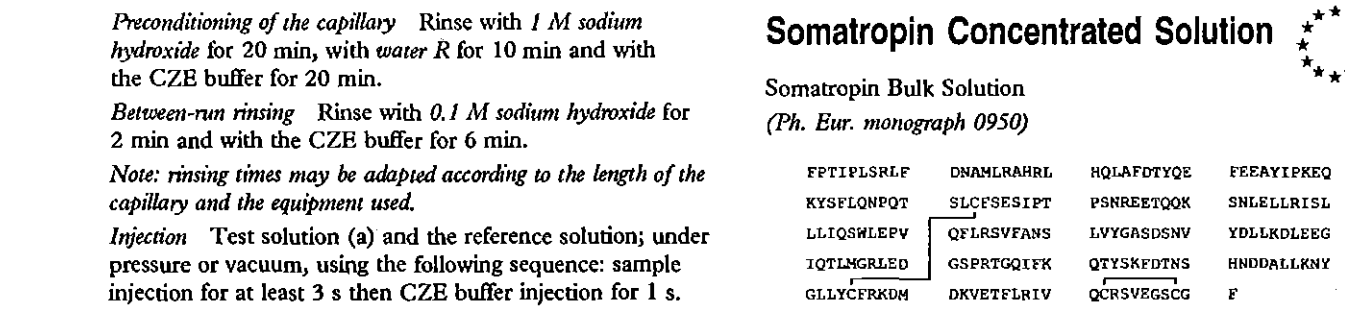
In an airtight container, at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-evident container.

Ph Eur

Somatropin Concentrated Solution

Somatropin Bulk Solution

(Ph. Eur. monograph 0950)



$C_{990}H_{1528}N_{262}O_{300}S_7$ 22 125

Action and use
Growth hormone.

Preparation
Somatropin Injection

Ph Eur

DEFINITION

Solution containing a protein having the structure (191 amino-acid residues) of the major component of growth hormone produced by the human pituitary. It may contain buffer salts and other auxiliary substances.

Content
91.0 per cent to 105.0 per cent of the amount of somatropin stated on the label.

By convention, for the purpose of labelling somatropin preparations, 1 mg of anhydrous somatropin ($C_{990}H_{1528}N_{262}O_{300}S_7$) is equivalent to 3.0 IU of biological activity.

PRODUCTION

Somatropin concentrated solution is produced by a method based on recombinant DNA (rDNA) technology. During the course of product development, it must be demonstrated that the manufacturing process produces a product having a biological activity of at least 2.5 IU/mg, using a validated bioassay based on growth promotion and approved by the competent authority.

Somatropin concentrated solution complies with the following additional requirements.

Host-cell-derived proteins
The limit is approved by the competent authority.

Host-cell- and vector-derived DNA
The limit is approved by the competent authority.

CHARACTERS

Appearance
Clear or slightly turbid, colourless solution.

IDENTIFICATION

A. Capillary electrophoresis (*2.2.47*) as described in the test for charged variants with the following modifications.

Injection Test solution (b); under pressure or vacuum, using the following sequence: sample injection for at least 3 s then CZE buffer injection for 1 s.

Results In the electropherogram obtained, only 1 principal peak, corresponding to somatropin, is detected: no doubling of this peak is observed.

B. Examine the chromatograms obtained in the test for related proteins.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to

the principal peak in the chromatogram obtained with the reference solution.

C. Peptide mapping (*2.2.55*).

SELECTIVE CLEAVAGE OF THE PEPTIDE BONDS

Test solution Dilute the solution to be examined with *0.05 M tris-hydrochloride buffer solution pH 7.5 R* so that it contains 2.0 mg/mL of somatropin and transfer about 1.0 mL to a tube made from a suitable material such as polypropylene. Prepare a 1 mg/mL solution of *trypsin for peptide mapping R* in *0.05 M tris-hydrochloride buffer solution pH 7.5 R* and add 30 µL to the solution of the substance to be examined. Cap the tube and place in a water-bath at 37 °C for 4 h. Remove from the water-bath and stop the reaction immediately, for example by freezing. If analysed immediately using an automatic injector, maintain at 2-8 °C.

Note: If a 2 mg/mL somatropin concentration is not obtainable, a similar digest relationship (micrograms of trypsin per milligram of somatropin) may be used.

Reference solution Prepare at the same time and in the same manner as for the test solution, but using *somatropin CRS* instead of the substance to be examined.

CHROMATOGRAPHIC SEPARATION Liquid chromatography (*2.2.29*).

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octylsilyl silica gel for chromatography R* (5-10 µm) with a pore size of 30 nm;
- *temperature*: 30 °C.

Mobile phase:
- *mobile phase A*: dilute 1 mL of *trifluoroacetic acid R* to 1000 mL with *water for chromatography R*;
- *mobile phase B*: to 100 mL of *water for chromatography R*, add 1 mL of *trifluoroacetic acid R* and dilute to 1000 mL with *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	100 → 80	0 → 20
20 - 40	80 → 75	20 → 25
40 - 65	75 → 50	25 → 50
65 - 70	50 → 20	50 → 80

Flow rate 1 mL/min.

Detection Spectrophotometer at 214 nm.

Injection 100 µL.

System suitability The chromatogram obtained with the reference solution is qualitatively similar to the chromatogram of somatropin digest supplied with *somatropin CRS*.

Results The profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.

D. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with the reference solution.

TESTS

Related proteins

Liquid chromatography (*2.2.29*): use the normalisation procedure. *Maintain the solutions at 2-8 °C and use within 24 h. If an automatic injector is used, maintain it at 2-8 °C.*

Test solution Dilute the solution to be examined in *0.05 M tris-hydrochloride buffer solution pH 7.5 R*, so as to contain 2.0 mg/mL of somatropin. A weaker solution may be prepared, in which case the injection volume is adjusted accordingly.

Reference solution Prepare a solution of *somatropin CRS* in *0.05 M tris-hydrochloride buffer solution pH 7.5 R*, containing 2.0 mg/mL of somatropin.

Resolution solution Dissolve the contents of a vial of *somatropin/desamidosomatropin resolution mixture CRS* in *0.05 M tris-hydrochloride buffer solution pH 7.5 R* to obtain a concentration of 2 mg/mL of somatropin.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped butylsilyl silica gel for chromatography R* (5 µm) with a pore size of 30 nm; a silica saturation column is placed between the pump and the injector valve;
- *temperature*: 45 °C.

Mobile phase *propanol R, 0.05 M tris-hydrochloride buffer solution pH 7.5 R* (29:71 *V/V*).

Flow rate 0.5 mL/min.

Detection Spectrophotometer at 220 nm.

Preconditioning of the column Rinse with 200-500 mL of a 0.1 per cent *V/V* solution of *trifluoroacetic acid R* in a 50 per cent *V/V* solution of *acetonitrile R*; repeat as necessary, to improve column performance.

Injection 20 µL.

Relative retention With reference to somatropin (retention time = about 33 min; if necessary adjust the concentration of *propanol R* in the mobile phase): desamidosomatropin = about 0.85.

System suitability Resolution solution:
- *resolution*: minimum 1.0 between the peaks due to desamidosomatropin and somatropin;
- *symmetry factor*: 0.9 to 1.8 for the peak due to somatropin.

Limit:
- *total*: maximum 6.0 per cent.

Dimer and related substances of higher molecular mass

Size-exclusion chromatography (*2.2.30*): use the normalisation procedure.

Test solution Dilute the solution to be examined in *0.025 M phosphate buffer solution pH 7.0 R*, so as to contain 1.0 mg/mL of somatropin.

Reference solution Dissolve the contents of a vial of *somatropin CRS* in *0.025 M phosphate buffer solution pH 7.0 R* and dilute with the same solution to obtain a concentration of 1.0 mg/mL.

Resolution solution Place 1 vial of *somatropin CRS* in an oven at 50 °C for a period sufficient to generate 1-2 per cent of dimer (typically 12-24 h). Dissolve its contents in *0.025 M phosphate buffer solution pH 7.0 R* and dilute with the same solution to obtain a concentration of 1.0 mg/mL.

Column:
- *size*: *l* = 0.30 m, Ø = 7.8 mm;
- *stationary phase*: *hydrophilic silica gel for chromatography R* of a grade suitable for fractionation of globular proteins in the relative molecular mass range of 5000 to 150 000.

Mobile phase *2-propanol R, 0.063 M phosphate buffer solution pH 7.0 R* (3:97 *V/V*); filter and degas.

Flow rate 0.6 mL/min.

Detection Spectrophotometer at 214 nm.

Injection 20 µL.

Relative retention With reference to somatropin monomer (retention time = 12 min to 17 min): related substances of higher molecular mass = about 0.65; somatropin dimer = about 0.9.

System suitability Resolution solution:
— *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to the dimer and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to the monomer.

Limit:
— *sum of the peaks with retention times less than that of the principal peak*: maximum 4.0 per cent.

Charged variants
Capillary electrophoresis (*2.2.47*).

Test solution (a) Dilute the solution to be examined so as to obtain a concentration of 1 mg/mL of somatropin.

Test solution (b) Mix equal volumes of test solution (a) and the reference solution.

Reference solution Dissolve the contents of a vial of *somatropin CRS* in *water R* and dilute with the same solvent to obtain a concentration of 1 mg/mL.

Capillary:
— *material*: uncoated fused silica;
— *size*: effective length = at least 70 cm, Ø = 50 µm.

Temperature 30 °C.

CZE buffer 13.2 g/L solution of *ammonium phosphate R* adjusted to pH 6.0 with *phosphoric acid R* and filtered.

Detection Spectrophotometer at 200 nm.

Set the autosampler to store the samples at 4 °C during analysis.

Preconditioning of the capillary Rinse with *1 M sodium hydroxide* for 20 min, with *water R* for 10 min and with the CZE buffer for 20 min.

Between-run rinsing Rinse with *0.1 M sodium hydroxide* for 2 min and with the CZE buffer for 6 min.

Note: rinsing times may be adapted according to the length of the capillary and the equipment used.

Injection Test solution (a) and the reference solution; under pressure or vacuum, using the following sequence: sample injection for at least 3 s then CZE buffer injection for 1 s. The injection time and pressure may be adapted in order to meet the system suitability criteria.

Migration Apply a field strength of 217 V/cm (20 kV for capillaries of 92 cm total length) for 80 min, using the CZE buffer as the electrolyte in both buffer reservoirs.

Relative migration With reference to somatropin: deamidated forms = 1.02 to 1.11.

System suitability Reference solution:
— the electropherogram obtained is similar to the electropherogram of somatropin supplied with *somatropin CRS*; 2 peaks (I_1, I_2) eluting prior to the principal peak and at least 2 peaks (I_3, I_4) eluting after the principal peak are clearly visible.

Note: peak I_2 corresponds to the cleaved form and peak I_4 corresponds to the deamidated forms, eluting as a doublet.

Limits:
— *deamidated forms*: maximum 5.0 per cent;
— *any other impurity*: for each impurity, maximum 2.0 per cent;
— *total*: maximum 10.0 per cent.

Bacterial endotoxins (*2.6.14*)
Less than 5 IU in the volume that contains 1 mg of somatropin, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for removal of bacterial endotoxins.

ASSAY
Size-exclusion chromatography (*2.2.30*) as described in the test for dimer and related substances of higher molecular mass.

Calculate the content of somatropin ($C_{990}H_{1528}N_{262}O_{300}S_7$) from the declared content of $C_{990}H_{1528}N_{262}O_{300}S_7$ in *somatropin CRS*.

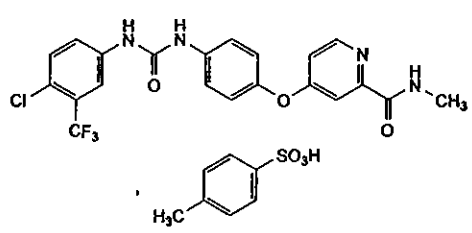
STORAGE
In an airtight container at a temperature of −20 °C. Avoid repeated freezing and thawing. If the solution is sterile, store in a sterile, airtight, tamper-evident container.

LABELLING
The label states:
— the content of somatropin in milligrams per millilitre;
— the name and concentration of any auxiliary substance.

Ph Eur

Sorafenib Tosilate

(Ph. Eur. monograph 2931)



$C_{28}H_{24}ClF_3N_4O_6S$ 637 *475207-59-1*

Action and use
Inhibitor of multiple tyrosine kinases; antineoplastic.

Preparation
Sorafenib Tablets

Ph Eur

DEFINITION
4-[4-[[[4-Chloro-3-(trifluoromethyl)phenyl]carbamoyl]amino] phenoxy]-*N*-methylpyridine-2-carboxamide 4-methylbenzene-1-sulfonate.

Content
97.5 per cent to 102.0 per cent (anhydrous substance).

PRODUCTION
It is considered that alkyl toluenesulfonate esters are genotoxic and are potential impurities in sorafenib tosilate. The manufacturing process should be developed taking into consideration the principles of quality risk management, together with considerations of the quality of starting materials, process capability and validation. The general method *2.5.40. Methyl, ethyl and isopropyl toluenesulfonate in active substances* is available to assist manufacturers.

CHARACTERS
Appearance
White or slightly yellowish or brownish powder.

Solubility
Practically insoluble in water, slightly soluble in anhydrous ethanol, practically insoluble in heptane.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *sorafenib tosilate CRS.*

TESTS

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Solvent mixture *phosphoric acid R, acetonitrile R, dimethyl sulfoxide R* (0.1:15:85 *V/V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dissolve 50.0 mg of *sorafenib tosilate CRS* in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 2 mg of *sorafenib impurity H CRS* in 100 mL of the solvent mixture using sonication. Dilute 1 mL of the solution to 20 mL with the test solution.

Reference solution (d) Dissolve 5 mg of *sorafenib for peak identification CRS* (containing impurities A and D) in the solvent mixture and dilute to 5 mL with the solvent mixture.

Column:
- *size: l* = 0.15 m, Ø = 2.1 mm;
- *stationary phase: end-capped extra-dense bonded octylsilyl silica gel for chromatography R* (3.5 µm);
- *temperature*: 75 °C.

Mobile phase:
- *mobile phase A*: dissolve 1.0 g of *potassium dihydrogen phosphate R* and 1.0 mL of *phosphoric acid R* in *water for chromatography R* and dilute to 1000 mL with the same solvent;
- *mobile phase B*: *anhydrous ethanol R, acetonitrile for chromatography R* (40:60 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	95	5
2 - 24	95 → 56.5	5 → 43.5
24 - 32	56.5 → 10	43.5 → 90
32 - 37	10	90

Flow rate 0.6 mL/min.

Detection Spectrophotometer at 235 nm.

Injection 3 µL of the test solution and reference solutions (b), (c) and (d).

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peak due to impurity H; use the chromatogram supplied with *sorafenib for peak identification CRS* and the chromatogram obtained with reference solution (d) to identify the peaks due to impurities A and D.

Relative retention With reference to sorafenib (retention time = about 27 min): toluenesulfonic acid = about 0.07; impurity A = about 0.1; impurity D = about 0.7; impurity H = about 0.98.

System suitability Reference solution (c):
- *resolution*: minimum 2.5 between the peaks due to impurity H and sorafenib.

Calculation of percentage contents:
- *correction factor*: multiply the peak area of impurity D by 0.7;
- for each impurity, use the concentration of sorafenib tosilate in reference solution (b).

Limits:
- *impurities A, D*: for each impurity, maximum 0.15 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.5 per cent;
- *reporting threshold*: 0.05 per cent; disregard the peak due to toluenesulfonic acid.

Water (*2.5.32*)
Maximum 1.0 per cent, determined on 0.200 g by direct sample introduction.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

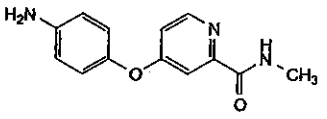
Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{28}H_{24}ClF_3N_4O_6S$ taking into account the assigned content of *sorafenib tosilate CRS.*

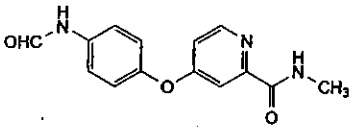
IMPURITIES

Specified impurities *A, D.*

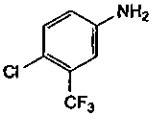
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, E, F, G, H.*



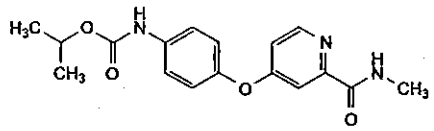
A. 4-(4-aminophenoxy)-*N*-methylpyridine-2-carboxamide,



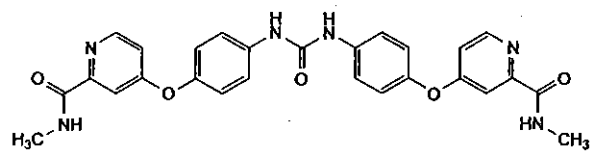
B. 4-(4-formamidophenoxy)-*N*-methylpyridine-2-carboxamide,



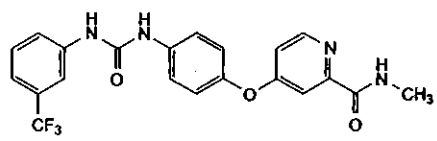
C. 4-chloro-3-(trifluoromethyl)aniline,



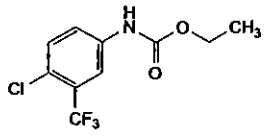
D. propan-2-yl [4-[[2-(methylcarbamoyl)pyridin-4-yl]oxy] phenyl]carbamate,



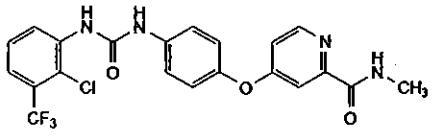
E. 4,4′-[carbonylbis(azanediyl-4,1-phenyleneoxy)]bis(*N*-methylpyridine-2-carboxamide),



F. *N*-methyl-4-[4-[[[3-(trifluoromethyl)phenyl] carbamoyl]amino]phenoxy]pyridine-2-carboxamide,



G. ethyl [4-chloro-3-(trifluoromethyl)phenyl]carbamate,

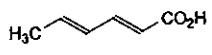


H. 4-[4-[[[2-chloro-3-(trifluoromethyl)phenyl] carbamoyl]amino]phenoxy]-*N*-methylpyridine-2-carboxamide.

Ph Eur

Sorbic Acid

(Ph. Eur. monograph 0592)



$C_6H_8O_2$ 112.1 *110-44-1*

Action and use
Antimicrobial preservative.

Ph Eur

DEFINITION
(*E,E*)-Hexa-2,4-dienoic acid.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Slightly soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION
First identification: A, C.
Second identification: A, B, D.

A. Melting point (*2.2.14*): 132 °C to 136 °C.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 50.0 mg in *water R* and dilute to 250.0 mL with the same solvent. Dilute 2.0 mL of this solution to 200.0 mL with *0.1 M hydrochloric acid.*

Spectral range 230-350 nm.

Absorption maximum At 264 nm.

Specific absorbance at the absorption maximum 2150 to 2550.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *sorbic acid CRS.*

D. Dissolve 0.2 g in 2 mL of *ethanol (96 per cent) R* and add 0.2 mL of *bromine water R.* The solution is decolorised.

TESTS

Solution S
Dissolve 1.25 g in *ethanol (96 per cent) R* and dilute to 25 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Aldehydes
Maximum 0.15 per cent, calculated as C_2H_4O.

Dissolve 1.0 g in a mixture of 30 mL of *water R* and 50 mL of *2-propanol R*, adjust to pH 4 with *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* and dilute to 100 mL with *water R.* To 10 mL of this solution add 1 mL of *decolorised fuchsin solution R* and allow to stand for 30 min. Any colour in the solution is not more intense than that in a standard prepared at the same time by adding 1 mL of *decolorised fuchsin solution R* to a mixture of 1.5 mL of *acetaldehyde standard solution (100 ppm C_2H_4O) R*, 4 mL of *2-propanol R* and 4.5 mL of *water R.*

Water (*2.5.12*)
Maximum 1.0 per cent, determined on 2.000 g.

Sulfated ash (*2.4.14*)
Maximum 0.2 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.1000 g in 20 mL of *ethanol (96 per cent) R.* Using 0.2 mL of *phenolphthalein solution R* as indicator, titrate with *0.1 M sodium hydroxide* until a pink colour is obtained.

1 mL of *0.1 M sodium hydroxide* is equivalent to 11.21 mg of $C_6H_8O_2$.

STORAGE
Protected from light.

Ph Eur

Sorbitan Laurate

(Ph. Eur. monograph 1040)

Action and use
Non-ionic surfactant.

When sorbitan monolaurate is demanded, Sorbitan Laurate shall be supplied.

Ph Eur

DEFINITION
Mixture usually obtained by partial esterification of sorbitol and its mono- and di-anhydrides with lauric (dodecanoic) acid.

CHARACTERS
Appearance
Brownish-yellow, viscous liquid.

Solubility
Practically insoluble but dispersible in water, miscible with ethanol (96 per cent).

Relative density
About 0.98.

IDENTIFICATION
A. Hydroxyl value (see Tests).

B. Iodine value (see Tests).

C. Composition of fatty acids (see Tests).

TESTS
Acid value (*2.5.1*)
Maximum 7.0, determined on 5.0 g.

Hydroxyl value (*2.5.3, Method A*)
330 to 358.

Iodine value (*2.5.4*)
Maximum 10.

Peroxide value (*2.5.5*)
Maximum 5.0.

Saponification value (*2.5.6*)
158 to 170.

Carry out the saponification for 1 h.

Composition of fatty acids
Gas chromatography (*2.4.22, Method C*).

Prepare reference solution (a) as indicated in tables 2.4.22.-1 and 2.4.22.-2.

Composition of the fatty acid fraction of the substance:
— *caproic acid*: maximum 1.0 per cent;
— *caprylic acid*: maximum 10.0 per cent;
— *capric acid*: maximum 10.0 per cent;
— *lauric acid*: 40.0 per cent to 60.0 per cent;
— *myristic acid*: 14.0 per cent to 25.0 per cent;
— *palmitic acid*: 7.0 per cent to 15.0 per cent;
— *stearic acid*: maximum 7.0 per cent;
— *oleic acid*: maximum 11.0 per cent;
— *linoleic acid*: maximum 3.0 per cent.

Water (*2.5.12*)
Maximum 1.5 per cent, determined on 1.00 g.

Total ash (*2.4.16*)
Maximum 0.5 per cent.

STORAGE
Protected from light.

FUNCTIONALITY-RELATED CHARACTERISTICS
This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for sorbitan laurate used as emulsifier and co-solubiliser in creams.

Composition of fatty acids
(see Tests).

Hydroxyl value
(see Tests).

Ph Eur

Sorbitan Oleate

(Ph. Eur. monograph 1041)

Action and use
Non-ionic surfactant.

When sorbitan mono-oleate is demanded, Sorbitan Oleate shall be supplied.

Ph Eur

DEFINITION
Mixture usually obtained by esterification of 1 mole of sorbitol and its mono-and di-anhydrides per mole of oleic ((9*Z*)-octadec-9-enoic) acid. A suitable antioxidant may be added.

CHARACTERS
Appearance
Brownish-yellow, viscous liquid.

Solubility
Practically insoluble but dispersible in water, soluble in fatty oils producing a hazy solution, miscible with ethanol (96 per cent).

Relative density
About 0.99.

IDENTIFICATION
A. Hydroxyl value (see Tests).

B. Iodine value (see Tests).

C. Composition of fatty acids (see Tests).

Margaric acid Maximum 0.2 per cent for oleic acid of vegetable origin and maximum 4.0 per cent for oleic acid of animal origin.

TESTS
Acid value (*2.5.1*)
Maximum 8.0, determined on 5.0 g.

Hydroxyl value (*2.5.3, Method A*)
190 to 210.

Iodine value (*2.5.4*)
62 to 76.

Peroxide value (*2.5.5*)
Maximum 10.0.

Saponification value (*2.5.6*)
145 to 160.

Carry out the saponification for 1 h.

Composition of fatty acids
Gas chromatography (*2.4.22, Method C*).

Composition of the fatty acid fraction of the substance:
— *myristic acid*: maximum 5.0 per cent;
— *palmitic acid*: maximum 16.0 per cent;
— *palmitoleic acid*: maximum 8.0 per cent;
— *stearic acid*: maximum 6.0 per cent;
— *oleic acid*: 65.0 per cent to 88.0 per cent;
— *linoleic acid*: maximum 18.0 per cent;
— *linolenic acid*: maximum 4.0 per cent;
— *fatty acids with chain length greater than C_{18}*: maximum 4.0 per cent.

Water (*2.5.12*)
Maximum 1.5 per cent, determined on 1.00 g.

Total ash (*2.4.16*)
Maximum 0.5 per cent, determined on 1.5 g.

STORAGE
Protected from light.

LABELLING
The label states the origin of the oleic acid used (animal or vegetable).

FUNCTIONALITY-RELATED CHARACTERISTICS
This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for sorbitan oleate used as emulsifier and co-solubiliser in creams.

Composition of fatty acids
(see Tests).

Hydroxyl value
(see Tests).

Ph Eur

Sorbitan Palmitate

(Ph. Eur. monograph 1042)

Action and use
Non-ionic surfactant.

When sorbitan monopalmitate is demanded, Sorbitan Palmitate shall be supplied.

Ph Eur

DEFINITION
Mixture usually obtained by partial esterification of sorbitol and its mono- and di-anhydrides with palmitic (hexadecanoic) acid.

CHARACTERS

Appearance
Yellow or yellowish powder, waxy flakes or hard masses.

Solubility
Practically insoluble in water, soluble in fatty oils, slightly soluble in ethanol (96 per cent).

IDENTIFICATION
A. Melting point (*2.2.15*): 44 °C to 51 °C.

Introduce the melted substance into the glass capillary tubes and allow to stand at a temperature below 10 °C for 24 h.

B. Hydroxyl value (see Tests).

C. Composition of fatty acids (see Tests).

TESTS

Acid value (*2.5.1*)
Maximum 8.0, determined on 5.0 g.

Hydroxyl value (*2.5.3, Method A*)
270 to 305.

Peroxide value (*2.5.5*)
Maximum 5.0.

Saponification value (*2.5.6*)
140 to 155.

Carry out the saponification for 1 h.

Composition of fatty acids
Gas chromatography (*2.4.22, Method C*).

Composition of the fatty acid fraction of the substance:
— *palmitic acid*: minimum 92.0 per cent;
— *stearic acid*: maximum 6.0 per cent.

Water (*2.5.12*)
Maximum 1.5 per cent, determined on 1.00 g.

Total ash (*2.4.16*)
Maximum 0.5 per cent.

STORAGE
Protected from light.

FUNCTIONALITY-RELATED CHARACTERISTICS
This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can

also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for sorbitan palmitate used as emulsifier and co-solubiliser in creams.

Composition of fatty acids
(see Tests).

Hydroxyl value
(see Tests).

Ph Eur

Sorbitan Sesquioleate

(Ph. Eur. monograph 1916)

Action and use
Non-ionic surfactant.

Ph Eur

DEFINITION
Mixture usually obtained by esterification of 2 moles of sorbitol and its mono- and di-anhydrides per 3 moles of oleic ((9*Z*)-octadec-9-enoic) acid. A suitable antioxidant may be added.

CHARACTERS
Appearance
Pale yellow or slightly brownish-yellow paste, which becomes a viscous, oily, brownish-yellow liquid at about 25 °C.

Solubility
Dispersible in water, soluble in fatty oils, slightly soluble in ethanol (96 per cent).

Relative density
About 0.99.

IDENTIFICATION
A. Hydroxyl value (see Tests).

B. Iodine value (see Tests).

C. Composition of fatty acids (see Tests).

Margaric acid Maximum 0.2 per cent for oleic acid of vegetable origin and maximum 4.0 per cent for oleic acid of animal origin.

TESTS
Acid value (*2.5.1*)
Maximum 16.0, determined on 5.0 g.

Hydroxyl value (*2.5.3, Method A*)
180 to 215.

Iodine value (*2.5.4*)
70 to 95.

Peroxide value (*2.5.5*)
Maximum 10.0.

Saponification value (*2.5.6*)
145 to 166.

Carry out the saponification for 1 h.

Composition of fatty acids
Gas chromatography (*2.4.22, Method C*).

Composition of the fatty acid fraction of the substance:
— *myristic acid*: maximum 5.0 per cent;
— *palmitic acid*: maximum 16.0 per cent;
— *palmitoleic acid*: maximum 8.0 per cent;
— *stearic acid*: maximum 6.0 per cent;
— *oleic acid*: 65.0 per cent to 88.0 per cent;
— *linoleic acid*: maximum 18.0 per cent;
— *linolenic acid*: maximum 4.0 per cent;
— *fatty acids with chain length greater than C_{18}*: maximum 4.0 per cent.

Water (*2.5.12*)
Maximum 1.5 per cent, determined on 1.00 g.

Total ash (*2.4.16*)
Maximum 0.5 per cent, determined on 1.5 g.

STORAGE
Protected from light.

LABELLING
The label states the origin of the oleic acid used (animal or vegetable).

FUNCTIONALITY-RELATED CHARACTERISTICS
This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for sorbitan sesquioleate used as emulsifier and co-solubiliser in creams.

Composition of fatty acids
(see Tests).

Hydroxyl value
(see Tests).

Ph Eur

Sorbitan Stearate

(Ph. Eur. monograph 1043)

Action and use
Non-ionic surfactant.

When sorbitan monostearate is demanded, Sorbitan Stearate shall be supplied.

Ph Eur

DEFINITION
Mixture usually obtained by partial esterification of sorbitol and its mono- and di-anhydrides with *Stearic acid 50 (1474)* or *Stearic acid 70 (1474)*.

CHARACTERS
Appearance
Pale yellow, waxy solid.

Solubility
Practically insoluble but dispersible in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION
A. Melting point (*2.2.15*): 50 °C to 60 °C.

Introduce the melted substance into the capillary tubes and allow to stand at a temperature below 10 °C for 24 h.

B. Hydroxyl value (see Tests).

C. Composition of fatty acids (see Tests).

TESTS

Acid value (*2.5.1*)
Maximum 10.0, determined on 5.0 g.

Hydroxyl value (*2.5.3, Method A*)
235 to 260.

Peroxide value (*2.5.5*)
Maximum 5.0.

Saponification value (*2.5.6*)
147 to 157.

Carry out the saponification for 1 h.

Composition of fatty acids
Gas chromatography (*2.4.22, Method C*).

Composition of the fatty acid fraction of the substance:

	Type of fatty acid used	Composition of fatty acids
Sorbitan stearate (type I)	Stearic acid 50	*Stearic acid*: 40.0 per cent to 60.0 per cent, *Sum of the contents of palmitic and stearic acids*: minimum 90.0 per cent.
Sorbitan stearate (type II)	Stearic acid 70	*Stearic acid*: 60.0 per cent to 80.0 per cent, *Sum of the contents of palmitic and stearic acids*: minimum 90.0 per cent.

Water (*2.5.12*)
Maximum 1.5 per cent, determined on 1.00 g.

Total ash (*2.4.16*)
Maximum 0.5 per cent.

STORAGE
Protected from light.

LABELLING
The label states the type of sorbitan stearate.

FUNCTIONALITY-RELATED CHARACTERISTICS
This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for sorbitan stearate used as emulsifier and co-solubiliser in creams.

Composition of fatty acids
(see Tests).

Hydroxyl value
(see Tests).

Ph Eur

Sorbitan Trioleate

(Ph. Eur. monograph 1044)

Action and use
Non-ionic surfactant.

Ph Eur

DEFINITION
Mixture usually obtained by esterification of 1 mole of sorbitol and its mono-anhydride per 3 moles of oleic ((9*Z*)-octadec-9-enoic) acid. A suitable antioxidant may be added.

CHARACTERS

Appearance
Pale yellow, light yellowish or brown solid, which becomes a viscous, oily, brownish-yellow liquid at about 25 °C.

Solubility
Practically insoluble but dispersible in water, soluble in fatty oils, slightly soluble in ethanol (96 per cent).

Relative density
About 0.98.

IDENTIFICATION

A. Hydroxyl value (see Tests).

B. Iodine value (see Tests).

C. Composition of fatty acids (see Tests).

Margaric acid Maximum 0.2 per cent for oleic acid of vegetable origin and maximum 4.0 per cent for oleic acid of animal origin.

TESTS

Acid value (*2.5.1*)
Maximum 16.0, determined on 5.0 g.

Hydroxyl value (*2.5.3, Method A*)
55 to 75.

Iodine value (*2.5.4*)
76 to 90.

Peroxide value (*2.5.5*)
Maximum 10.0.

Saponification value (*2.5.6*)
170 to 190.

Carry out the saponification for 1 h.

Composition of fatty acids
Gas chromatography (*2.4.22, Method C*).

Composition of the fatty acid fraction of the substance:
— *myristic acid*: maximum 5.0 per cent;
— *palmitic acid*: maximum 16.0 per cent;
— *palmitoleic acid*: maximum 8.0 per cent;
— *stearic acid*: maximum 6.0 per cent;
— *oleic acid*: 65.0 per cent to 88.0 per cent;
— *linoleic acid*: maximum 18.0 per cent;
— *linolenic acid*: maximum 4.0 per cent;
— *fatty acids with chain length greater than C_{18}*: maximum 4.0 per cent.

Water (*2.5.12*)
Maximum 1.5 per cent, determined on 1.00 g.

Total ash (*2.4.16*)
Maximum 0.5 per cent, determined on 1.5 g.

STORAGE
Protected from light.

LABELLING
The label states the origin of the oleic acid used (animal or vegetable).

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for sorbitan trioleate used as emulsifier and co-solubiliser in creams.

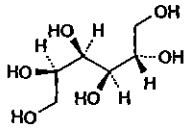
Composition of fatty acids
(see Tests).

Hydroxyl value
(see Tests).

Ph Eur

Sorbitol

(Ph. Eur. monograph 0435)



$C_6H_{14}O_6$ 182.2 *50-70-4*

Action and use
Used for parenteral nutrition.

Ph Eur

DEFINITION
D-Glucitol (D-sorbitol).

Content
97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Very soluble in water, practically insoluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification: A.

Second identification: B, C, D.

A. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

B. Dissolve 0.5 g with heating in a mixture of 0.5 mL of *pyridine R* and 5 mL of *acetic anhydride R*. After 10 min, pour the solution into 25 mL of *water R* and allow to stand in iced water for 2 h. The precipitate, recrystallised from a small volume of *ethanol (96 per cent) R* and dried *in vacuo*, melts (*2.2.14*) at 98 °C to 104 °C.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 25 mg of the substance to be examined in *water R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dissolve 25 mg of *sorbitol CRS* in *water R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 25 mg of *mannitol CRS* and 25 mg of *sorbitol CRS* in *water R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *water R, ethyl acetate R, propanol R* (10:20:70 *V/V/V*).

Application 2 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *4-aminobenzoic acid solution R*; dry in a current of cold air until the acetone is removed; heat at 100 °C for 15 min; allow to cool and spray with a 2 g/L solution of *sodium periodate R*; dry in a current of cold air; heat at 100 °C for 15 min.

System suitability Reference solution (b):
— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Specific optical rotation (*2.2.7*): + 4.0 to + 7.0 (anhydrous substance).

Dissolve 5.00 g of the substance to be examined and 6.4 g of *disodium tetraborate R* in 40 mL of *water R*. Allow to stand for 1 h, shaking occasionally, and dilute to 50.0 mL with *water R*. Filter if necessary.

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 5 g in *water R* and dilute to 50 mL with the same solvent.

Conductivity (*2.2.38*)
Maximum 20 $\mu S \cdot cm^{-1}$.

Dissolve 20.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100.0 mL with the same solvent. Measure the conductivity of the solution while gently stirring with a magnetic stirrer.

Reducing sugars
Maximum 0.2 per cent, expressed as glucose equivalent.

Dissolve 5.0 g in 6 mL of *water R* with the aid of gentle heat. Cool and add 20 mL of *cupri-citric solution R* and a few glass beads. Heat so that boiling begins after 4 min and maintain boiling for 3 min. Cool rapidly and add 100 mL of a 2.4 per cent *V/V* solution of *glacial acetic acid R* and 20.0 mL of *0.025 M iodine*. With continuous shaking, add 25 mL of a mixture of 6 volumes of *hydrochloric acid R* and 94 volumes of *water R* and, when the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulfate* using 1 mL of *starch solution R*, added towards the end of the titration, as indicator. Not less than 12.8 mL of *0.05 M sodium thiosulfate* is required.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 5.0 g of the substance to be examined in 20 mL of *water R* and dilute to 100.0 mL with the same solvent.

Reference solution (a) Dissolve 0.50 g of *sorbitol CRS* in 2 mL of *water R* and dilute to 10.0 mL with the same solvent.

Reference solution (b) Dilute 2.0 mL of the test solution to 100.0 mL with *water R*.

Reference solution (c) Dilute 5.0 mL of reference solution (b) to 100.0 mL with *water R*.

Reference solution (d) Dissolve 0.5 g of *sorbitol R* and 0.5 g of *mannitol R* (impurity A) in 5 mL of *water R* and dilute to 10 mL with the same solvent.

Column:
— *size*: l = 0.3 m, Ø = 7.8 mm;
— *stationary phase*: *strong cation-exchange resin (calcium form) R* (9 µm);
— *temperature*: 85 ± 1 °C.

Mobile phase Degassed *water for chromatography R*.

Flow rate 0.5 mL/min.

Detection Differential refractometer maintained at a constant temperature (e.g. 35 °C).

Injection 20 µL of the test solution and reference solutions (b), (c) and (d).

Run time Twice the retention time of sorbitol.

Relative retention With reference to sorbitol (retention time = about 27 min): impurity C = about 0.6; impurity A = about 0.8; impurity B = about 1.1.

System suitability Reference solution (d):
— *resolution*: minimum 2.0 between the peaks due to impurity A and sorbitol.

Limits:
— *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);
— *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent);
— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Water (*2.5.12*)
Maximum 1.5 per cent, determined on 1.00 g. Use a mixture of 1 volume of *formamide R1* and 2 volumes of *anhydrous methanol R* as solvent.

Microbial contamination
If intended for use in the manufacture of parenteral preparations:
— TAMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

If not intended for use in the manufacture of parenteral preparations:
— TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*);
— TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*);
— absence of *Escherichia coli* (*2.6.13*);
— absence of *Salmonella* (*2.6.13*).

Bacterial endotoxins (*2.6.14*)
If intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins:
— less than 4 IU/g for parenteral preparations having a concentration of less than 100 g/L of sorbitol;
— less than 2.5 IU/g for parenteral preparations having a concentration of 100 g/L or more of sorbitol.

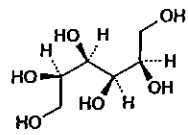
ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

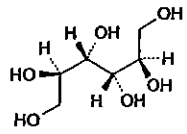
Calculate the percentage content of $C_6H_{14}O_6$ taking into account the assigned content of *sorbitol CRS*.

LABELLING
The label states:
— where applicable, the maximum concentration of bacterial endotoxins;
— where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

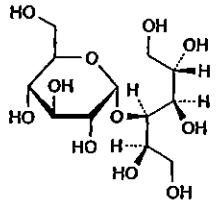
IMPURITIES



A. D-mannitol,



B. D-iditol,



C. 4-*O*-α-D-glucopyranosyl-D-glucitol (D-maltitol).

FUNCTIONALITY-RELATED CHARACTERISTICS
This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for sorbitol used as filler and binder in tablets.

Particle-size distribution (*2.9.31* or *2.9.38*)

Powder flow (*2.9.36*)

Partially Dehydrated Liquid Sorbitol

(Sorbitol, Liquid, Partially Dehydrated, Ph. Eur. monograph 2048)

Action and use
Excipient.

Ph Eur

DEFINITION
Partially dehydrated liquid sorbitol is obtained by acid-catalysed partial internal dehydration of liquid sorbitol. It contains not less than 68.0 per cent *m/m* and not more than 85.0 per cent *m/m* of anhydrous substances, composed of a mixture of mainly D-sorbitol and 1,4-sorbitan, with mannitol, hydrogenated oligo- and disaccharides, and sorbitans.

Content
(nominal value):
— 1,4-sorbitan ($C_6H_{12}O_5$): minimum 15.0 per cent (anhydrous substance);
— D-sorbitol ($C_6H_{14}O_6$): minimum 25.0 per cent (anhydrous substance).

The contents of 1,4-sorbitan and D-sorbitol are within 95.0 per cent to 105.0 per cent of the nominal values.

CHARACTERS
Appearance
Clear, colourless, syrupy liquid.

Solubility
Miscible with water, practically insoluble in mineral oils and vegetable oils.

IDENTIFICATION
Examine the chromatograms obtained in the assay.

Results The 2 principal peaks in the chromatogram obtained with the test solution are similar in retention time and size to the peaks in the chromatogram obtained with reference solution (a).

TESTS
Solution S
Dilute the substance to be examined with *carbon dioxide-free water R* prepared from *distilled water R* to obtain a solution containing 50.0 per cent *m/m* of anhydrous substance.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Conductivity (*2.2.38*)
Maximum 20 $\mu S \cdot cm^{-1}$.

Measure the conductivity of solution S, while gently stirring with a magnetic stirrer.

Reducing sugars
Maximum 0.3 per cent, calculated as glucose (anhydrous substance).

To an amount of the substance to be examined equivalent to 3.3 g of anhydrous substance, add 3 mL of *water R*, 20.0 mL of *cupri-citric solution R* and a few glass beads. Heat so that boiling begins after 4 min. Maintain boiling for 3 min. Cool rapidly and add 100 mL of a 2.4 per cent *V/V* solution of *glacial acetic acid R* and 20.0 mL of *0.025 M iodine*. With continuous shaking, add 25 mL of a mixture of 6 mL of *hydrochloric acid R* and 94 mL of *water R*. When the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulfate* using 2 mL of *starch solution R*, added towards the end of the titration, as indicator. Not less than 12.8 mL of *0.05 M sodium thiosulfate* is required.

Water (*2.5.12*)
15.0 per cent to 32.0 per cent, determined on 0.100 g.

Microbial contamination
TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*).
TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).
Absence of *Escherichia coli* (*2.6.13*).
Absence of *Salmonella* (*2.6.13*).

ASSAY
Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.400 g of the substance to be examined in *water R* and dilute to 20.0 mL with the same solvent.

Reference solution (a) Dissolve 50.0 mg of *sorbitol CRS* and 20.0 mg of *1,4-sorbitan CRS* in *water R* and dilute to 5.0 mL with the same solvent.

Reference solution (b) Dissolve 0.100 g of *mannitol R* and 0.100 g of *sorbitol R* in *water R* and dilute to 10 mL with the same solvent.

Column:
— *size*: *l* = 0.3 m, Ø = 7.8 mm;
— *stationary phase*: *strong cation-exchange resin (calcium form) R* (9 µm);
— *temperature*: 80 ± 5 °C.

Mobile phase Degassed *water for chromatography R*.

Flow rate 0.5 mL/min.

Detection Differential refractometer maintained at a constant temperature (e.g. 30-35 °C).

Injection 40 µL.

Relative retention With reference to D-sorbitol (retention time = about 25 min): 1,4-sorbitan = about 0.5; mannitol = about 0.8.

System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the peaks due to mannitol and D-sorbitol.

Calculate the percentage contents of 1,4-sorbitan ($C_6H_{12}O_5$) and D-sorbitol ($C_6H_{14}O_6$) taking into account the assigned contents of *1,4-sorbitan CRS* and *sorbitol CRS*.

LABELLING
The label states the content of D-sorbitol and the content of 1,4-sorbitan (= nominal values).

Ph Eur

Liquid Sorbitol (Crystallising)

Sorbitol Solution (70 per cent) (Crystallising)
(Ph. Eur. monograph 0436)

Action and use
Excipient.

Ph Eur

DEFINITION
Aqueous solution of a hydrogenated, partly hydrolysed starch.

Content
— anhydrous substance: 68.0 per cent *m/m* to 72.0 per cent *m/m*,

— D-glucitol (D-sorbitol, $C_6H_{14}O_6$): 92.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Clear, colourless, syrupy liquid, miscible with water.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

B. To 7.0 g add 40 mL of *water R* and 6.4 g of *disodium tetraborate R*, allow to stand for 1 h, shaking occasionally, and dilute to 50.0 mL with *water R*. Filter if necessary. The angle of rotation (*2.2.7*) is 0° to + 1.5°.

C. It is a clear, syrupy liquid at a temperature of 25 °C.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dilute 7.0 g to 50 mL with *water R*.

Conductivity (*2.2.38*)

Maximum 10 $\mu S \cdot cm^{-1}$ measured on the undiluted liquid sorbitol (crystallising) while gently stirring with a magnetic stirrer.

Reducing sugars

Maximum 0.2 per cent calculated as glucose equivalent.

To 5.0 g add 6 mL of *water R*, 20 mL of *cupri-citric solution R* and a few glass beads. Heat so that boiling begins after 4 min and maintain boiling for 3 min. Cool rapidly and add 100 mL of a 2.4 per cent *V/V* solution of *glacial acetic acid R* and 20.0 mL of *0.025 M iodine*. With continuous shaking, add 25 mL of a mixture of 6 volumes of *hydrochloric acid R* and 94 volumes of *water R* and, when the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulfate* using 1 mL of *starch solution R*, added towards the end of the titration, as indicator. Not less than 12.8 mL of *0.05 M sodium thiosulfate* is required.

Water (*2.5.12*)

28.0 per cent to 32.0 per cent *m/m*, determined on 0.100 g.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution Mix 1.00 g of the substance to be examined with 20 mL of *water R* and dilute to 50.0 mL with the same solvent.

Reference solution (a) Dissolve 65.0 mg of *sorbitol CRS* in 2 mL of *water R* and dilute to 5.0 mL with the same solvent.

Reference solution (b) Dissolve 65 mg of *mannitol R* and 65 mg of *sorbitol R* in 2 mL of *water R* and dilute to 5 mL with the same solvent.

Column:
— *size*: l = 0.3 m, Ø = 7.8 mm,
— *stationary phase*: *strong cation-exchange resin (calcium form) R* (9 µm),
— *temperature*: 85 ± 1 °C.

Mobile phase Degassed *water for chromatography R*.

Flow rate 0.5 mL/min.

Detection Differential refractometer maintained at a constant temperature (e.g. 35 °C).

Injection 20 µL.

Run time Twice the retention time of sorbitol.

Relative retention With reference to sorbitol (retention time = about 27 min): mannitol = about 0.8.

System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the peaks due to mannitol and to sorbitol.

Calculate the percentage content of $C_6H_{14}O_6$ taking into account the assigned content of *sorbitol CRS*.

Ph Eur

Liquid Sorbitol (Non-crystallising)

Sorbitol Solution (70 per cent) (Non-crystallising)

(Ph. Eur. monograph 0437)

Action and use

Excipient.

Ph Eur

DEFINITION

Aqueous solution of a hydrogenated, partly hydrolysed starch.

Content

— anhydrous substance: 68.0 per cent *m/m* to 72.0 per cent *m/m*,
— D-glucitol (D-sorbitol, $C_6H_{14}O_6$): 72.0 per cent to 92.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Clear, colourless, syrupy liquid, miscible with water.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

B. To 7.0 g add 40 mL of *water R* and 6.4 g of *disodium tetraborate R*. Allow to stand for 1 h, shaking occasionally, and dilute to 50.0 mL with *water R*. Filter if necessary. The angle of rotation (*2.2.7*) is + 1.5° to + 3.5°.

C. It is a clear, syrupy liquid at 25 °C.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dilute 7.0 g to 50 mL with *water R*.

Conductivity (*2.2.38*)

Maximum 10 $\mu S \cdot cm^{-1}$ measured on the undiluted liquid sorbitol (non crystallising) while gently stirring with a magnetic stirrer.

Reducing sugars

Maximum 0.2 per cent calculated as glucose equivalent.

To 5.0 g add 6 mL of *water R*, 20 mL of *cupri-citric solution R* and a few glass beads. Heat so that boiling begins after 4 min and maintain boiling for 3 min. Cool rapidly and add 100 mL of a 2.4 per cent *V/V* solution of *glacial acetic acid R* and 20.0 mL of *0.025 M iodine*. With continuous shaking, add 25 mL of a mixture of 6 volumes of *hydrochloric acid R* and 94 volumes of *water R* and, when the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulfate* using 1 mL of *starch solution R*, added towards the

end of the titration, as indicator. Not less than 12.8 mL of *0.05 M sodium thiosulfate* is required.

Reducing sugars after hydrolysis
Maximum 9.3 per cent calculated as glucose equivalent.

To 6.0 g add 35 mL of *water R*, 40 mL of *1 M hydrochloric acid* and a few glass beads. Boil under a reflux condenser for 4 h. Cool and neutralise with *dilute sodium hydroxide solution R* using 0.2 mL of *bromothymol blue solution R1* as indicator. Cool and dilute to 100.0 mL with *water R*.
To 3.0 mL of the solution add 5 mL of *water R*, 20 mL of *cupri-citric solution R* and a few glass beads. Heat so that boiling begins after 4 min and maintain boiling for 3 min. Cool rapidly and add 100 mL of a 2.4 per cent *V/V* solution of *glacial acetic acid R* and 20.0 mL of *0.025 M iodine*. With continuous shaking, add 25 mL of a mixture of 6 volumes of *hydrochloric acid R* and 94 volumes of *water R*. When the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulfate* using 1 mL of *starch solution R*, added towards the end of the titration, as indicator. Not less than 8.0 mL of *0.05 M sodium thiosulfate* is required.

Water (*2.5.12*)
28.0 per cent to 32.0 per cent *m/m*, determined on 0.100 g.

ASSAY
Liquid chromatography (*2.2.29*).

Test solution Mix 1.00 g of the substance to be examined with 20 mL of *water R* and dilute to 50.0 mL with the same solvent.

Reference solution (a) Dissolve 55.0 mg of *sorbitol CRS* in 2 mL of *water R* and dilute to 5.0 mL with the same solvent.

Reference solution (b) Dissolve 55 mg of *mannitol R* and 55 mg of *sorbitol R* in 2 mL of *water R* and dilute to 5 mL with the same solvent.

Column:
— *size*: *l* = 0.3 m, Ø = 7.8 mm,
— *stationary phase*: *strong cation-exchange resin (calcium form) R* (9 µm),
— *temperature*: 85 ± 1 °C.

Mobile phase Degassed *water for chromatography R*.

Flow rate 0.5 mL/min.

Detection Differential refractometer maintained at a constant temperature (e.g. 35 °C).

Injection 20 µL.

Run time Twice the retention time of sorbitol.

Relative retention With reference to sorbitol (retention time = about 27 min): mannitol = about 0.8.

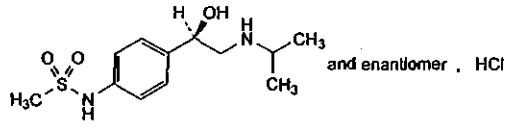
System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the peaks due to mannitol and to sorbitol.

Calculate the percentage content of $C_6H_{14}O_6$ taking into account the assigned content of *sorbitol CRS*.

Ph Eur

Sotalol Hydrochloride

(Ph. Eur. monograph 2004)



$C_{12}H_{21}ClN_2O_3S$ 308.8 *959-24-0*

Action and use
Beta-adrenoceptor antagonist; class II and class III antiarrhythmic.

Ph Eur

DEFINITION
N-[4-[(1*RS*)-1-Hydroxy-2-[(propan-2-yl)amino]ethyl]phenyl] methanesulfonamide hydrochloride.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white powder.

Solubility
Freely soluble in water, soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *sotalol hydrochloride CRS*.
B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS
Solution S
Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50.0 mL with the same solvent.

Appearance of solution
Solution S is not more opalescent than reference suspension III (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

pH (*2.2.3*)
4.0 to 5.0.

Dilute 5.0 mL of solution S to 10.0 mL with *carbon dioxide-free water R*.

Optical rotation (*2.2.7*)
−0.10° to + 0.10°.

Dilute 25.0 mL of solution S to 50.0 mL with *water R*.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 3.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 8.0 mg of *sotalol impurity B CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (c) Dilute 1.0 mL of reference solution (b) to 100.0 mL with the mobile phase.

Reference solution (d) Dilute 1.5 mL of reference solution (b) to 100 mL with the mobile phase. To 1 mL of this solution add 1 mL of reference solution (a).

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 2 g of *sodium octanesulfonate R* in 790 mL of *water for chromatography R*, adjust to pH 3.0 with *phosphoric acid R* and add 210 mL of *acetonitrile for chromatography R*.

Flow rate 1 mL/min.

Detection Spectrophotometer at 228 nm.

Injection 10 µL of the test solution and reference solutions (a), (c) and (d).

Run time 2.5 times the retention time of sotalol.

System suitability Reference solution (d):
— *resolution*: minimum 4.0 between the peaks due to sotalol and impurity B.

Limits:
— *impurity B*: not more than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent);
— *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent), and not more than 1 such peak has an area greater than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *total of other impurities*: not more than 1.65 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *disregard limit*: 0.17 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

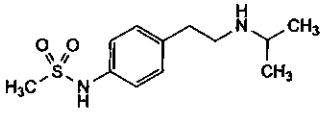
In order to avoid overheating during the titration, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.

Dissolve 0.250 g in 10 mL of *anhydrous formic acid R*, using sonication if necessary. Add 40 mL of *acetic anhydride R* and titrate immediately with *0.1 M perchloric acid*. Determine the end-point potentiometrically (*2.2.20*).

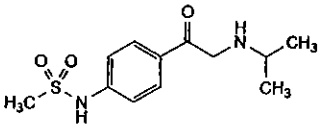
1 mL of *0.1 M perchloric acid* is equivalent to 30.88 mg of $C_{12}H_{21}ClN_2O_3S$.

STORAGE
Protected from light.

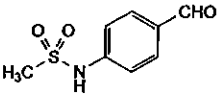
IMPURITIES



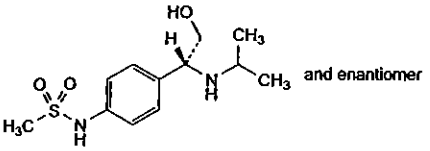
A. *N*-[4-[2-[(propan-2-yl)amino]ethyl]phenyl] methanesulfonamide,



B. *N*-[4-[[(propan-2-yl)amino]acetyl]phenyl] methanesulfonamide,



C. *N*-(4-formylphenyl)methanesulfonamide,



D. *N*-[4-[(1*RS*)-2-hydroxy-1-[(propan-2-yl)amino]ethyl] phenyl]methanesulfonamide.

Ph Eur

Hydrogenated Soya Oil

Hydrogenated Soyabean Oil
(Hydrogenated Soya-bean Oil, Ph. Eur. monograph 1265)

Action and use
Excipient.

Ph Eur

DEFINITION
Product obtained by refining, bleaching, hydrogenation and deodorisation of oil obtained from seeds of *Glycine max* (L.) Merr. (*G. hispida* (Moench) Maxim.). The product consists mainly of triglycerides of palmitic and stearic acids.

CHARACTERS

Appearance
White or almost white mass or powder which melts to a clear, pale yellow liquid when heated.

Solubility
Practically insoluble in water, freely soluble in methylene chloride, in light petroleum (bp: 65-70 °C) after heating and in toluene, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION
A. Melting point (see Tests).
B. Composition of fatty acids (see Tests).

TESTS

Melting point (*2.2.15*)
66 °C to 72 °C.

Acid value (*2.5.1*)
Maximum 0.5.

Dissolve 10.0 g in 50 mL of a hot mixture of equal volumes of *ethanol (96 per cent) R* and *toluene R*, previously neutralised with *0.1 M potassium hydroxide* using 0.5 mL of *phenolphthalein solution R1* as indicator. Titrate the solution immediately while still hot.

Peroxide value (*2.5.5, Method A*)
Maximum 5.0.

Unsaponifiable matter (*2.5.7*)
Maximum 1.0 per cent, determined on 5.0 g.

Alkaline impurities (*2.4.19*)
Dissolve 2.0 g with gentle heating in a mixture of 1.5 mL of *ethanol (96 per cent) R* and 3 mL of *toluene R*. Add 0.05 mL of a 0.4 g/L solution of *bromophenol blue R* in *ethanol (96 per cent) R*. Not more than 0.4 mL of *0.01 M hydrochloric acid* is required to change the colour to yellow.

Composition of fatty acids
Gas chromatography (*2.4.22, Method A*) with the following modifications. Use the mixture of calibrating substances in Table 2.4.22.-3.

Column:
— *material*: fused silica;
— *size*: l = 25 m, Ø = 0.25 mm;
— *stationary phase*: *cyanopropylpolysiloxane R* (film thickness 0.2 µm).

Carrier gas *helium for chromatography R.*

Flow rate 0.65 mL/min.

Split ratio 1:100.

Temperature:
— *column*: 180 °C for 20 min;
— *injection port and detector*: 250 °C.

Detection Flame ionisation.

Composition of the fatty-acid fraction of the substance:
— *saturated fatty acids of chain length less than C_{14}*: maximum 0.1 per cent;
— *myristic acid*: maximum 0.5 per cent;
— *palmitic acid*: 9.0 per cent to 16.0 per cent;
— *stearic acid*: 79.0 per cent to 89.0 per cent;
— *oleic acid and isomers*: maximum 4.0 per cent;
— *linoleic acid and isomers*: maximum 1.0 per cent;
— *linolenic acid and isomers*: maximum 0.2 per cent;
— *arachidic acid*: maximum 1.0 per cent;
— *behenic acid*: maximum 1.0 per cent.

STORAGE
Protected from light.

Ph Eur

Refined Soya Oil

Refined Soyabean Oil

(Refined Soya-bean Oil, Ph. Eur. monograph 1473)

Action and use
Excipient.

When Soya Oil, Soyabean Oil or Soya-bean Oil is demanded, Refined Soya Oil shall be supplied.

When intended for use in the manufacture of a parenteral dosage form, Refined Soya Oil complying with the requirement for Water below should be used.

Ph Eur

DEFINITION
Fatty oil obtained from seeds of *Glycine max* (L.) Merr. (*Glycine hispida* (Moench) Maxim.) by extraction and subsequent refining. It may contain a suitable antioxidant.

PRODUCTION
The oil is prepared using materials and methods designed to ensure that the content of brassicasterol (*2.4.23*) in the sterol fraction of the oil is not greater than 0.3 per cent.

CHARACTERS

Appearance
Clear, pale yellow liquid.

Solubility
Practically insoluble in ethanol (96 per cent), miscible with light petroleum (bp: 50-70 °C).

Relative density
About 0.922.

Refractive index
About 1.475.

IDENTIFICATION
First identification: B.

Second identification: A.

A. Identification of fatty oils by thin-layer chromatography (*2.3.2*).

Results The chromatogram obtained is similar to the corresponding chromatogram shown in Figure 2.3.2.-1.

B. Composition of fatty acids (see Tests).

TESTS

Acid value (*2.5.1*)
Maximum 0.5.

Peroxide value (*2.5.5, Method A*)
Maximum 10.0, or maximum 5.0 if intended for use in the manufacture of parenteral preparations.

Unsaponifiable matter (*2.5.7*)
Maximum 1.5 per cent, determined on 5.0 g.

Alkaline impurities (*2.4.19*)
It complies with the test.

Composition of fatty acids (*2.4.22, Method A*)
Use the mixture of calibrating substances in Table 2.4.22.-3.

Composition of the fatty-acid fraction of the oil:
— *saturated fatty acids of chain length less than C_{14}*: maximum 0.1 per cent;
— *myristic acid*: maximum 0.2 per cent;
— *palmitic acid*: 9.0 per cent to 13.0 per cent;
— *palmitoleic acid*: maximum 0.3 per cent;
— *stearic acid*: 2.5 per cent to 5.0 per cent;
— *oleic acid and isomer*: 17.0 per cent to 30.0 per cent;
— *linoleic acid*: 48.0 per cent to 58.0 per cent;
— *linolenic acid*: 5.0 per cent to 11.0 per cent;
— *arachidic acid*: maximum 1.0 per cent;
— *eicosenoic acid*: maximum 1.0 per cent;
— *behenic acid*: maximum 1.0 per cent.

Water (*2.5.32*)
Maximum 0.1 per cent, determined on 1.00 g.

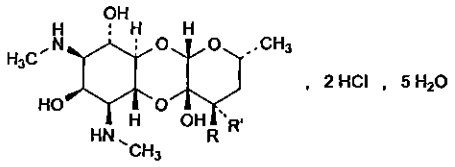
STORAGE
In a well-filled container, protected from light, at a temperature not exceeding 25 °C. If intended for use in the manufacture of parenteral preparations, store under an inert gas.

LABELLING
The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral preparations and the name of the inert gas.

Ph Eur

Spectinomycin Dihydrochloride Pentahydrate

(Ph. Eur. monograph 1152)



, 2 HCl , 5 H_2O

Compound	R	R'	Molec. Formula	M_r
spectinomycin	R + R' = O		$C_{14}H_{26}Cl_2N_2O_7,5H_2O$	495.4
(4*R*)-dihydro-spectinomycin	OH	H	$C_{14}H_{28}Cl_2N_2O_7,5H_2O$	497.4

Action and use
Aminocyclotol antibacterial.

Ph Eur

DEFINITION

Mixture of (2*R*,4a*R*,5a*R*,6*S*,7*S*,8*R*,9*S*,9a*R*,10a*S*)4a,7,9-trihydroxy-2-methyl-6,8-bis(methylamino)decahydro-4*H*-pyrano[2,3-*b*][1,4]benzodioxin-4-one dihydrochloride pentahydrate (spectinomycin dihydrochloride pentahydrate) and of (2*R*,4*R*,4a*S*,5a*R*,6*S*,7*S*,8*R*,9*S*,9a*R*,10a*S*)-2-methyl-6,8-bis(methylamino)decahydro-2*H*-pyrano[2,3-*b*][1,4]benzodioxine-4,4a,7,9-tetrol dihydrochloride pentahydrate ((4*R*)-dihydrospectinomycin dihydrochloride pentahydrate).

It is produced by *Streptomyces spectabilis* or by any other means.

Content
— *(4R)-dihydrospectinomycin dihydrochloride*: maximum 9.0 per cent (anhydrous substance);
— *sum of the contents of spectinomycin dihydrochloride and (4R)-dihydrospectinomycin dihydrochloride*: 93.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, slightly hygroscopic powder.

Solubility
Freely soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *spectinomycin hydrochloride CRS.*

B. Dilute 1.0 mL of solution S (see Tests) to 10 mL with *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S
Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 25.0 mL with the same solvent.

Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dilute 2.0 mL of solution S to 20.0 mL with *water R*.

pH (*2.2.3*)
3.8 to 5.6 for solution S.

Specific optical rotation (*2.2.7*)
+ 15.0 to + 21.0 (anhydrous substance), determined on solution S within 20 min of preparation.

Related substances
Liquid chromatography (*2.2.29*). *In order to avoid formation of anomers, prepare the solutions immediately before use.*

Test solution Dissolve 15.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dissolve 3 mg of *spectinomycin for system suitability CRS* in the mobile phase and dilute to 20 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase.

Reference solution (c) Dilute 3.0 mL of reference solution (b) to 10.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: ambient and constant.

Mobile phase Dissolve 4.2 g of *oxalic acid R* and 2.0 mL of *heptafluorobutyric acid R* in *water R* and dilute to 1000 mL with *water R*; adjust to pH 3.2 with *sodium hydroxide solution R*, add 105 mL of *acetonitrile R* and mix; filter through a 0.45 µm filter and degas with *helium for chromatography R* for 10 min.

Flow rate 1.0 mL/min.

Post-column solution *carbonate-free sodium hydroxide solution R* diluted with *carbon dioxide-free water R* to obtain a final concentration of NaOH of 21 g/L. Degas the solution with *helium for chromatography R* for 10 min before use. Add it pulse-less to the column effluent using a 375 µL polymeric mixing coil.

Post-column flow rate 0.5 mL/min.

Detection Pulsed amperometric detection or equivalent with a gold indicator electrode having preferably a diameter of 1.4 mm or greater, a suitable reference electrode and a stainless steel counter electrode, held at + 0.12 V detection, + 0.70 V oxidation and −0.60 V reduction potentials respectively, with pulse durations according to the instrument used. Keep the detection cell at ambient and constant temperature. Clean the gold indicator electrode with an eraser and damp precision wipe prior to start-up of the system to enhance the detector sensitivity and increase the signal-to-noise ratio.

Injection 20 µL.

Run time 1.5 times the retention time of spectinomycin.

Identification of impurities Use the chromatogram supplied with *spectinomycin for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, D and E.

Relative retention With reference to spectinomycin (retention time = 11 min to 20 min): impurity A = about 0.5; impurity F = about 0.53; impurity G = about 0.6; impurity D = about 0.7; impurity E = about 0.9; (4*R*)-dihydrospectinomycin = about 1.3; impurity C = about 1.4.

System suitability Reference solution (a):
— *resolution*: minimum 1.5 between the peaks due to impurity E and spectinomycin.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity A by 0.4;

— *impurities A, C, F, G*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
— *impurities D, E*: for each impurity, not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (4.0 per cent);
— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
— *total*: not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (6.0 per cent);
— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent); disregard the peak due to (4*R*)-dihydrospectinomycin.

Water (*2.5.12*)
16.0 per cent to 20.0 per cent, determined on 0.100 g.

Sulfated ash (*2.4.14*)
Maximum 1.0 per cent, determined on 1.0 g.

Bacterial endotoxins (*2.6.14*)
Less than 0.09 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins. Prepare the solutions using a 0.42 per cent *m/m* solution of *sodium hydrogen carbonate R*.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Test solution Dissolve 40.0 mg of the substance to be examined in *water R* and dilute to 50.0 mL with the same solvent. Allow to stand for not less than 15 h and not more than 72 h (formation of anomers). Dilute 5.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution Dissolve 40.0 mg of *spectinomycin hydrochloride CRS* (containing (4*R*)-dihydrospectinomycin) in *water R* and dilute to 50.0 mL with the same solvent. Allow to stand for the same period of time as the test solution (formation of anomers). Dilute 5.0 mL of this solution to 50.0 mL with the mobile phase.

System suitability:
— *repeatability*: maximum relative standard deviation of 3.0 per cent for the principal peak after 6 injections of the reference solution.

Calculate the sum of the percentage contents of spectinomycin dihydrochloride and (4*R*)-dihydrospectinomycin dihydrochloride from the declared contents of $C_{14}H_{26}Cl_2N_2O_7$ and $C_{14}H_{28}Cl_2N_2O_7$ in *spectinomycin hydrochloride CRS*.

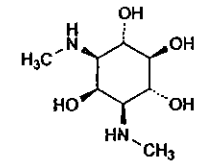
STORAGE

In an airtight container. If the substance is sterile, store in a sterile, airtight, tamper-evident container.

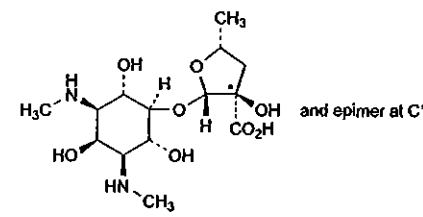
IMPURITIES

Specified impurities *A, C, D, E, F, G.*

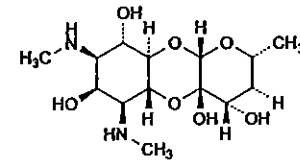
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B.*



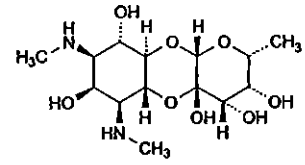
A. 1,3-dideoxy-1,3-bis(methylamino)-*myo*-inositol (actinamine),



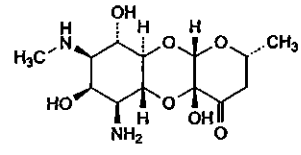
B. (2*S*,3*RS*,5*R*)-3-hydroxy-5-methyl-2-[[(1*r*,2*R*,3*S*,4*r*,5*R*,6*S*)-2,4,6-trihydroxy-3,5-bis(methylamino)cyclohexyl] oxy]tetrahydrofuran-3-carboxylic acid (actinospectinoic acid),



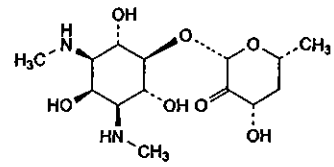
C. (2*R*,4*S*,4a*S*,5a*R*,6*S*,7*S*,8*R*,9*S*,9a*R*,10a*S*)-2-methyl-6,8-bis (methylamino)decahydro-2*H*-pyrano[2,3-*b*][1,4] benzodioxine-4,4a,7,9-tetrol ((4*S*)-dihydrospectinomycin),



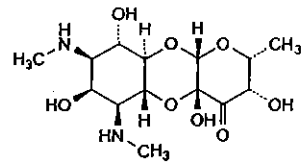
D. (2*R*,3*R*,4*S*,4a*S*,5a*R*,6*S*,7*S*,8*R*,9*S*,9a*R*,10a*S*)-2-methyl-6,8-bis(methylamino)decahydro-2*H*-pyrano[2,3-*b*][1,4] benzodioxine-3,4,4a,7,9-pentol (dihydroxyspectinomycin),



E. (2*R*,4a*R*,5a*R*,6*S*,7*R*,8*R*,9*S*,9a*R*,10a*S*)-6-amino-4a,7,9-trihydroxy-2-methyl-8-(methylamino)decahydro-4*H*-pyrano[2,3-*b*][1,4]benzodioxin-4-one (*N*-desmethylspectinomycin),



F. (2*S*,4*S*,6*R*)-4-hydroxy-6-methyl-2-[[(1*r*,2*R*,3*S*,4*r*,5*R*,6*S*)-2,4,6-trihydroxy-3,5-bis(methylamino)cyclohexyl] oxy]dihydro-2*H*-pyran-3(4*H*)-one (triol spectinomycin),

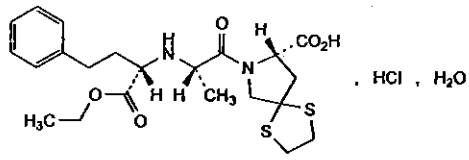


G. (2*R*,3*S*,4a*R*,5a*R*,6*S*,7*S*,8*R*,9*S*,9a*R*,10a*S*)-3,4a,7,9-tetrahydroxy-2-methyl-6,8-bis(methylamino)decahydro-4*H*-pyrano[2,3-*b*][1,4]benzodioxin-4-one (tetrahydroxyspectinomycin).

Ph Eur

Spirapril Hydrochloride Monohydrate

(Ph. Eur. monograph 1766)



$C_{22}H_{31}ClN_2O_5S_2,H_2O$ 521.1

Action and use
Angiotensin-converting enzyme inhibitor.

Ph Eur

DEFINITION
(8*S*)-7-[(2*S*)-2-[[(1*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]propanoyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid hydrochloride monohydrate.

Content
97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, fine crystalline powder.

Solubility
Very slightly soluble in water, soluble in methanol, slightly soluble in acetonitrile, practically insoluble in methylene chloride.

IDENTIFICATION
A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *spirapril hydrochloride monohydrate CRS.*

C. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Specific optical rotation (*2.2.7*)
−13.0 to −11.0 (anhydrous substance).

Dissolve 0.200 g in *dimethylformamide R* and dilute to 20.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R1, water R* (20:80 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 20.0 mL with the solvent mixture.

Reference solution (a) Dissolve 6 mg of *spirapril for system suitability CRS* (containing impurities B and D) in the solvent mixture and dilute to 20 mL with the solvent mixture.

Reference solution (b) Dilute 5.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 5.0 mL of this solution to 50.0 mL with the solvent mixture.

Reference solution (c) Dilute 1.0 mL of reference solution (b) to 10.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.125 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 70 °C.

Mobile phase:
— *mobile phase A*: dissolve 4.5 g of *tetramethylammonium hydroxide R* in 900 mL of *water R*, add 100 mL of *acetonitrile R1* and adjust to pH 2.2 with *phosphoric acid R*;
— *mobile phase B*: dissolve 4.5 g of *tetramethylammonium hydroxide R* in 400 mL of *water R*, add 600 mL of *acetonitrile R1* and adjust to pH 2.2 with *phosphoric acid R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 4	90	10
4 - 14	90 → 10	10 → 90
14 - 20	10	90

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µL.

Relative retention With reference to spirapril (retention time = about 10 min): impurity C = about 0.6; impurity B = about 0.7; impurity A = about 1.26; impurity D = about 1.38.

System suitability Reference solution (a):
— *resolution*: minimum 3.5 between the peaks due to impurity B and spirapril, and minimum 5.5 between the peaks due to spirapril and impurity D.

Limits:
— *impurity D*: not more than 0.8 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent);
— *impurity B*: not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
— *impurities A, C*: for each impurity, not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
— *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
— *disregard limit*: area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Water (*2.5.12*)
3.0 per cent to 4.0 per cent, determined on 0.200 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*).

Solvent mixture Mix equal volumes of *acetonitrile R1* and *water R*.

Test solution Dissolve 20.0 mg of the substance to be examined in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Reference solution (a) Dissolve 20.0 mg of *spirapril hydrochloride monohydrate CRS* in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Reference solution (b) Dissolve 6.0 mg of *spirapril for system suitability CRS* (spirapril spiked with impurity B and impurity D) in a mixture of 2 volumes of *acetonitrile R* and 8 volumes of *water R* and dilute to 20 mL with the same mixture of solvents.

Solution A Dissolve 4.5 g of *tetramethylammonium hydroxide R* in 900 mL of *water R*, adjust to pH 1.75 with *phosphoric acid R* and add 100 mL of *acetonitrile R1*.

Solution B Dissolve 4.5 g of *tetramethylammonium hydroxide R* in 400 mL of *water R*, adjust to pH 1.75 with *phosphoric acid R* and add 600 mL of *acetonitrile R1*.

Column:
- *size*: *l* = 0.125 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 70 °C.

Mobile phase Solution A, solution B (45:55 *V/V*).

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µL.

Retention time Spirapril = 1.6 min to 2.9 min; impurity D = about 13 min. Adjust the proportion of solution B in the mobile phase if necessary.

System suitability Reference solution (b):
- *resolution*: minimum 15 between the peaks due to spirapril and impurity D.

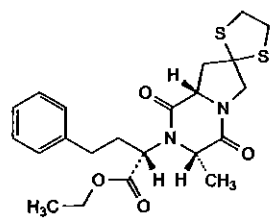
Calculate the percentage content of $C_{22}H_{31}ClN_2O_5S_2$ using the chromatogram obtained with reference solution (a) and taking into account the assigned content of *spirapril hydrochloride monohydrate CRS*.

STORAGE

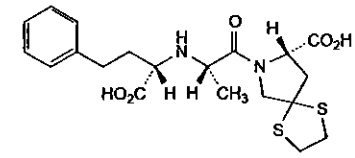
In an airtight container, protected from light.

IMPURITIES

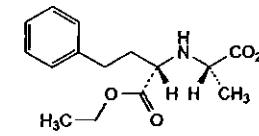
Specified impurities *A, B, C, D.*



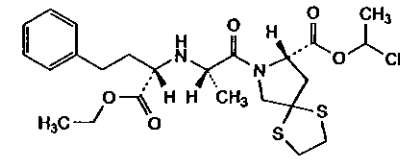
A. ethyl (2*S*)-2-[(3′*S*,8′a*S*)-3′-methyl-1′,4′-dioxohexahydrospiro[1,3-dithiolane-2,7′(6′*H*)-pyrrolo[1,2-*a*]pyrazin]-2′-yl]-4-phenylbutanoate,



B. (8*S*)-7-[(2*S*)-2-[[(1*S*)-1-carboxy-3-phenylpropyl]amino] propanoyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid (spiraprilat),



C. (2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino] propanoic acid,

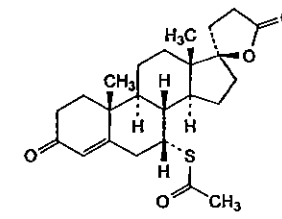


D. 1-methylethyl (8*S*)-7-[(2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]propanoyl]-1,4-dithia-7-azaspiro[4.4] nonane-8-carboxylate.

Ph Eur

Spironolactone

(Ph. Eur. monograph 0688)



$C_{24}H_{32}O_4S$ 416.6 *52-01-7*

Action and use

Aldosterone receptor antagonist; potassium-sparing diuretic.

Preparations

Spironolactone Oral Suspension

Spironolactone Tablets

Ph Eur

DEFINITION

S-[(2′*R*)-3,5′-Dioxo-3′,4′-dihydro-5′*H*-spiro[androst-4-ene-17,2′-furan]-7α-yl] ethanethioate.

Content

97.5 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish-white powder.

Solubility
Practically insoluble in water, soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification: A.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *spironolactone CRS.*

If the spectra obtained in the solid state shows differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *methanol R*, evaporate to dryness and record new spectra using the residues.

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 20 mg of the substance to be examined in *methylene chloride R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 20 mg of *spironolactone CRS* in *methylene chloride R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *water R, cyclohexane R, ethyl acetate R* (1:24:75 *V/V/V*).

Application 5 µL.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. To about 10 mg add 2 mL of a 50 per cent *V/V* solution of *sulfuric acid R* and shake. An orange solution with an intense yellowish-green fluorescence is produced. Heat the solution gently; the colour becomes deep red and hydrogen sulfide, which blackens *lead acetate paper R*, is evolved. Add the solution to 10 mL of *water R*; a greenish-yellow solution is produced, showing opalescence or a precipitate.

TESTS

Specific optical rotation (*2.2.7*)
−46 to −41 (dried substance).

Dissolve 0.100 g in *ethanol (96 per cent) R* and dilute to 10.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Solvent mixture *acetonitrile R, water R* (50:50 *V/V*).

Test solution (a) Dissolve 50.0 mg of the substance to be examined in 2.5 mL of *tetrahydrofuran R* and dilute to 25.0 mL with the solvent mixture.

Test solution (b) Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of test solution (b) to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve with the aid of ultrasound the contents of a vial of *spironolactone for system suitability CRS* (containing impurities A, C, D, E and I) in 1.0 mL of the solvent mixture.

Reference solution (c) Dissolve 50.0 mg of *spironolactone CRS* in 2.5 mL of *tetrahydrofuran R* and dilute to 25.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 100.0 mL with the solvent mixture.

Reference solution (d) Dissolve 5.0 mg of *canrenone CRS* (impurity F) in 2.5 mL of *tetrahydrofuran R* and dilute to 25.0 mL with the solvent mixture. Dilute 3.0 mL of this solution to 100.0 mL with the solvent mixture.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm);
- *temperature*: 40 °C.

Mobile phase *acetonitrile R, tetrahydrofuran R, methanol R1, water for chromatography R* (15:20:425:540 *V/V/V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL of test solution (a) and reference solutions (a), (b) and (d).

Run time 2.5 times the retention time of spironolactone.

Identification of impurities Use the chromatogram supplied with *spironolactone for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, C, D, E and I; use the chromatogram obtained with reference solution (d) to identify the peak due to impurity F.

Relative retention With reference to spironolactone (retention time = about 26 min): impurity A = about 0.95; impurity F = about 1.2; impurity C = about 1.5; impurity D = about 1.6; impurity E = about 1.7; impurity I = about 1.9.

System suitability Reference solution (b):
- *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to spironolactone.

Limits:
- *correction factor*: for the calculation of content, multiply the peak area of impurity F by 2.3;
- *impurity I*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *impurities E, F*: for each impurity, not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
- *impurities A, C*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *impurity D*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 7 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.7 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Free thiol compounds
To 2.0 g add 20 mL of *water R*, shake for 1 min and filter. To 10 mL of the filtrate add 0.05 mL of *0.01 M iodine* and 0.1 mL of *starch solution R* and mix. A blue colour develops.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (c).

Calculate the percentage content of $C_{24}H_{32}O_4S$ from the assigned content of *spironolactone CRS*.

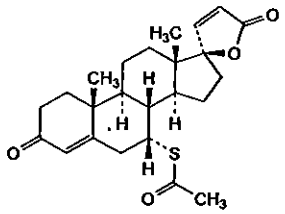
STORAGE

Protected from light.

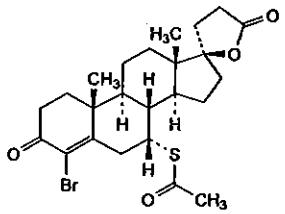
IMPURITIES

Specified impurities *A, C, D, E, F, I.*

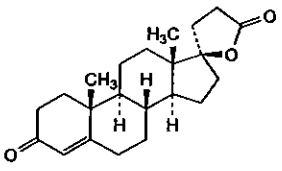
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, G, H.*



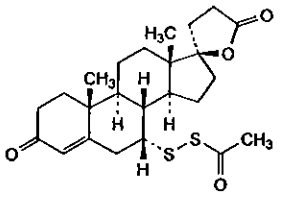
A. *S*-[(2′*R*)-3,5′-dioxo-5′*H*-spiro[androst-4-ene-17,2′-furan]-7α-yl] ethanethioate (Δ20-spironolactone),



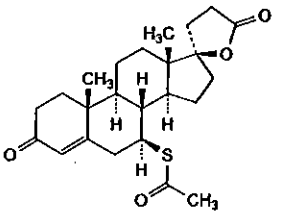
B. *S*-[(2′*R*)-4-bromo-3,5′-dioxo-3′,4′-dihydro-5′*H*-spiro[androst-4-ene-17,2′-furan]-7α-yl] ethanethioate (4-bromospironolactone),



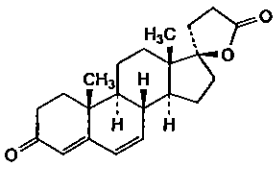
C. (2′*R*)-3′,4′-dihydro-5′*H*-spiro[androst-4-ene-17,2′-furan]-3,5′-dione (aldone),



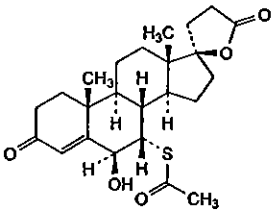
D. *S*-[(2′*R*)-3,5′-dioxo-3′,4′-dihydro-5′*H*-spiro[androst-4-ene-17,2′-furan]-7α-yl] ethane(dithioperoxate) (disulfanyl-spironolactone),



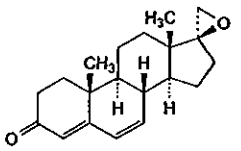
E. *S*-[(2′*R*)-3,5′-dioxo-3′,4′-dihydro-5′*H*-spiro[androst-4-ene-17,2′-furan]-7β-yl] ethanethioate (7β-spironolactone),



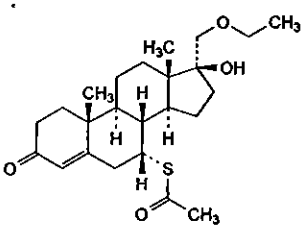
F. (2′*R*)-3′,4′-dihydro-5′*H*-spiro[androst-4,6-diene-17,2′-furan]-3,5′-dione (canrenone),



G. *S*-[(2′*R*)-6β-hydroxy-3,5′-dioxo-3′,4′-dihydro-5′*H*-spiro[androst-4-ene-17,2′-furan]-7α-yl] ethanethioate (6β-hydroxy-spironolactone),



H. (2′*S*)-spiro[androst-4,6-diene-17,2′-oxiran]-3-one,



I. *S*-[17α-(ethoxymethyl)-17β-hydroxy-3-oxoandrost-4-en-7α-yl] ethanethioate.

Products with Risk of Transmitting Agents of Animal Spongiform Encephalopathies

(Ph. Eur. monograph 1483)

Ph Eur

DEFINITION

Products with risk of transmitting agents of animal spongiform encephalopathies are those derived from tissues or secretions of animals susceptible to transmissible spongiform encephalopathies other than by experimental challenge. This monograph applies to all substances or preparations obtained from such animals and to all substances or preparations where products obtained from such animals are included as active substances or excipients or have been used during production, for example as raw or source materials, starting materials or reagents.

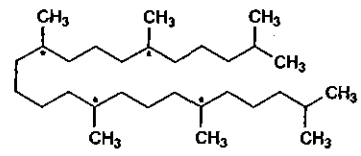
PRODUCTION

Production complies with chapter *5.2.8. Minimising the risk of transmitting animal spongiform encephalophathy agents via human and veterinary medicinal products.*

Ph Eur

Squalane

(Ph. Eur. monograph 1630)



$C_{30}H_{62}$ 422.8 *111-01-3*

Action and use
Excipient; emollient.

Ph Eur

DEFINITION

(6*E*,10*E*,15*E*,19*E*)-2,6,10,15,19,23-Hexamethyltetracosane (perhydrosqualene). It may be of vegetable (unsaponifiable matter of olive oil), animal (shark liver oil) or synthetic origin.

Content
96.0 per cent to 103.0 per cent.

PRODUCTION

The origin of the squalane (vegetable, animal or synthetic) is stated by the manufacturer.

CHARACTERS

Appearance
Clear, colourless, oily liquid.

Solubility
Practically insoluble in water, miscible with most fats and oils, freely soluble in acetone and in cyclohexane, practically insoluble in ethanol (96 per cent).

Relative density
About 0.815.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *squalane CRS.*
B. Refractive index (see Tests).

TESTS

Appearance
The substance to be examined is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Refractive index (*2.2.6*)
1.450 to 1.454.

Acid value (*2.5.1*)
Maximum 0.2.

Iodine value (*2.5.4, Method A*)
Maximum 4.0.

Saponification value (*2.5.6*)
Maximum 3.0.

Total ash (*2.4.16*)
Maximum 0.5 per cent, determined on 1.000 g.

ASSAY

Gas chromatography (*2.2.28*).

Internal standard solution To 1.0 mL of *dimethylacetamide R* add 100.0 mL of *heptane R.*

Test solution Dissolve 0.100 g of the substance to be examined in the internal standard solution and dilute to 25.0 mL with the same solution.

Reference solution (a) Dissolve 0.100 g of *squalane CRS* in the internal standard solution and dilute to 25.0 mL with the same solution.

Reference solution (b) To 0.1 mL of *methyl erucate R* add 0.100 g of the substance to be examined, dissolve in the internal standard solution and dilute to 25 mL with the same solution.

Column:
— *material:* fused silica;
— *size:* *l* = 30 m, Ø = 0.32 mm;
— *stationary phase:* *methylpolysiloxane R* (film thickness 1 µm).

Carrier gas *helium for chromatography R.*

Flow rate 1.7 mL/min.

Split ratio 1:12.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 39	60 → 290
	39 - 50	290
Injection port		275
Detector		300

Detection Flame ionisation.

Injection 1 µL.

Relative retention With reference to squalane (retention time = about 41 min): internal standard = about 0.2; methyl erucate = about 0.9; cyclosqualane = 1.05.

System suitability Reference solution (b):
— *resolution:* minimum 5 between the peaks due to methyl erucate and squalane.

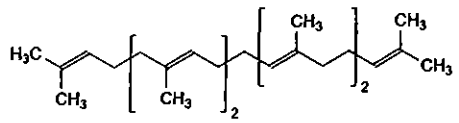
Calculate the percentage content of $C_{30}H_{62}$ using the chromatogram obtained with reference solution (a) and taking into account the assigned content of *squalane CRS.*

LABELLING
The label states the origin of squalane (vegetable, animal or synthetic).

Ph Eur

Squalene

(Ph. Eur. monograph 2805)



$C_{30}H_{50}$ 410.7 *111-02-4*

Ph Eur

DEFINITION
(6*E*,10*E*,14*E*,18*E*)-2,6,10,15,19,23-Hexamethyltetracosa-2,6,10,14,18,22-hexaene. It may be of vegetable or animal origin.

Content
97.0 per cent to 103.0 per cent (anhydrous substance).

This monograph applies to squalene used as an adjuvant in vaccines.

CHARACTERS
Appearance
Clear, colourless or light yellow, oily liquid.

Solubility
Practically insoluble in water, freely soluble in acetone and in cyclohexane, soluble in ethanol (96 per cent).

Relative density
About 0.86.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *squalene CRS.*

TESTS
Appearance
The substance to be examined is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Refractive index (*2.2.6*)
1.491 to 1.499.

Acid value (*2.5.1*)
Maximum 1.0.

Iodine value (*2.5.4, Method B*)
350 to 450, determined on 0.06 g.

Peroxide value (*2.5.5, Method A*)
Maximum 5.0.

Saponification value (*2.5.6*)
Maximum 5.0.

Water (*2.5.32*)
Maximum 0.2 per cent, determined on 2.00 g.

ASSAY
Gas chromatography (*2.2.28*). *Prepare the solutions immediately before use.*

Test solution Dissolve 0.100 g of the substance to be examined in *heptane R* and dilute to 100.0 mL with the same solvent.

Reference solution (a) Dissolve 0.100 g of *squalene CRS* in *heptane R* and dilute to 100.0 mL with the same solvent.

Reference solution (b) Dissolve 0.100 g of the substance to be examined and 0.100 g of *methyl lignocerate R* in *heptane R* and dilute to 100.0 mL with the same solvent.

Column:
— *material:* fused silica;
— *size:* *l* = 30 m, Ø = 0.32 mm;
— *stationary phase:* *methylpolysiloxane R* (film thickness 1 µm).

Carrier gas *helium for chromatography R.*

Flow rate 1.7 mL/min.

Split ratio 1:12.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 12	290
Injection port		275
Detector		300

Detection Flame ionisation.

Injection 1 µL.

Relative retention With reference to squalene (retention time = about 8.1 min): methyl lignocerate = about 0.9.

System suitability Reference solution (b):
— *resolution:* minimum 5.0 between the peaks due to methyl lignocerate and squalene.

Calculate the percentage content of $C_{30}H_{50}$ taking into account the assigned content of *squalene CRS.*

STORAGE
In an airtight container, protected from light.

LABELLING
The label states the origin of squalene (vegetable or animal).

Ph Eur

Stannous Chloride Dihydrate

(Ph. Eur. monograph 1266)

$SnCl_2,2H_2O$ 225.6 *10025-69-1*

Ph Eur

DEFINITION
Content
98.0 per cent to 102.0 per cent.

CHARACTERS
Appearance
White or almost white, crystalline powder or colourless crystals, efflorescent in air.

Solubility
Freely soluble in water (the solution becomes cloudy after standing or on dilution), freely soluble in ethanol (96 per cent). It dissolves in dilute hydrochloric acid.

IDENTIFICATION
A. To 1 mL of solution S1 (see Tests) add 5 mL of *water R* and 0.05 mL of *mercuric chloride solution R.* A blackish-grey precipitate is formed.

B. Dissolve 1.0 g in 3.0 mL of *water R.* Add 0.5 mL of *dilute sodium hydroxide solution R* to the cloudy solution; a yellowish flocculent precipitate is formed. Add 6.5 mL of *water R.*

To 1.0 mL of the previously shaken suspension add 1.0 mL of *strong sodium hydroxide solution R*; the precipitate dissolves and the resulting solution is clear and colourless.

C. Dissolve 10 mg in 2 mL of *dilute nitric acid R*.
The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S1
To 0.40 g add 1 mL of *dilute hydrochloric acid R* and dilute to 20 mL with *distilled water R*.

Solution S2
Dissolve 1.0 g in *dilute hydrochloric acid R* and dilute to 30 mL with the same acid. Heat to boiling. Add 30 mL of *thioacetamide solution R* and boil for 15 min (solution A). Take 5 mL, filter and heat the filtrate to boiling. Add 5 mL of *thioacetamide solution R* and boil for 15 min. If a precipitate is formed, add the remainder of solution A (solution A′) to the mixture. Add 10 mL of *thioacetamide solution R* and boil. Repeat the series of operations from "Take 5 mL" until a precipitate is no longer formed on addition of *thioacetamide solution R* to the filtrate obtained from the 5 mL of solution A (solution A′, solution A″, etc. respectively). If no precipitate is formed or if no more precipitate is formed combine the solution obtained with the remainder of solution A (solution A′, solution A″, etc. respectively), filter and wash the precipitate with 10 mL of *water R*. Heat the filtrate until the resulting vapour no longer turns a moistened piece of *lead acetate paper R* blackish-grey. Allow to cool and dilute to 50 mL with *water R*.

Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 10.0 g in *dilute hydrochloric acid R* and dilute to 20 mL with the same acid.

Substances not precipitated by thioacetamide
Maximum 0.2 per cent.

Evaporate 25 mL of solution S2 to dryness and ignite at 600 ± 50 °C. The residue weighs a maximum of 1 mg.

Sulfates (*2.4.13*)
Maximum 500 ppm, determined on solution S1.

Iron (*2.4.9*)
Maximum 100 ppm.

Dilute 5 mL of solution S2 to 10 mL with *water R*.

ASSAY
Dissolve 0.100 g in 50 mL of *water R*, freed from oxygen by purging with carbon dioxide or nitrogen for 15 min. Add 1.5 mL of *hydrochloric acid R1*, 5 g of *sodium potassium tartrate R*, 10 g of *sodium hydrogen carbonate R* and 1 mL of *starch solution R*. Titrate immediately with *0.05 M iodine*. Carry out a blank titration.

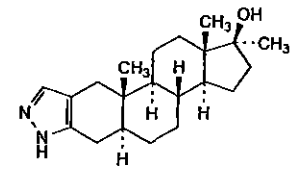
1 mL of *0.05 M iodine* is equivalent to 11.28 mg of $SnCl_2,2H_2O$.

STORAGE
In an airtight container.

Ph Eur

Stanozolol

(Ph. Eur. monograph 1568)



$C_{21}H_{32}N_2O$ 328.5 *10418-03-8*

Action and use
Anabolic steroid; androgen.

Ph Eur

DEFINITION
17-Methyl-2′*H*-5α-androst-2-eno[3,2-*c*]pyrazol-17β-ol.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, hygroscopic, crystalline powder.

Solubility
Practically insoluble in water, soluble in dimethylformamide, slightly soluble in ethanol (96 per cent), very slightly soluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *stanozolol CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *methylene chloride R*, evaporate to dryness at room temperature under an air-stream and record new spectra using the residues.

B. Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (c).

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (c).

TESTS

Specific optical rotation (*2.2.7*)
+ 37 to + 41 (dried substance).

Dissolve 60.0 mg in *methanol R* and dilute to 20.0 mL with the same solvent.

Impurities A and B
Thin-layer chromatography (*2.2.27*).

Solvent mixture *methanol R1, methylene chloride R* (10:90 *V/V*).

Test solution Dissolve 20 mg of the substance to be examined in 1.0 mL of the solvent mixture.

Reference solution Dissolve 2 mg of *stanozolol CRS*, 2.0 mg of *stanozolol impurity A CRS* and 2.0 mg of *stanozolol impurity B CRS* in 1.0 mL of the solvent mixture. Dilute 0.1 mL of the solution to 2.0 mL with the solvent mixture.

Plate *TLC silica gel plate R*.

Mobile phase *glacial acetic acid R, ethyl acetate R, cyclohexane R* (2:48:50 *V/V/V*).

Application 10 µL.

Development Over 3/4 of the plate.

Drying In air.

Detection Spray with *vanillin reagent R* and heat at 120 °C.

System suitability Reference solution:

— the chromatogram shows 3 clearly separated spots, due to stanozolol, impurity A and impurity B, in order of increasing R_F value.

Limits:

— *impurity A*: any spot due to impurity A is not more intense than the corresponding spot in the chromatogram obtained with the reference solution (0.5 per cent);

— *impurity B*: any spot due to impurity B is not more intense than the corresponding spot in the chromatogram obtained with the reference solution (0.5 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 15.0 mg of the substance to be examined in *methanol R* and dilute to 5.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *methanol R*. Dilute 1.0 mL of this solution to 10.0 mL with *methanol R*.

Reference solution (b) Dissolve 1 mg of *stanozolol CRS* and 1 mg of *stanozolol impurity B CRS* in *methanol R* and dilute to 20 mL with the same solvent.

Reference solution (c) Dissolve 15.0 mg of *stanozolol CRS* in *methanol R* and dilute to 5.0 mL with the same solvent.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R1* (5 µm).

Mobile phase 1 g/L solution of *sodium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*, *methanol R1* (30:70 *V/V*).

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 228 nm.

Injection 25 µL of the test solution and reference solutions (a) and (b).

Run time 3 times the retention time of stanozolol.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity B.

Relative retention With reference to stanozolol (retention time = about 12 min): impurity B = about 1.3.

System suitability Reference solution (b):

— *resolution*: minimum 4.0 between the peaks due to stanozolol and impurity B.

Limits:

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying at 105 °C at a pressure not exceeding 0.7 kPa.

ASSAY

Dissolve 0.250 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

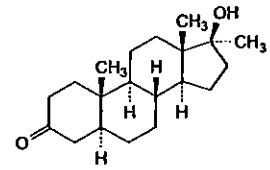
1 mL of *0.1 M perchloric acid* is equivalent to 32.85 mg of $C_{21}H_{32}N_2O$.

STORAGE

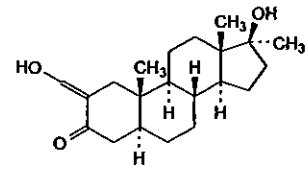
In an airtight container, protected from light.

IMPURITIES

Specified impurities *A*, *B*.



A. 17β-hydroxy-17-methyl-5α-androstan-3-one (mestanolone),

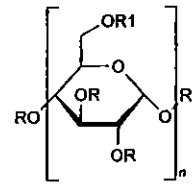


B. 17β-hydroxy-2-(hydroxymethylene)-17-methyl-5α-androstan-3-one (oxymetholone).

Ph Eur

Hydroxyethyl Starches

(Ph. Eur. monograph 1785)



R = -[CH_2CH_2O]$_n$H (*n* = 0, 1, 2...)

R1 = -[CH_2CH_2O]$_n$-H (*n* = 0 or 1) or glucose

[$C_6H_{10}O_5(C_2H_4O)_x$]$_n$ with *x* = molar substitution *9005-27-0*

Ph Eur

DEFINITION

Hydroxyethyl starches are partially substituted poly(2-hydroxyethyl)ethers of waxy maize starch or potato starch, which primarily consist of amylopectine. The type of hydroxyethyl starch is defined by 2 numbers: the mean molecular weight (*Mw*) and the number of hydroxyethyl groups per anhydroglucose unit expressed as the molar substitution (*MS*). Hydroxyethyl starch is also characterised by the number of hydroxyethyl groups located at the C2 group over the number of hydroxyethyl groups located at C6, expressed as the C2/C6 ratio. The parameters *Mw*, *MS* and C2/C6 ratio are determined by the reaction conditions of the production.

PRODUCTION

Hydroxyethyl starches are produced from waxy maize starch or potato starch by acidic hydrolysis and reaction with ethylene oxide and purified by ultrafiltration.

CHARACTERS

Appearance

White or almost white powder.

Solubility

Freely soluble in water and in dimethyl sulfoxide, practically insoluble in anhydrous ethanol.

Hydroxyethyl starches are hygroscopic until they reach a water content of about 12 per cent to 15 per cent.

IDENTIFICATION

First identification: A, C.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *medium Mw hydroxyethyl starch CRS.*

Results The spectrum obtained shows the same absorption bands as the spectrum obtained with *medium Mw hydroxyethyl starch CRS*. Due to the difference in the substitution of the substance, the intensity of some absorption bands can vary.

B. To 5 mL of solution S (see Tests), add 0.1 mL of *0.05 M iodine*. A reddish-brown or blue-violet colour appears.

C. Molecular weight (see Tests).

TESTS

Solution S

Dissolve 5.0 g of the substance to be examined (dried substance) in *carbon dioxide-free water R* and dilute to 100.0 mL with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II (*2.2.1*).

pH (*2.2.3*)

4.5 to 7.0.

To 25 mL of solution S, add 0.2 mL of a saturated solution of *potassium chloride R*.

Absorbance (*2.2.25*)

Maximum 0.025, determined at 400 nm on solution S filtered through a 0.2 µm filter.

Molecular weight (*Mw*) and molecular weight distribution

Size-exclusion chromatography (*2.2.30*).

Buffer solution Dissolve 54.34 g of *sodium acetate R* in *water R*, add 100.0 mL of *glacial acetic acid R* and dilute to 1000.0 mL with *water R*.

Test stock solution Dissolve 2.0 g of the substance to be examined (dried substance) in *water R* and dilute to 50 mL with the same solvent. Add 10.0 mL of the buffer solution and dilute to 100.0 mL with *water R*.

Reference solution (a) To prepare reference solution (a):

— if the nominal *Mw* of the substance to be examined is below 300 000, use *medium Mw hydroxyethyl starch CRS*;

— if the nominal *Mw* of the substance to be examined is above 300 000, use *high Mw hydroxyethyl starch CRS*.

Dissolve 0.4 g of *medium Mw hydroxyethyl starch CRS* or *high Mw hydroxyethyl starch CRS* in 10 mL of *water R*. Add 2.0 mL of the buffer solution and dilute to 20.0 mL with *water R*.

Reference solution (b) Dilute 10.0 mL of reference solution (a) to 20.0 mL with the mobile phase.

Reference solution (c) Dilute 10.0 mL of reference solution (b) to 20.0 mL with the mobile phase.

Reference solution (d) Dilute 10.0 mL of reference solution (c) to 20.0 mL with the mobile phase.

Column:

— *stationary phase: hydroxylated polymethacrylate gel R*;

— 4 columns to be connected in series:

Length in m	Internal diameter in mm	Particle size in µm	Pore size in nm
0.30	7.5	17	> 100
0.30	7.5	17	100
0.30	7.5	10	20
0.30	7.5	10	12.5

Mobile phase Dilute 100.0 mL of the buffer solution to 1000 mL with *water for chromatography R*.

Flow rate 0.5-1.0 mL/min.

Detection Multiple-angle light scattering (MALS) detector and refractometer maintained at a constant temperature, connected in series.

Injection volume 50 µL.

Determine the suitable working solution as follows: inject reference solutions (a) and (b), the mean *Mw* determined with reference solution (b) does not deviate by more than 3 per cent from the mean *Mw* determined with reference solution (a). If the deviation meets the requirement, use reference solution (a) to check the system suitability criterion.

If the deviation is higher, inject reference solution (c) and determine the mean *Mw*. The mean *Mw* determined with reference solution (c) does not deviate by more than 3 per cent from the mean *Mw* determined with reference solution (b). If the deviation meets the requirement, use reference solution (b) to check the system suitability criterion.

If the deviation is higher, inject reference solution (d) and determine the mean *Mw*. The mean *Mw* determined with reference solution (d) does not deviate by more than 3 per cent from the mean *Mw* determined with reference solution (c). If the deviation meets the requirement, use reference solution (c) to check the system suitability criterion.

System suitability:

— *mean Mw*: within 5 per cent of the value assigned to the *medium Mw hydroxyethyl starch CRS* or *high Mw hydroxyethyl starch CRS*.

If necessary, dilute the test stock solution in order to have the same concentration as that of the reference solution used to check the system suitability.

Results Use a suitable integrator to determine the mean *Mw* and the *Mw* of the lowest and highest 10 per cent mass fraction.

Low *Mw* 2000 - 100 000	Medium *Mw* 100 000 - 300 000	High *Mw* 300 000 - 900 000
Determined *Mw* = nominal *Mw* ± 15 per cent		
Mw at 10 per cent lowest fraction > 10 per cent of nominal *Mw*	*Mw* at 10 per cent lowest fraction > 15 000	*Mw* at 10 per cent lowest fraction > 15 000
Mw at 10 per cent highest fraction < 300 per cent of nominal *Mw*	*Mw* at 10 per cent highest fraction < 300 per cent of nominal *Mw*	*Mw* at 10 per cent highest fraction < 500 per cent of nominal *Mw*

C2/C6 ratio

Gas chromatography (*2.2.28*).

Solution A Mix equal volumes of *dilute sulfuric acid R* and *water R*.

Test solution Introduce 0.18 g of the substance to be examined into a 5 mL vial. Add 3.0 mL of solution A, cap, seal the vial and shake until dissolution. Heat the vials for 4 h in a heating block already preheated to 100 °C, shaking them from time to time. Cool to room temperature. Open the vial and carefully add 0.9 g of *barium carbonate R*. Shake carefully and then centrifuge at about 9000 *g* for about 15 min. Test the clear supernatant for neutral pH with pH paper. If the solution is still acid, add more *barium carbonate R* in portions of 0.2 g until the solution is neutral. Filter the clear supernatant (pore size 0.45 µm). Introduce 0.5 mL of the filtrate into a autosampler vial and evaporate to dryness at 40 °C (several hours are usually needed). Take up the residue with 0.50 mL of *pyridine R*, 0.25 mL of *N,O-bis(trimethylsilyl)acetamide R* and 25 µL of *chlorotrimethylsilane R*. Seal the vial and heat to 40 °C for 1 h shaking from time to time. Cool to room temperature. Place the vial into the autosampler and perform 3 injections from each vial. Prepare in duplicate.

Reference solution Prepare as prescribed for the test solution but using *medium Mw hydroxyethyl starch CRS* instead of the substance to be examined.

Column:
- *size*: *l* = 15 m, Ø = 0.32 mm;
- *stationary phase*: *methylpolysiloxane R* (film thickness 0.25 µm).

Carrier gas *hydrogen for chromatography R* at a constant pressure of 69 kPa.

Split ratio 1:20.

	Time (min)	Temperature (°C)
Column	0 - 1	150
	1 - 25	150 → 270
	25 - 28	270
Injection port		250
Detector		300

Detection Flame ionisation.

Injection 1 µL.

Identification of peaks Use the chromatogram supplied with *medium Mw hydroxyethyl starch CRS* and the chromatogram obtained with the reference solution to identify the peaks due to derivatised product 1, derivatised product 2, derivatised product 3, 2-*O*-hydroxyethyl-α-D-glucose, 6-*O*-hydroxyethyl-α-D-glucose, 2-*O*-hydroxyethyl-β-D-glucose and 6-*O*-hydroxyethyl-β-D-glucose.

System suitability Reference solution:
- *resolution*: minimum 1.5 between the peaks due to 2-*O*-hydroxyethyl-β-D-glucose and 6-*O*-hydroxyethyl-β-D-glucose;
- *symmetry factor*: 0.6 to 1.5 for the peak due to derivatised product 1;
- *repeatability*: maximum relative standard deviation of 5.0 per cent for derivatised product 1 after 3 injections.

Calculate the C2/C6 ratio using the following expression:

$$\frac{A_1 + A_2 + A_3 + A_4 + A_5}{A_6 + A_7}$$

A_1 = area of the peak due to derivatised product 1;
A_2 = area of the peak due to derivatised product 2;
A_3 = area of the peak due to derivatised product 3;
A_4 = area of the peak due to 2-*O*-hydroxyethyl-α-D-glucose;
A_5 = area of the peak due to 2-*O*-hydroxyethyl-β-D-glucose;
A_6 = area of the peak due to 6-*O*-hydroxyethyl-α-D-glucose;
A_7 = area of the peak due to 6-*O*-hydroxyethyl-β-D-glucose.

Calculate the mean C2/C6 ratio from the values obtained with the 2 test solutions.

The test is not valid unless the difference of the 2 values is not more than 5 per cent.

Limit Within 20.0 per cent of the nominal value.

Molar substitution (*MS*)

Gas chromatography (*2.2.28*).

The content of hydroxyethyl groups is determined after hydrolysis with hydriodic acid as iodoethane.

Internal standard solution Dilute 1.0 mL of *toluene R* to 200.0 mL with *xylene R*.

Test solution Introduce 50.0 mg of the substance to be examined and about 0.10-0.15 g of *adipic acid R* in a 5 mL vial. Add 1.0 mL of the internal standard solution and 2.0 mL of *hydriodic acid R*. Tightly seal and cap the vial with a septum and an aluminium, centre tear-off seal. Prepare the test solution 5 times.

Reference solutions In each of 7 vials of 5 mL, introduce about 0.10-0.15 g of *adipic acid R*. To each vial add 1.0 mL of the internal standard solution and 2.0 mL of *hydriodic acid R*. Tightly seal and cap the vials with a septum and an aluminium, centre tear-off seal. Weigh the vials with an accuracy of 0.01 mg. Introduce respectively 10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg and 70 mg of *iodoethane R* with a 100 µL syringe piercing the septa carefully. Weigh the vials again with an accuracy of 0.01 mg and calculate the exact amount of *iodoethane R* added.

Determine the mass of the vials to the nearest 1 mg. Place the vials for 10 h into a heating block already preheated to 150 °C. After cooling to room temperature, determine the mass of each vial to the nearest 1 mg. Disregard any vial with a loss in mass of more than 5 mg. From 4 vials of the test solution and 5 of the reference solutions, take-up 100 µL of the upper layer. Introduce in an autosampler vial and dilute with 1.0 mL of *xylene R*. Seal immediately the vials and shortly shake.

Column:
- *material*: fused silica;
- *size*: *l* = 30 m, Ø = 0.53 mm;
- *stationary phase*: *cyanopropyl(3)phenyl(3)methyl(94) polysiloxane R* (film thickness 3 µm).

Carrier gas *helium for chromatography R*.

Flow rate 8 mL/min.

Split ratio 1:20.

	Time (min)	Temperature (°C)
Column	0 - 4	50
	4 - 16	50 → 230
	16 - 20	230
Injection port		200
Detector		280

Detection Flame ionisation.

Injection volume 1 µL; inject each solution twice.

Relative retention With reference to toluene (retention time = about 7.5 min): iodoethane = about 0.5.

System suitability Reference solutions:
— *resolution*: minimum 1.5 between the peaks due to iodoethane and toluene;
— calculate the ratio of the area of the peak due to *iodoethane R* to the area of the peak due to the internal standard for each chromatogram. Calculate the linear regression curve plotting the ratios calculated for the reference solutions against the quantity of *iodoethane R* added (in milligrams). The coefficient of determination R^2 is not less than 0.990.

Results Calculate the quantity (T) of iodoethane in milligrams present in the test solution using the following expression:

$$\frac{A-B}{M}$$

A = ratio of the area of the peak due to iodoethane to the area of the peak due to the internal standard in the chromatogram obtained with the test solution;
B = y-intercept of the curve;
M = slope of the curve.

Then calculate the percentage content of ethylene oxide (C) using the following expression:

$$\frac{44.05 \times T \times 100}{155.97 \times m}$$

m = mass of the substance to be examined, in milligrams;
44.05 = molecular mass of ethylene oxide;
155.97 = molecular mass of iodoethane.

Then calculate the *MS* using the following expression:

$$\frac{C \times 162.14}{(100-C) \times 44.05}$$

162.14 = molecular mass of anhydroglucose;
44.05 = molecular mass of ethylene oxide.

Calculate the mean *MS* from the values obtained with the 4 test solutions.

Limit 0.05 to 2.4, and within 8.0 per cent of the nominal value.

Ethylene glycol

Liquid chromatography (*2.2.29*).

Test solution Dissolve 1.0 g of the substance to be examined (dried substance) in *water R* and dilute to 50.0 mL with the same solvent.

Reference solution Dissolve 0.800 g of *ethylene glycol R* in *water R* and dilute to 100.0 mL with the same solvent. Dilute 2.0 mL of the solution to 200.0 mL with *water R*. Dilute 2.0 mL of this solution to 200.0 mL with *water R*.

Precolumn:
— *size*: l = 0.01 m, Ø = 4.0 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography compatible with 100 per cent aqueous mobile phases R* (5 µm).

Column:
— *size*: l = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography compatible with 100 per cent aqueous mobile phases R* (5 µm);
— *temperature*: 30 °C.

Mobile phase *water for chromatography R*.

Flow rate 1.0 mL/min.

Post-column solution Dilute 750 mL of *2 M sodium hydroxide R* to 1000 mL with *water for chromatography R*.

Flow rate of post-column solution 0.2 mL/min.

Detection Pulsed amperometric detector.

Injection 20 µL.

Run time 2.5 times the retention time of ethylene glycol.

Retention time Ethylene glycol = about 4 min.

System suitability Reference solution:
— *signal-to-noise ratio*: minimum 10 for the principal peak;
— *repeatability*: maximum relative standard deviation of 10.0 per cent determined on 6 injections.

After a maximum of 8 sample injections, wash the column using the following program.

Rinsing solution *acetonitrile for chromatography R, water for chromatography R* (20:80 *V/V*).

Time (min)	Mobile phase (per cent *V/V*)	Rinsing solution (per cent *V/V*)
0 - 15	75	25
15 - 20	75 → 0	25 → 100
20 - 25	0	100
25 - 30	0 → 100	100 → 0
30 - 100	100	0

Limit:
— *ethylene glycol*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (40 ppm).

2-Chloroethanol

Gas chromatography (*2.2.28*).

Solvent mixture *methanol R, acetonitrile R* (25:75 *V/V*).

Internal standard solution Dissolve 0.250 g of *2,6-dimethylaniline R* in the solvent mixture and dilute to 50.0 mL with the solvent mixture. Dilute 0.5 mL of the solution to 50.0 mL with the solvent mixture.

Test solution Introduce 1.0 g of the substance to be examined into a 20 mL vial. Add 10.0 mL of the solvent mixture. Close tightly. Treat in an ultrasonic bath for 3.5 h. Allow to cool to room temperature. To 1.0 mL of this solution add 0.8 mL of the internal standard solution.

Reference solution Dissolve 0.250 g of *2-chloroethanol R* in the solvent mixture and dilute to 50.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 100.0 mL with the solvent mixture. To 1.0 mL of this solution add 0.8 mL of the internal standard solution.

Precolumn:
— *material*: fused silica;
— *size*: l = 10 m, Ø = 0.53 mm;
— *stationary phase*: *polar-deactivated macrogol R*.

Column:
— *material*: fused silica;
— *size*: l = 30 m, Ø = 0.32 mm;
— *stationary phase*: *macrogol 20 000 R* (film thickness 0.25 µm).

Carrier gas *hydrogen for chromatography R*.

Flow rate 2.9 mL/min.

Split program:

Time (min)	Split state	Split ratio
initial	on	1:20
0.01	off	1:20
0.50	on	1:20

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 4	45
	4 - 23.5	45 → 240
	23.5 - 28.5	240
Injection port		250
Detector		270

Detection Flame ionisation.

Injection 1 µL.

System suitability Reference solution:
- *signal-to-noise ratio*: minimum 10 for the peak due to 2-chloroethanol;
- *repeatability*: maximum relative standard deviation of 10.0 per cent after 6 injections.

Limit:
- *2-chloroethanol*: calculate the ratio (*R*) of the area of the peak due to 2-chloroethanol to the area of the peak due to the internal standard from the chromatogram obtained with the reference solution; calculate the ratio of the area of the peak due to 2-chloroethanol to the area of the peak due to the internal standard from the chromatogram obtained with the test solution: this ratio is not greater than *R* (5 ppm).

Ethylene oxide

Head-space gas chromatography (*2.2.28*).

Test solution Dissolve 1.0 g of the substance to be examined in 1.0 mL of *water R*. Close the vial tightly. Prepare in duplicate.

Reference stock solution Introduce 80 mL of *water R* in a 100 mL volumetric flask. Cool at about 4 °C for at least 30 min. Place the flask on an analytical balance and slowly introduce 1.0 g of *ethylene oxide R*. Determine the precise quantity of ethylene oxide by differential weighing. Dilute to 100.0 mL with *water R*. Store in the refrigerator and use within 4 weeks.

Reference solution (a) Dilute 1.0 mL of the reference stock solution to 100.0 mL with *water R*. Dilute 1.0 mL of this solution to 100.0 mL with *water R*. Use within 24 h.

Reference solution (b) Dissolve 1.0 g of the substance to be examined in 1.0 mL of reference solution (a). Close the vial tightly. Prepare in duplicate.

Column:
- *material*: quartz;
- *size*: *l* = 30 m, Ø = 0.32 mm;
- *stationary phase*: *cyanopropyl(3)phenyl(3)methyl(94) polysiloxane R* (film thickness 1.5 µm).

Carrier gas *helium for chromatography R* at a pressure of 110.3 kPa.

Split ratio 1:35.

Static head-space conditions which may be used:
- *equilibration temperature*: 80 °C;
- *equilibration time*: 40 min;
- *transfer-line temperature*: 150 °C;
- *pressurisation time*: 2.0 min;
- *injection time*: 3 s.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 20	40
	20 - 30	40 → 240
	30 - 40	240
Injection port		140
Detector		250

Detection Flame ionisation.

Injection Inject a suitable volume of the gaseous phase of the test solution and reference solution (b).

System suitability:
- *signal-to-noise ratio*: minimum 10 for the peak due to ethylene oxide in the chromatogram obtained with reference solution (b).

Limit:
- *ethylene oxide*: not more than 0.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (1 ppm).

Sodium chloride

Maximum 0.1 per cent.

Test solution In a 250 mL conical flask, dissolve 10.0 g of the substance to be examined in 100 mL of *water R*. Add 2 mL of *dilute nitric acid R* and 5.0 mL of a 9 g/L solution of *sodium chloride R*.

Reference solution In a 250 mL conical flask, dilute 5.0 mL of a 9 g/L solution of *sodium chloride R* with 100 mL of *water R*. Add 2 mL of *dilute nitric acid R*.

Carry out a potentiometric titration (*2.2.20*) with *0.1 M silver nitrate*. Calculate the percentage content of sodium chloride using the following expression:

$$\frac{(n_1 - n_2) \times 5.844 \times 100}{m}$$

n_1 = volume of *0.1 M silver nitrate* used for the test solution, in millilitres;
n_2 = volume of *0.1 M silver nitrate* used for the reference solution, in millilitres;
m = mass of the substance to be examined in the test solution, in milligrams.

Loss on drying (*2.2.32*)

Maximum 15.0 per cent, determined on 1.000 g by drying at 105 °C.

Bacterial endotoxins (*2.6.14*)

Less than 2.5 IU/g.

Microbial contamination

TAMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

LABELLING

The label states the mean molecular weight, molar substitution and C2/C6 ratio (nominal values).

Ph Eur

Hydroxypropyl Starch

(Ph. Eur. monograph 2165)

9049-76-7

Ph Eur

DEFINITION

Hydroxypropyl starch is a partially substituted 2-hydroxypropylether of *Maize starch (0344)*, *Potato starch (0355)*, cassava starch, *Rice starch (0349)* or *Pea starch (2403)* chemically modified by etherification with the reagent propylene oxide. In addition, this starch may be partially hydrolysed using acids or enzymes to obtain 'thinned starch' with reduced viscosity.

Content

— *hydroxypropyl groups*: 0.5 per cent to 7.0 per cent.

PRODUCTION

The production of hydroxypropyl starch shall be in compliance with the requirements of the European legislation for food additives.

Mixing of starches from different botanical sources prior to chemical modification is not allowed.

CHARACTERS

Appearance

White or slightly yellowish powder.

Solubility

Practically insoluble in cold water and in ethanol (96 per cent).

IDENTIFICATION

A. Examined under a microscope, using not less than 20 × magnification and using a mixture of equal volumes of *glycerol R* and *water R*, it appears as follows according to the botanical source stated on the label.

— *Maize-based hydroxypropyl starch*: it presents either angular polyhedral granules of irregular sizes with diameters of about 2-23 µm or rounded or spheroidal granules of irregular sizes with diameters of about 25-35 µm; the central hilum consists of a distinct cavity or 2-to-5-rayed cleft and there are no concentric striations; between orthogonally orientated polarising plates or prisms, the starch granules show a distinct black cross intersecting at the hilum.

— *Potato-based hydroxypropyl starch*: it presents granules, either irregularly shaped, ovoid or pear-shaped, usually 30-100 µm in size but occasionally exceeding 100 µm, or rounded, 10-35 µm in size; there are occasional compound granules having 2-4 components; the ovoid and pear-shaped granules have an eccentric hilum and the rounded granules a centric or slightly eccentric hilum; all granules show clearly visible concentric striations; between orthogonally orientated polarising plates or prisms, the starch granules show a distinct black cross intersecting at the hilum.

— *Cassava-based hydroxypropyl starch*: it presents spherical granules with one truncated side, typically 5-35 µm in diameter and having a circular or several-rayed central cleft; some granules may also be egg-shaped or cap-shaped; the hilum is centric, sometimes slightly fissured; between orthogonally orientated polarising plates or prisms, the starch granules show a distinct black cross intersecting at the hilum.

— *Rice-based hydroxypropyl starch*: it presents polyhedral, simple granules 1-10 µm, mostly 4-6 µm, in size; these simple granules often gather in ellipsoidal, compound granules 50-100 µm in diameter; the granules have a poorly visible central hilum and there are no concentric striations; between orthogonally orientated polarising plates or prisms, the starch granules show a distinct black cross intersecting at the hilum.

— *Pea-based hydroxypropyl starch*: it presents a majority of large elliptical granules, 25-45 µm in size, sometimes irregular or reniform; it also presents a minority of small rounded granules, 5-8 µm in size; granules can present cracks or irregularities; sometimes, granules show barely visible concentric striations; exceptionally, granules show a slit along the main axis; between orthogonally orientated polarising plates or prisms, the starch granules show a distinct black cross.

B. Suspend 1 g in 50 mL of *water R*, boil for 1 min and cool. A translucent or clear mucilage is formed.

C. To 1 mL of the mucilage obtained in identification test B add 0.05 mL of *iodine solution R1*. An orange-red or dark blue colour is produced, which disappears on heating.

D. Introduce 0.1 g into a 100 mL volumetric flask and add 12.5 mL of *dilute sulfuric acid R*. Place the flask in a water-bath and heat until the sample is dissolved. Cool and dilute to 100 mL with *water R*. Introduce 1 mL of this solution into a 25 mL graduated test-tube with glass stopper and, with the tube immersed in cold water, add dropwise 8 mL of *sulfuric acid R*. Mix well and place the tube in a water-bath for exactly 3 min. Immediately transfer the tube to an ice-bath until the solution is chilled. Add 0.6 mL of *ninhydrin solution R2*, carefully allowing the reagent to run down the walls of the test-tube. Immediately shake well, and place the tube in a water-bath at 25 °C for 100 min. Dilute to 25 mL with *sulfuric acid R* and mix by inverting the tube several times. Do not shake. A violet colour develops within 5 min.

TESTS

pH *(2.2.3)*

4.5 to 8.0.

Shake 5.0 g with 25.0 mL of *carbon dioxide-free water R* for 60 s. Allow to stand for 15 min.

Foreign matter

Examined under a microscope using a mixture of equal volumes of *glycerol R* and *water R*, not more than traces of matter other than starch granules are present.

Oxidising substances *(2.5.30)*

Maximum 20 ppm, calculated as H_2O_2.

Sulfur dioxide *(2.5.29)*

Maximum 50 ppm.

Iron *(2.4.9)*

— For hydroxypropyl starch obtained from maize, potato, cassava or rice: maximum 20 ppm.

Shake 1.0 g with 20 mL of *dilute hydrochloric acid R*. Filter. The filtrate complies with the test for iron.

— For hydroxypropyl starch obtained from pea: maximum 50 ppm.

Shake 1.0 g with 50 mL of *dilute hydrochloric acid R*. Filter. The filtrate complies with the test for iron.

Loss on drying

(2.2.32) determined on 1.000 g by drying in an oven at 130 °C for 90 min:

— maximum 15.0 per cent for hydroxypropyl starch obtained from maize, cassava, rice or pea;

— maximum 20.0 per cent for hydroxypropyl starch obtained from potato.

Sulfated ash (*2.4.14*)
Maximum 0.6 per cent, determined on 1.0 g.

Microbial contamination
TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*).

TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

Absence of *Escherichia coli* (*2.6.13*).

Absence of *Salmonella* (*2.6.13*).

ASSAY
Nuclear magnetic resonance spectrometry (*2.2.33*).

Internal standard solution Disperse 50.0 mg of *3-trimethylsilyl-1-propanesulfonic acid sodium salt CRS* in about 5 g of *deuterium oxide R1*, weighed to the nearest 0.1 mg. Store in a sealed bottle.

Test solution Disperse 20 g of the substance to be examined in 200.0 mL of *carbon dioxide-free water R* at room temperature. Agitate for 15 min and filter. Repeat the operation twice. If problems of poor dispersibility or slow filtration are encountered, use cooled *carbon dioxide-free water R* for the washing operation. Dry the washed starch for at least 4 h in an oven *in vacuo* at 30 ± 5 °C. Determine the moisture content (*W*) on 5 g of this washed and dried sample using the test for loss on drying. Weigh 12.0 mg (dried substance) of the washed and dried sample in a 5 mm NMR tube. Add 0.1 mL of *deuterium chloride solution R* and 0.75 mL of *deuterium oxide R1*. Cap the tube, mix, and place it in a water-bath until a clear solution is obtained (3 min to 1 h maximum). When a clear solution is obtained, allow to cool to room temperature. Dry the exterior of the tube and weigh to the nearest 0.1 mg. Add 0.05 mL of the internal standard solution and weigh to the nearest 0.1 mg. Determine the mass of the internal standard solution introduced. Mix thoroughly.

Apparatus FT-NMR spectrometer at minimum 300 MHz.

Acquisition of ^1H NMR spectra The following parameters may be used:
— *sweep width*: 8 ppm (−1.0 to + 7 ppm);
— *irradiation frequency offset*: none;
— *time domain*: 64 K at least;
— *pulse width*: 90°;
— *pulse delay*: 10 s;
— *dummy scans*: 0;
— *number of scans*: 8.

Use the CH_3 signal of the internal standard for shift referencing. The shift of the singlet is set to 0 ppm.

Record the FID signal.

Call the integration sub-routine after phase corrections and baseline correction between −0.5 ppm and + 6 ppm.

Measure the peak areas of the doublet from the methyl groups of the hydroxypropyl function at + 1.2 ppm (A_2), and of the methyl groups at 0 ppm of the internal standard (A_1) without ^{13}C-satellites.

Results Measure the signal coming from the 3 protons of the methyl group in the hydroxypropyl function; calculate the hydroxypropyl groups content as a percentage *m/m* (dried substance) using the following expression:

$$\frac{3A_2}{A_1} \times \frac{P}{100} \times \frac{W_1 \times m_1}{218} \times 59 \times \frac{100}{m} \times \frac{100}{100 - W}$$

3	=	numerical value representing the 3 methyl groups in the internal standard;
A_1	=	area of the methyl groups in the internal standard;
A_2	=	area of the methyl groups of hydroxypropyl;
P	=	percentage content of *3-trimethylsilyl-1-propanesulfonic acid sodium salt CRS*;
W_1	=	mass fraction of the internal standard in the internal standard solution, in milligrams per gram;
m_1	=	mass of the internal standard solution in the NMR tube, in grams;
218	=	molar mass of the internal standard, in grams per mole;
59	=	molar mass of the hydroxypropyl group, in grams per mole;
m	=	mass of the washed and dried sample in the NMR tube, in milligrams;
W	=	moisture content, as a percentage *m/m*.

LABELLING
The label states the botanical source of the starch and the type of modification.

Ph Eur

Pregelatinised Hydroxypropyl Starch

(Ph. Eur. monograph 2645)

Ph Eur

DEFINITION
Pregelatinised hydroxypropyl starch is prepared from *Starch, hydroxypropyl (2165)* by mechanical processing in the presence of water, with or without heat, to rupture all or part of the starch granules, and subsequent drying.

Content
— *hydroxypropyl groups*: 0.5 per cent to 7.0 per cent.

PRODUCTION
The production of pregelatinised hydroxypropyl starch shall be in compliance with the requirements of the European legislation for food additives.

CHARACTERS
Appearance
White or slightly yellowish powder.

IDENTIFICATION
A. It swells in cold water.

B. Disperse 0.5 g in 2 mL of *water R*, without heating, and add 0.05 mL of *iodine solution R1*. A reddish-violet or greyish-blue colour is produced.

C. To 0.1 g add 12.5 mL of *dilute sulfuric acid R*. Heat in a water-bath until the sample is dissolved. Cool and dilute to 100 mL with *water R*. Introduce 1 mL of the solution into a 25 mL graduated test-tube with a ground-glass stopper and, with the tube immersed in cold water, add dropwise 8 mL of *sulfuric acid R*. Mix well and place the tube in a water-bath for exactly 3 min. Immediately transfer the tube to an ice-bath until the solution is chilled. Add 0.6 mL of *ninhydrin solution R2*, carefully allowing the reagent to run down the walls of the test-tube. Immediately shake well, and place the tube in a water-bath at 25 °C for 100 min. Dilute to 25 mL with *sulfuric acid R* and mix by inverting the tube several times. Do not shake. A violet colour develops within 5 min.

TESTS
pH (*2.2.3*)
4.5 to 8.0.

Progressively add 3.0 g to 100.0 mL of *carbon dioxide-free water R*, stirring continuously. Determine the pH when a homogeneous solution is obtained.

Impurity A
Gas chromatography (*2.2.28*).

Internal standard solution Mix 50.0 mg of *propane-1,3-diol R* with *anhydrous pyridine R* and dilute to 100.0 mL with the same solvent.

Test solution To 0.200 g of the substance to be examined add 1.0 mL of the internal standard solution and 9.0 mL of *anhydrous pyridine R*. Heat under a reflux condenser for 20 min. Allow to cool. Transfer 1.0 mL of this solution to a 2 mL vial with a screw cap fitted with a septum. Add 0.1 mL of *chlorotrimethylsilane R* and 0.2 mL of *hexamethyldisilazane R*. Close and mix. Allow to stand for 15 min.

Reference solution Mix 50.0 mg of *propane-1,3-diol R* and 50.0 mg of *propylene glycol CRS* (impurity A) with *anhydrous pyridine R* and dilute to 100.0 mL with the same solvent. Transfer 0.1 mL of the solution to a 2 mL vial with a screw cap fitted with a septum. Add 0.1 mL of *chlorotrimethylsilane R*, 0.2 mL of *hexamethyldisilazane R* and 0.9 mL of *anhydrous pyridine R*. Close and mix. Allow to stand for 15 min.

Column:
- *material*: fused silica;
- *size*: l = 30 m, Ø = 0.32 mm;
- *stationary phase*: *methylpolysiloxane R* (film thickness 0.25 µm).

NOTE: the column must be desorbed regularly. Conditions: temperature program of 70 °C to 300 °C at a rate of 7 °C/min. Maintain for 10 min at 300 °C.

Carrier gas *helium for chromatography R*.

Flow rate 3 mL/min.

Split ratio 1:30.

Temperature:
- *column*: 70 °C;
- *injection port and detector*: 250 °C.

Detection Flame ionisation.

Injection 1 µL.

Relative retention With reference to the trimethylsilyl derivative of propane-1,3-diol (retention time = about 8.5 min): trimethylsilyl derivative of propylene glycol = about 0.7.

System suitability Reference solution:
- *resolution*: minimum 5.0 between the peaks due to the trimethylsilyl derivative of propylene glycol and the trimethylsilyl derivative of propane-1,3-diol.

Calculation of percentage contents Use the internal standard method.

Limit:
- *impurity A*: maximum 0.1 per cent.

Oxidising substances (*2.5.30*)
Maximum 20 ppm, calculated as H_2O_2.

Use a mixture of equal volumes of *methanol R* and *water R* as solvent.

Sulfur dioxide (*2.5.29*)
Maximum 50 ppm.

Iron (*2.4.9*)
- For pregelatinised hydroxypropyl starch obtained from maize, potato, cassava or rice: maximum 20 ppm.

Dissolve the residue obtained in the test for sulfated ash in 20 mL of *dilute hydrochloric acid R* and filter. The filtrate complies with the test for iron.
- For pregelatinised hydroxypropyl starch obtained from pea: maximum 50 ppm.

Dissolve the residue obtained in the test for sulfated ash in 50 mL of *dilute hydrochloric acid R* and filter. The filtrate complies with the test for iron.

Loss on drying
(*2.2.32*) determined on 1.000 g by drying in an oven at 130 °C for 90 min:
- maximum 15.0 per cent for pregelatinised hydroxypropyl starch obtained from maize, cassava, rice or pea;
- maximum 20.0 per cent for pregelatinised hydroxypropyl starch obtained from potato.

Sulfated ash (*2.4.14*)
Maximum 0.6 per cent, determined on 1.0 g.

Microbial contamination
TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*).

TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

Absence of *Escherichia coli* (*2.6.13*).

Absence of *Salmonella* (*2.6.13*).

ASSAY

Nuclear magnetic resonance spectrometry (*2.2.33*).

Internal standard solution Disperse 50.0 mg of *3-trimethylsilyl-1-propanesulfonic acid sodium salt CRS* in about 5 g of *deuterium oxide R1*, weighed to the nearest 0.1 mg. Store in a sealed bottle.

Test solution Dry 5.000 g of the substance to be examined at 130 °C for 90 min. Weigh 12.0 mg of the dried substance in a 5 mm NMR tube. Add 0.1 mL of *deuterium chloride solution R* and 0.75 mL of *deuterium oxide R1*. Cap the tube, mix, and place it in a water-bath until a clear solution is obtained (3 min to maximum 1 h). When a clear solution is obtained, allow to cool to room temperature. Dry the exterior of the tube and weigh to the nearest 0.1 mg. Add 0.05 mL of the internal standard solution and weigh to the nearest 0.1 mg. Determine the mass of the internal standard solution introduced. Mix thoroughly.

Apparatus FT-NMR spectrometer operating at minimum 300 MHz.

Acquisition of ^1H NMR spectra The following parameters may be used:
- *sweep width*: 8 ppm (−1.0 to + 7 ppm);
- *irradiation frequency offset*: none;
- *time domain*: at least 64 K;
- *pulse width*: 90°;
- *pulse delay*: 10 s;
- *dummy scans*: 0;
- *number of scans*: 8.

Use the CH_3 signal of the internal standard for shift referencing. The shift of the singlet is set to 0 ppm.

Record the FID signal.

Call the integration sub-routine after phase corrections and baseline correction between −0.5 ppm and + 6 ppm.

Measure the peak areas of the doublet from the methyl groups of the hydroxypropyl function at + 1.2 ppm (A_2), and of the methyl groups at 0 ppm of the internal standard (A_1) without ^{13}C-satellites.

Results Measure the signal coming from the 3 protons of the methyl group in the hydroxypropyl function; calculate the percentage content of hydroxypropyl groups using the following expression:

$$\frac{3A_2}{A_1} \times \frac{P}{100} \times \frac{W_1 \times m_1}{218} \times 59 \times \frac{100}{m}$$

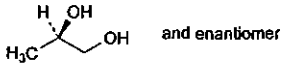
3 = numerical value representing the 3 methyl groups in the internal standard;

A_1	=	area of the methyl groups in the internal standard;
A_2	=	area of the methyl groups of hydroxypropyl;
P	=	percentage content of *3-trimethylsilyl-1-propanesulfonic acid sodium salt CRS*;
W_1	=	mass fraction of the internal standard in the internal standard solution, in milligrams per gram;
m_1	=	mass of the internal standard solution in the NMR tube, in grams;
218	=	molar mass of the internal standard, in grams per mole;
59	=	molar mass of the hydroxypropyl group, in grams per mole;
m	=	mass of the substance to be examined in the NMR tube, in milligrams.

LABELLING

The label states the botanical source of the starch and the type of modification.

IMPURITIES



and enantiomer

A. (2*RS*)-propane-1,2-diol (propylene glycol).

Ph Eur

Maize Starch[1]

(Ph. Eur. monograph 0344)

Action and use

Excipient.

When Starch is specified and the type is not indicated, Maize Starch, Potato Starch, Rice Starch, Wheat Starch or, in tropical countries where these are not available, Tapioca Starch may be supplied or used.

Ph Eur

DEFINITION

Maize starch is obtained from the caryopsis of *Zea mays* L.

♦CHARACTERS

Appearance

Matt, white to slightly yellowish, very fine powder that creaks when pressed between the fingers.

Solubility

Practically insoluble in cold water and in ethanol (96 per cent).

The presence of granules with cracks or irregularities on the edge is exceptional.♦

IDENTIFICATION

A. Microscopic examination (*2.8.23*), using a 50 per cent *V/V* solution of *glycerol R*. It appears as either angular polyhedral granules of irregular sizes with diameters ranging from about 2 µm to about 23 µm or as rounded or spheroidal granules of irregular sizes with diameters ranging from about 25 µm to about 35 µm (Figure 0344.-1). The central hilum consists of a distinct cavity or 2- to 5-rayed cleft and there are no concentric striations. Between orthogonally orientated polarising plates or prisms, the starch granules show a distinct black cross intersecting at the hilum.

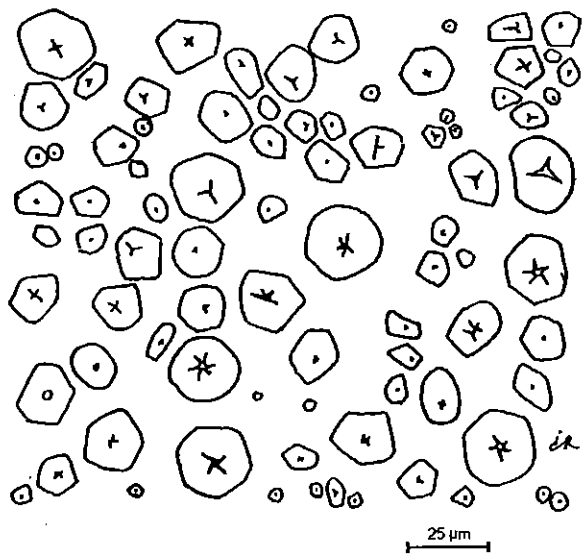


Figure 0344.-1. – *Illustration for identification test A of maize starch*

B. Suspend 1 g in 50 mL of *water R*, boil for 1 min and cool. A thin, cloudy mucilage is formed.

C. To 1 mL of the mucilage obtained in identification test B add 0.05 mL of *iodine solution R1*. An orange-red to dark blue colour is produced, which disappears on heating.

TESTS

pH (*2.2.3*)

4.0 to 7.0.

To 5.0 g add 25.0 mL of *carbon dioxide-free water R*. Agitate continuously at a moderate rate for 60 s. Stop the agitation and allow to stand for 15 min.

◊Foreign matter

Examined under a microscope using a 50 per cent *V/V* solution of *glycerol R*, not more than traces of matter other than starch granules are present. No starch grains of any other origin are present.◊

Oxidising substances (*2.5.30*)

Maximum 20 ppm, calculated as H_2O_2.

Sulfur dioxide (*2.5.29*)

Maximum 50 ppm.

Iron (*2.4.9*)

Maximum 10 ppm.

Shake 1.5 g with 15 mL of *dilute hydrochloric acid R*. Filter. The filtrate complies with the test.

Loss on drying (*2.2.32*)

Maximum 15.0 per cent, determined on 1.000 g by drying in an oven at 130 °C for 90 min.

Sulfated ash (*2.4.14*)

Maximum 0.6 per cent, determined on 1.0 g.

Microbial contamination

TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*).

TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

Absence of *Escherichia coli* (*2.6.13*).

◊Absence of *Salmonella* (*2.6.13*).◊

Ph Eur

[1] *This monograph has undergone pharmacopoeial harmonisation. See chapter 5.8 Pharmacopoeial harmonisation.*

Pea Starch

(Ph. Eur. monograph 2403)

Ph Eur

DEFINITION

Pea starch is obtained from the seeds of *Pisum sativum* L.

CHARACTERS

Appearance
White or almost white, very fine powder.

Solubility
Practically insoluble in cold water and in ethanol (96 per cent).

IDENTIFICATION

A. Microscopic examination (*2.8.23*) using a 50 per cent *V/V* solution of *glycerol R*. It presents a majority of large elliptical granules, 25-45 µm in size, sometimes irregular, or reniform. It also presents a minority of small rounded granules, 5-8 µm in size (Figure 2403.-1). Granules can present cracks or irregularities. Sometimes, granules show barely visible concentric striations. Exceptionally, granules show a slit along the main axis. Between orthogonally oriented polarising plates or prisms, the granules show a distinct black cross.

B. Suspend 1 g in 50 mL of *water R*, boil for 1 min and cool. A thin, cloudy mucilage is formed.

C. To 1 mL of the mucilage obtained in identification test B, add 0.05 mL of *iodine solution R1*. A dark blue colour is produced, which disappears on heating.

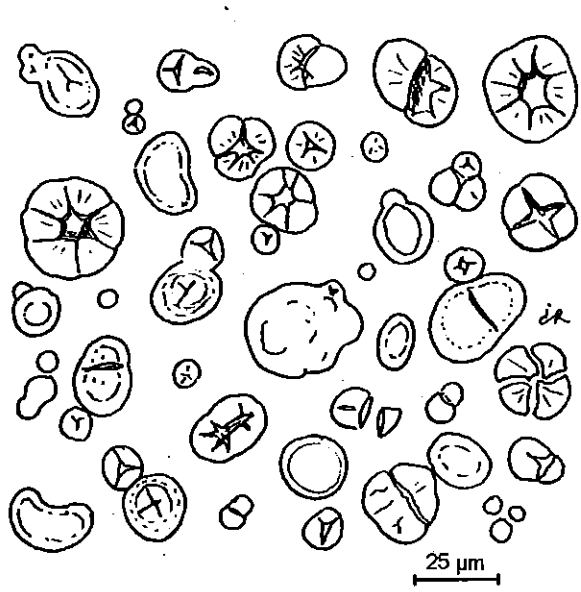


Figure 2403.-1. – *Illustration for identification test A of pea starch*

TESTS

pH (*2.2.3*)
5.0 to 8.0.

Shake 5.0 g with 25.0 mL of *carbon dioxide-free water R* for 60 s. Allow to stand for 15 min and shake again.

Foreign matter
Examined under a microscope using a 50 per cent *V/V* solution of *glycerol R*, not more than traces of matter other than starch granules are present. No starch granules of any other origin are present.

Oxidising substances (*2.5.30*)
Maximum 20 ppm, calculated as H_2O_2.

Sulfur dioxide (*2.5.29*)
Maximum 50 ppm.

Iron (*2.4.9*)
Maximum 50 ppm.

Shake 1.0 g with 50 mL of *dilute hydrochloric acid R*. Filter. The filtrate complies with the test for iron.

Loss on drying (*2.2.32*)
Maximum 16.0 per cent, determined on 1.000 g by drying in an oven at 130 °C for 90 min.

Sulfated ash (*2.4.14*)
Maximum 0.6 per cent, determined on 1.0 g.

Microbial contamination
TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*).
TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).
Absence of *Escherichia coli* (*2.6.13*).
Absence of *Salmonella* (*2.6.13*).

Ph Eur

Potato Starch[1]

(Ph. Eur. monograph 0355)

Action and use
Excipient.

When Starch is specified and the type is not indicated, Maize Starch, Potato Starch, Rice Starch, Wheat Starch or, in tropical countries where these are not available, Tapioca Starch may be supplied or used.

Ph Eur

DEFINITION

Potato starch is obtained from the tuber of *Solanum tuberosum* L.

♦CHARACTERS

Appearance
Very fine, white or almost white powder which creaks when pressed between the fingers.

Solubility
Practically insoluble in cold water and in ethanol (96 per cent).

Potato starch does not contain starch grains of any other origin. It may contain a minute quantity, if any, of tissue fragments of the original plant.♦

IDENTIFICATION

A. Microscopic examination (*2.8.23*) using a 50 per cent *V/V* solution of *glycerol R*. It presents granules, either irregularly shaped, ovoid or pear-shaped, usually 30-100 µm in size but occasionally exceeding 100 µm, or rounded, 10-35 µm in size. There are occasional compound granules having 2-4 components (Figure 0355.-1). The ovoid and pear-shaped granules have an eccentric hilum and the rounded granules acentric or slightly eccentric hilum. All granules show clearly visible concentric striations. Between orthogonally orientated polarising plates or prisms, the granules show a distinct black cross intersecting at the hilum.

[1] *This monograph has undergone pharmacopoeial harmonisation. See chapter 5.8 Pharmacopoeial harmonisation.*

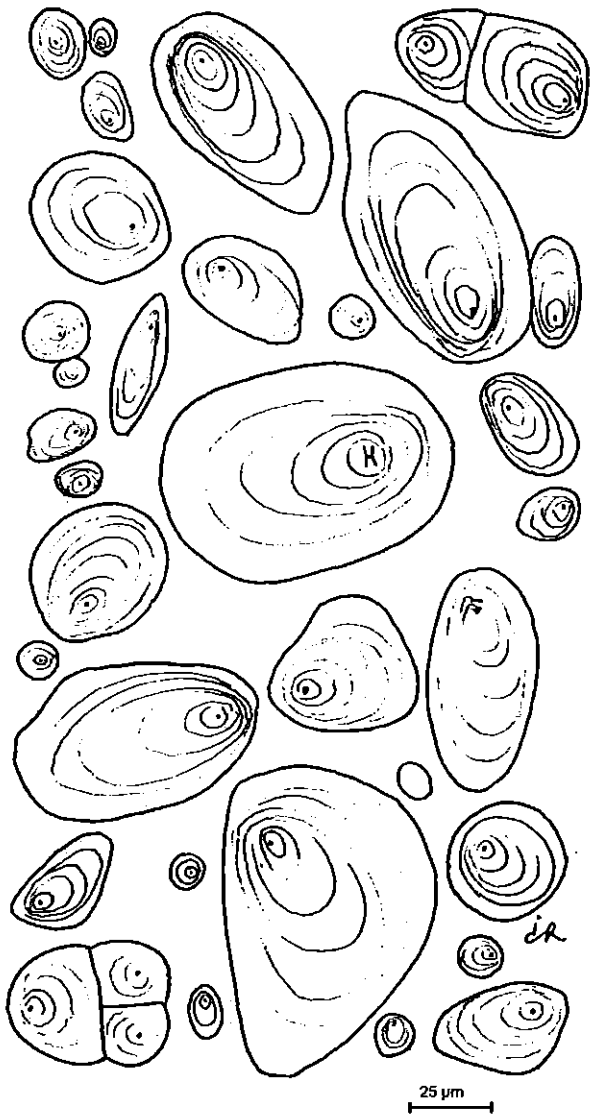


Figure 0355.-1. – *Illustration for identification test A of potato starch*

B. Suspend 1 g in 50 mL of *water R*, boil for 1 min and cool. A thick, opalescent mucilage is formed.

C. To 1 mL of the mucilage obtained in identification test B, add 0.05 mL of *iodine solution R1*. An orange-red to dark blue colour is produced which disappears on heating.

TESTS

pH (*2.2.3*)
5.0 to 8.0.

Shake 5.0 g with 25.0 mL of *carbon dioxide-free water R* for 60 s. Allow to stand for 15 min.

◊Foreign matter
Examined under a microscope using a 50 per cent *V/V* solution of *glycerol R*, not more than traces of matter other than starch granules are present. No starch grains of any other origin are present.◊

Oxidising substances (*2.5.30*)
Maximum 20 ppm, calculated as H_2O_2.

Sulfur dioxide (*2.5.29*)
Maximum 50 ppm.

Iron (*2.4.9*)
Maximum 10 ppm.

Shake 1.5 g with 15 mL of *dilute hydrochloric acid R*. Filter. The filtrate complies with the limit test for iron.

Loss on drying (*2.2.32*)
Maximum 20.0 per cent, determined on 1.000 g by drying in an oven at 130 °C for 90 min.

Sulfated ash (*2.4.14*)
Maximum 0.6 per cent, determined on 1.0 g.

Microbial contamination
TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*).
TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).
Absence of *Escherichia coli* (*2.6.13*).
◊Absence of *Salmonella* (*2.6.13*).◊

Ph Eur

Pregelatinised Starch

Pregelatinised Maize Starch
(Ph. Eur. monograph 1267)
When Pregelatinised Starch is prepared *Zea mays*, the title Pregelatinised Maize Starch may be used.

Ph Eur

DEFINITION
Pregelatinised starch is prepared from *Maize starch (0344)*, *Potato starch (0355)* or *Rice starch (0349)* by mechanical processing in the presence of water, with or without heat, to rupture all or part of the starch granules, and subsequent drying. It contains no added substances but it may be modified to render it compressible and to improve its flow characteristics.

CHARACTERS

Appearance
White or yellowish-white powder.

It swells in cold water.

IDENTIFICATION
A. Examined under a microscope using a mixture of equal volumes of *glycerol R* and *water R* it presents irregular, translucent, white or yellowish-white flakes or pieces with an uneven surface. Under polarised light (between crossed nicol prisms), starch granules with a distinct black cross intersecting at the hilum may be seen.

B. Disperse 0.5 g in 2 mL of *water R* without heating and add 0.05 mL of *iodine solution R1*. A reddish-violet or blue colour is produced.

TESTS

pH (*2.2.3*)
4.5 to 7.0.

Progressively add 3.0 g to 100.0 mL of *carbon dioxide-free water R*, stirring continuously. Determine the pH when a homogeneous solution is obtained.

Oxidising substances (*2.5.30*)
It complies with the test for oxidising substances. Use a mixture of equal volumes of *methanol R* and *water R* as solvent.

Sulfur dioxide (*2.5.29*)
Maximum 50 ppm.

Iron (*2.4.9*)
Maximum 20 ppm.

Dissolve the residue obtained in the test for sulfated ash in 20 mL of *dilute hydrochloric acid R*. Filter. The filtrate complies with the test.

Foreign matter
Examined under a microscope using a mixture of equal volumes of *glycerol R* and *water R*, not more than traces of matter other than starch granules are present.

Loss on drying (*2.2.32*)
Maximum 15.0 per cent, determined on 1.000 g by drying in an oven at 130 °C for 90 min.

Sulfated ash (*2.4.14*)
Maximum 0.6 per cent, determined on 1.0 g.

Microbial contamination
TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*).

TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

Absence of *Escherichia coli* (*2.6.13*).

Absence of *Salmonella* (*2.6.13*).

LABELLING
The label states the type of starch used as starting material.

FUNCTIONALITY-RELATED CHARACTERISTICS
This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for pregelatinised starch used as filler, binder or disintegrant in tablets and in hard capsules.

Cold-water-soluble matter
Transfer 100 mL of *water R* at 25 ± 1 °C into a beaker and add 1.000-3.000 g of the substance to be examined while stirring. Continue to stir for 10 min. Transfer 35 mL of the dispersion to a centrifuge tube and centrifuge at 3000 *g* for 15 min. Transfer 25 mL of the supernatant to a crucible that has previously been dried in an oven at 120 ± 2 °C for 4 h and weighed to the nearest 0.1 mg. Evaporate to dryness on a water-bath, then place the crucible in an oven at 120 ± 2 °C for 4 h. Allow to cool in a desiccator. Weigh the crucible to the nearest 0.1 mg again.

Determine the percentage of cold-water-soluble matter using the following expression:

$$\frac{(B-A)\times\frac{100}{25}\times 100}{S\times\frac{100-C}{100}}$$

A = initial crucible mass, in grams;
B = final crucible mass, in grams;
C = loss on drying, in per cent;
S = sample mass, in grams.

Particle-size distribution (*2.9.31* or *2.9.38*)

Powder flow (*2.9.36*)

Ph Eur

Rice Starch[1]

(Ph. Eur. monograph 0349)

Action and use
Excipient.

When Starch is specified and the type is not indicated, Maize Starch, Potato Starch, Rice Starch, Wheat Starch or, in tropical countries where these are not available, Tapioca Starch may be supplied or used.

Ph Eur

DEFINITION
Rice starch is obtained from the caryopsis of *Oryza sativa* L.

♦CHARACTERS
Appearance
Very fine, white or almost white powder, which creaks when pressed between the fingers.

Solubility
Practically insoluble in cold water and in ethanol (96 per cent).

Rice starch does not contain starch grains of any other origin. It may contain traces of, if any, fragments of the endosperm tissue of the fruit.♦

IDENTIFICATION
A. Microscopic examination (*2.8.23*) using a 50 per cent *V/V* solution of *glycerol R*. It presents polyhedral, simple grains 1-10 µm (mostly 4-6 µm) in size (Figure 0349.-1). These simple grains often gather in ellipsoidal, compound grains 50-100 µm in diameter. The grains have a poorly visible central hilum and there are no concentric striations. Between orthogonally orientated polarising plates or prisms, the starch grains show a distinct black cross intersecting at the hilum.

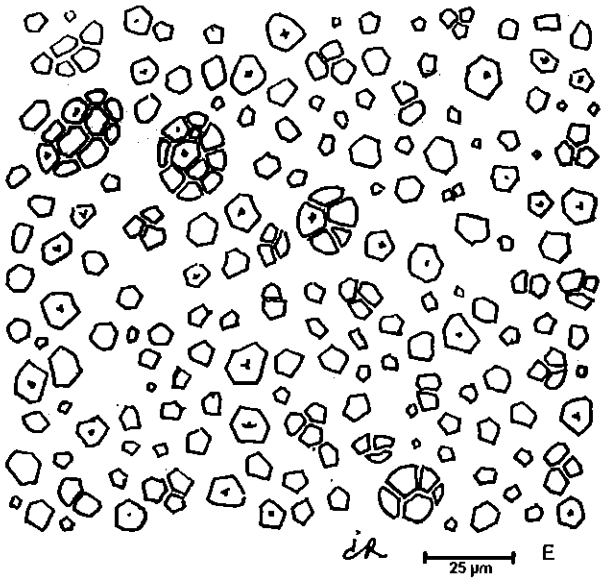


Figure 0349.-1. – *Illustration for identification test A of rice starch*

B. Suspend 1 g in 50 mL of *water R*, boil for 1 min and cool. A thin, cloudy mucilage is formed.

[1] *This monograph has undergone pharmacopoeial harmonisation. See chapter 5.8 Pharmacopoeial harmonisation.*

C. To 1 mL of the mucilage obtained in identification test B add 0.05 mL of *iodine solution R1*. An orange-red to dark blue colour is produced, which disappears on heating.

TESTS

pH (*2.2.3*)
5.0 to 8.0.

Shake 5.0 g with 25.0 mL of *carbon dioxide-free water R* for 60 s. Allow to stand for 15 min.

♦Foreign matter
Examined under a microscope using a 50 per cent *V/V* solution of *glycerol R*, not more than traces of matter other than starch granules are present. No starch grains of any other origin are present.♦

Oxidising substances (*2.5.30*)
Maximum 20 ppm, calculated as H_2O_2.

Sulfur dioxide (*2.5.29*)
Maximum 50 ppm.

Iron (*2.4.9*)
Maximum 10 ppm.

Shake 1.5 g with 15 mL of *dilute hydrochloric acid R*. Filter. The filtrate complies with the test.

Loss on drying (*2.2.32*)
Maximum 15.0 per cent, determined on 1.00 g by drying in an oven at 130 °C for 90 min.

Sulfated ash (*2.4.14*)
Maximum 0.6 per cent, determined on 1.0 g.

Microbial contamination
TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*).

TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

Absence of *Escherichia coli* (*2.6.13*).

◊Absence of *Salmonella* (*2.6.13*).◊

Ph Eur

Tapioca Starch

Cassava Starch

Action and use
Excipient.

When Starch is specified and the type is not indicated, Maize Starch, Potato Starch, Rice Starch, Wheat Starch or, in tropical and sub-tropical countries where these are not available, Tapioca Starch may be supplied or used.

DEFINITION
Tapioca Starch is obtained from the rhizomes of *Manihot utilissima* Pohl.

CHARACTERISTICS
Very fine powder which creaks when pressed between the fingers.

Practically insoluble in cold *water* and in *ethanol (96%)*.

IDENTIFICATION
A. Principally simple granules, subspherical, muller-shaped or rounded polyhedral; smaller granules 5 to 10 µm, larger granules 20 to 35 µm in diameter; hilum, central, punctate, linear or triradiate; striations, faint, concentric; compound granules, few, of two to three unequal components.

B. Heat to boiling a suspension of 1 g in 50 mL of *water* for 1 minute and cool. A thin, cloudy mucilage is formed.

C. Mix 0.05 mL of *iodine solution R1* with 1 mL of the mucilage obtained in test B. A dark blue colour is produced which disappears on heating and reappears on cooling.

TESTS

Acidity
Add 10 g of the starch to 100 mL of *ethanol (70%)* previously neutralised to 0.5 mL of *phenolphthalein solution*, shake for 1 hour, filter and titrate 50 mL of the filtrate with *0.1M sodium hydroxide VS*. Not more than 2.0 mL is required to change the colour of the solution.

Foreign matter
Not more than traces of cell membranes and protoplasm are present.

Loss on drying
When dried to constant weight at 100° to 105°, loses not more than 15.0% of its weight. Use 1 g.

Sulfated ash
Not more than 0.6%, Appendix IX A, Method II. Use 1 g.

Microbial contamination
1.0 g is free from *Escherichia coli*, Appendix XVI B1.

STORAGE
Tapioca Starch should be kept in an airtight container.

Wheat Starch[1]

(Ph. Eur. monograph 0359)

Action and use
Excipient.

When Starch is specified and the type is not indicated, Maize Starch, Potato Starch, Rice Starch, Wheat Starch or, in tropical countries where these are not available, Tapioca Starch may be supplied or used.

Ph Eur

DEFINITION
Wheat starch is obtained from the caryopsis of *Triticum aestivum* L. (*T. vulgare* Vill.).

◊PRODUCTION
The gluten content is monitored and stated on the label.◊

♦CHARACTERS

Appearance
Very fine, white or almost white powder that creaks when pressed between the fingers.

Solubility
Practically insoluble in cold water and in ethanol (96 per cent).

Wheat starch does not contain starch granules of any other origin. It may contain a minute quantity, if any, of tissue fragments of the original plant.♦

IDENTIFICATION
A. Microscopic examination (*2.8.23*) using a 50 per cent *V/V* solution of *glycerol R*. It shows large and small granules, and, very rarely, intermediate sizes (Figure 0359.-1). The large granules, 10-60 µm in diameter, are discoid or, more rarely, reniform in surface view. The central hilum and striations are invisible or barely visible and the granules sometimes show cracks on the edges. In side view, the granules are elliptical

[1] *This monograph has undergone pharmacopoeial harmonisation. See chapter 5.8 Pharmacopoeial harmonisation.*

and fusiform and the hilum appears as a slit along the main axis. The small granules, rounded or polyhedral, are 2-10 µm in diameter. Between orthogonally orientated polarising plates or prisms, the granules show a distinct black cross intersecting at the hilum.

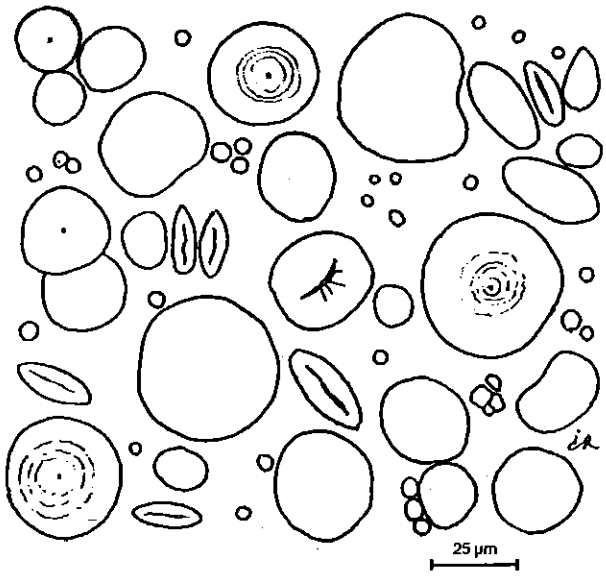


Figure 0359.-1. – *Illustration for identification test A of wheat starch*

B. Suspend 1 g in 50 mL of *water R*, boil for 1 min and cool. A thin, cloudy mucilage is formed.

C. To 1 mL of the mucilage obtained in identification test B add 0.05 mL of *iodine solution R1*. A dark blue colour is produced, which disappears on heating.

TESTS

pH (*2.2.3*)
4.5 to 7.0.

Shake 5.0 g with 25.0 mL of *carbon dioxide-free water R* for 60 s. Allow to stand for 15 min.

◊Foreign matter
Examined under a microscope using a 50 per cent *V/V* solution of *glycerol R*, not more than traces of matter other than starch granules are present. No starch granules of any other origin are present.◊

Total protein
Maximum 0.3 per cent (corresponding to 0.048 per cent of nitrogen).

Determine the nitrogen content by sulfuric acid digestion as follows, and calculate the quantity of protein by multiplying by 6.25.

Carry out a blank determination by placing 4 g of a powdered mixture of 100 g of *dipotassium sulfate R*, 3 g of *copper sulfate pentahydrate R* and 3 g of *titanium dioxide R*, and 3 glass beads in a combustion flask. Wash any adhering particles from the neck into the flask with 25 mL of *sulfuric acid R*, allowing it to run down the sides of the flask, and swirl to mix the contents. Close the mouth of the flask loosely, for example by means of a glass bulb with a short stem, to avoid excessive loss of sulfuric acid. Heat gradually at first, then increase the temperature until there is vigorous boiling with condensation of sulfuric acid in the neck of the flask; take precautions to prevent the upper part of the flask from becoming overheated. Continue heating until a clear solution is obtained. Cool, dissolve the solid material by cautiously adding 25 mL of *water R* to the mixture, cool again and place in a steam-distillation apparatus. Add a suitable volume of *strong sodium hydroxide solution R* to change the colour of the solution from bluish-green to brown or black, and distil immediately by passing steam through the mixture. Collect about 40 mL of distillate in 50.0 mL of *0.01 M hydrochloric acid*, adding enough *water R* if necessary to cover the tip of the condenser. Towards the end of the distillation, lower the receiver so that the tip of the condenser is above the surface of the acid. Take precautions to prevent any water on the outer surface of the condenser from reaching the contents of the receiver. Titrate the distillate with *0.025 M sodium hydroxide* (n_1 mL), using *methyl red mixed solution R* as indicator.

Repeat the test adding 3.0 g (m g) of the substance to be examined to the combustion flask, and using the same volume of *strong sodium hydroxide solution R*. Titrate the distillate as described for the blank determination with *0.025 M sodium hydroxide* (n_2 mL). Calculate the percentage content of nitrogen using the following expression:

$$\frac{0.03503(n_1 - n_2)}{m}$$

Oxidising substances (*2.5.30*)
Maximum 20 ppm, calculated as H_2O_2.

Sulfur dioxide (*2.5.29*)
Maximum 50 ppm.

Iron (*2.4.9*)
Maximum 10 ppm.

Shake 1.5 g with 15 mL of *dilute hydrochloric acid R*. Filter. The filtrate complies with the test.

Loss on drying (*2.2.32*)
Maximum 15.0 per cent, determined on 1.000 g by drying in an oven at 130 °C for 90 min.

Sulfated ash (*2.4.14*)
Maximum 0.6 per cent, determined on 1.0 g.

Microbial contamination
TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*).

TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

Absence of *Escherichia coli* (*2.6.13*).

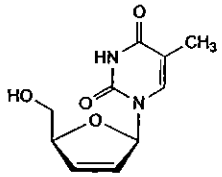
◊Absence of *Salmonella* (*2.6.13*).◊

◊LABELLING
The label states the gluten content.◊

Ph Eur

Stavudine

(Ph. Eur. monograph 2130)



$C_{10}H_{12}N_2O_4$ 224.2 *3056-17-5*

Action and use
Nucleoside reverse transciptase inhibitor; antiviral (HIV).

Ph Eur

DEFINITION

1-(2,3-Dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Soluble in water, sparingly soluble in ethanol (96 per cent), slightly soluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Specific optical rotation (*2.2.7*): −45.9 to −39.5 (anhydrous substance).

Dissolve 0.100 g in *water R* and dilute to 10.0 mL with the same solvent.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *stavudine CRS*.

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *anhydrous ethanol R*, evaporate to dryness and record new spectra using the residues.

TESTS

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use or store them at 2-8 °C until use.*

Test solution Dissolve 25.0 mg of the substance to be examined in *water R* and dilute to 50.0 mL with the same solvent.

Reference solution (a) Dilute 0.5 mL of the test solution to 100.0 mL with *water R*.

Reference solution (b) Dilute 20.0 mL of reference solution (a) to 100.0 mL with *water R*.

Reference solution (c) Dissolve 5 mg of *stavudine for system suitability CRS* (containing impurities A, B, C, E and G) in *water R* and dilute to 10.0 mL with the same solvent.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A*: mix 35 volumes of *acetonitrile for chromatography R* and 965 volumes of a 0.77 g/L solution of *ammonium acetate R*;
- *mobile phase B*: mix 250 volumes of *acetonitrile for chromatography R* and 750 volumes of a 0.77 g/L solution of *ammonium acetate R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	100	0
10 - 20	100 → 0	0 → 100
20 - 30	0	100

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µL.

Identification of impurities Use the chromatogram supplied with *stavudine for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, C, E and G.

Relative retention With reference to stavudine (retention time = about 10 min): impurity A = about 0.3; impurity B = about 0.50; impurity C = about 0.53; impurity E = about 1.1; impurity G = about 1.9.

System suitability Reference solution (c):
- *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity B; minimum 1.5, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to stavudine.

Calculation of percentage contents:
- *correction factor*: multiply the peak area of impurity A by 0.7;
- for impurity A, use the concentration of stavudine in reference solution (a);
- for impurities other than A, use the concentration of stavudine in reference solution (b).

Limits:
- *impurity A*: maximum 0.5 per cent;
- *impurity G*: maximum 0.2 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 1.0 per cent;
- *reporting threshold*: 0.05 per cent.

Impurity I

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use or store them at 2-8 °C until use.*

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 2 mg of *stavudine impurity I CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 20.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 30 volumes of *acetonitrile for chromatography R* and 70 volumes of a 1.15 g/L solution of *ammonium dihydrogen phosphate R* previously adjusted to pH 6.8 with *triethylamine R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 266 nm.

Injection 20 µL.

Run time 7 times the retention time of stavudine.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity I.

Relative retention With reference to stavudine (retention time = about 3 min): impurity I = about 6.0.

System suitability Reference solution (b):
- *signal-to-noise ratio*: minimum 40 for the principal peak.

Calculation of percentage content:
— *correction factor*: multiply the peak area of impurity I by 1.7;
— for impurity I, use the concentration of stavudine in reference solution (b).

Limit:
— *impurity I*: maximum 0.15 per cent.

Water (*2.5.12*)
Maximum 0.5 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use or store them at 2-8 °C until use.*

Test solution Dissolve 10.0 mg of the substance to be examined in *water R* and dilute to 100.0 mL with the same solvent. Dilute 10.0 mL of the solution to 50.0 mL with *water R*.

Reference solution (a) Dissolve 10.0 mg of *stavudine CRS* in *water R* and dilute to 100.0 mL with the same solvent. Dilute 10.0 mL of the solution to 50.0 mL with *water R*.

Reference solution (b) Dissolve 5 mg of *thymine R* (impurity A) and 7.5 mg of *thymidine R* (impurity C) in *water R* and dilute to 100.0 mL with the same solvent. Dilute 10.0 mL of the solution to 50.0 mL with *water R*.

Column:
— *size*: *l* = 0.033 m, Ø = 4.0 mm;
— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase Mix 5 volumes of *acetonitrile for chromatography R* and 95 volumes of a 0.77 g/L solution of *ammonium acetate R*.

Flow rate 0.7 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 25 µL.

Run time Twice the retention time of stavudine.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and C.

Relative retention With reference to stavudine (retention time = about 4 min): impurity A = about 0.4; impurity C = about 0.6.

System suitability:
— *resolution*: minimum 3.5 between the peaks due to impurities A and C in the chromatogram obtained with reference solution (b);
— *symmetry factor*: maximum 1.6 for the peak due to stavudine in the chromatogram obtained with reference solution (a).

Calculate the percentage content of $C_{10}H_{12}N_2O_4$ using the chromatograms obtained with the test solution and reference solution (a) and taking into account the assigned content of *stavudine CRS*.

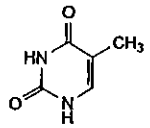
STORAGE

Protected from light and humidity.

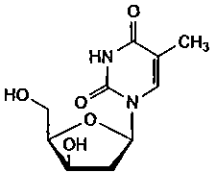
IMPURITIES

Specified impurities *A, G, I.*

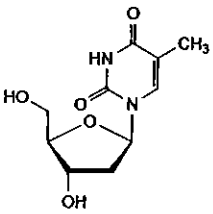
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D, E, F, H.*



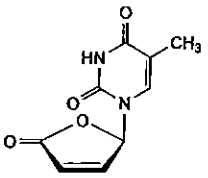
A. 5-methylpyrimidine-2,4(1*H*,3*H*)-dione (thymine),



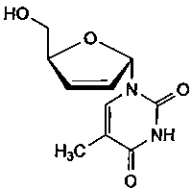
B. 1-(2-deoxy-β-D-*threo*-pentofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (3′-epithymidine),



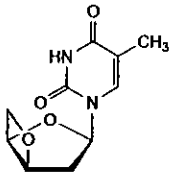
C. 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (thymidine),



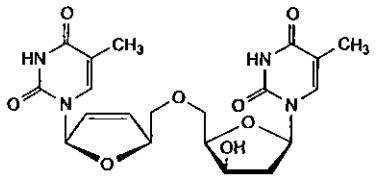
D. 1-[(2*R*)-5-oxo-2,5-dihydrofuran-2-yl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione,



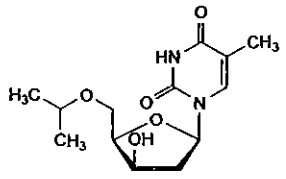
E. 1-(2,3-dideoxy-α-D-*glycero*-pent-2-enofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (stavudine anomer α),



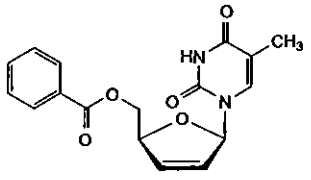
F. 1-(3,5-anhydro-2-deoxy-β-D-*threo*-pentofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione,



G. 1-[2-deoxy-5-*O*-[[(2*S*,5*R*)-5-[5-methyl-2,4-dioxo-3,4-dihydropyrimidine-1(2*H*)-yl]-2,5-dihydrofuran-2-yl]methyl]-β-D-*threo*-pentofuranosyl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione,



H. 1-[2-deoxy-5-*O*-(1-methylethyl)-β-D-*threo*-pentofuranosyl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione,



I. 1-(5-*O*-benzoyl-2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione.

Ph Eur

Stearic Acid[1]

(Ph. Eur. monograph 1474)

Action and use
Excipient.

Ph Eur

DEFINITION
Mixture consisting mainly of stearic (octadecanoic) acid ($C_{18}H_{36}O_2$; M_r 284.5) and palmitic (hexadecanoic) acid ($C_{16}H_{32}O_2$; M_r 256.4) obtained from fats or oils of vegetable or animal origin.

Content

Stearic acid 50	*Stearic acid*: 40.0 per cent to 60.0 per cent. *Sum of the contents of stearic and palmitic acids*: minimum 90.0 per cent.
Stearic acid 70	*Stearic acid*: 60.0 per cent to 80.0 per cent. *Sum of the contents of stearic and palmitic acids*: minimum 90.0 per cent.
Stearic acid 95	*Stearic acid*: minimum 90.0 per cent. *Sum of the contents of stearic and palmitic acids*: minimum 96.0 per cent.

[1] *This monograph has undergone pharmacopoeial harmonisation. See chapter 5.8 Pharmacopoeial harmonisation.*

♦CHARACTERS
Appearance
White or almost white, waxy, flaky crystals, white or almost white hard masses, or white or yellowish-white powder.

Solubility
Practically insoluble in water, soluble in ethanol (96 per cent) and in light petroleum (bp: 50-70 °C).♦

IDENTIFICATION
A. Freezing point (see Tests).

B. Acid value (*2.5.1*): 194 to 212, determined on 0.5 g.

C. Examine the chromatograms obtained in the assay.

Results The principal peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with the reference solution.

TESTS
◊Appearance
Heat the substance to be examined to about 75 °C.
The resulting liquid is not more intensely coloured than reference solution Y_7 or BY_7 (*2.2.2, Method I*). ◊

Acidity
Melt 5.0 g, shake for 2 min with 10 mL of hot *carbon dioxide-free water R*, cool slowly and filter. To the filtrate add 0.05 mL of *methyl orange solution R*. No red colour develops.

Iodine value (*2.5.4*)
See Table 1474.-1.

Freezing point (*2.2.18*)
See Table 1474.-1.

Table 1474.-1.

Type	Iodine value	Freezing point (°C)
Stearic acid 50	maximum 4.0	53 - 59
Stearic acid 70	maximum 4.0	57 - 64
Stearic acid 95	maximum 1.5	64 - 69

ASSAY
Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution In a conical flask fitted with a reflux condenser, dissolve 0.100 g of the substance to be examined in 5 mL of *boron trifluoride-methanol solution R*. Boil under reflux for 10 min. Add 4.0 mL of *heptane R* through the condenser and boil again under reflux for 10 min. Allow to cool. Add 20 mL of a saturated solution of *sodium chloride R*. Shake and allow the layers to separate. Remove about 2 mL of the organic layer and dry it over 0.2 g of *anhydrous sodium sulfate R*. Dilute 1.0 mL of this solution to 10.0 mL with *heptane R*.

Reference solution Prepare the reference solution in the same manner as the test solution using 50 mg of *palmitic acid CRS* and 50 mg of *stearic acid CRS* instead of the substance to be examined.

Column:
— *material*: fused silica;
— *size*: *l* = 30 m, Ø = 0.32 mm;
— *stationary phase*: *macrogol 20 000 R* (film thickness 0.5 µm).

Carrier gas *helium for chromatography R*.

Flow rate 2.4 mL/min.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 2	70
	2 - 36	70 → 240
	36 - 41	240
Injection port		220
Detector		260

Detection Flame ionisation.

Injection 1 µL.

Relative retention With reference to methyl stearate: methyl palmitate = about 0.9.

System suitability Reference solution:
- *resolution*: minimum 5.0 between the peaks due to methyl palmitate and methyl stearate;
- *repeatability*: maximum relative standard deviation of 3.0 per cent for the areas of the peaks due to methyl palmitate and methyl stearate, determined on 6 injections; maximum 1.0 per cent for the ratio of the areas of the peaks due to methyl palmitate to the areas of the peaks due to methyl stearate, determined on 6 injections.

LABELLING

The label states the type of stearic acid (50, 70, 95).

◊FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for stearic acid used as a lubricant in tablets and capsules.

Particle-size distribution (*2.9.31*)

Specific surface area
(*2.9.26, Method I*)◊.

Ph Eur

Stearoyl Macrogolglycerides

(Ph. Eur. monograph 1268)

Action and use
Excipient.

Ph Eur

DEFINITION

Mixtures of monoesters, diesters and triesters of glycerol and monoesters and diesters of macrogols with a mean relative molecular mass between 300 and 4000.

They are obtained by partial alcoholysis of saturated oils containing mainly triglycerides of stearic (octadecanoic) acid, using macrogol, or by esterification of glycerol and macrogol with saturated fatty acids, or by mixing glycerol esters and condensates of ethylene oxide with the fatty acids of these hydrogenated oils.

The hydroxyl value is within 15 units of the nominal value. The saponification value is within 10 units of the nominal value.

CHARACTERS

Appearance
Pale yellow waxy solid.

Solubility
Dispersible in warm water and in warm liquid paraffin, freely soluble in methylene chloride, soluble in warm anhydrous ethanol.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 1.0 g of the substance to be examined in *methylene chloride R* and dilute to 20 mL with the same solvent.

Plate *TLC silica gel plate R.*

Mobile phase *hexane R, ether R* (30:70 *V/V*).

Application 10 µL.

Development Over a path of 15 cm.

Drying In air.

Detection Spray with a 0.1 g/L solution of *rhodamine B R* in *ethanol (96 per cent) R* and examine in ultraviolet light at 365 nm.

Results The chromatogram shows a spot due to triglycerides with an R_F value of about 0.9 (R_{st} 1) and spots due to 1,3-diglycerides (R_{st} 0.7), to 1,2-diglycerides (R_{st} 0.6), to monoglycerides (R_{st} 0.1) and to esters of macrogol (R_{st} 0).

B. Hydroxyl value (see Tests).

C. Saponification value (see Tests).

D. Composition of fatty acids (see Tests).

TESTS

Acid value (*2.5.1*)
Maximum 2.0, determined on 2.0 g.

Hydroxyl value (*2.5.3, Method A*)
Within 15 units of the nominal value, determined on 1.0 g.

Peroxide value (*2.5.5, Method A*)
Maximum 6.0, determined on 2.0 g.

Saponification value (*2.5.6*)
Within 10 units of the nominal value, determined on 2.0 g.

Alkaline impurities
Introduce 5.0 g into a test-tube and carefully add a mixture, neutralised if necessary with *0.01 M hydrochloric acid* or with *0.01 M sodium hydroxide*, of 0.05 mL of a 0.4 g/L solution of *bromophenol blue R* in *ethanol (96 per cent) R*, 0.3 mL of *water R* and 10 mL of *ethanol (96 per cent) R*. Shake and allow to stand. Not more than 1.0 mL of *0.01 M hydrochloric acid* is required to change the colour of the upper layer to yellow.

Free glycerol
Maximum 3.0 per cent.

Dissolve 1.20 g in 25.0 mL of *methylene chloride R*. Heat if necessary. After cooling, add 100 mL of *water R*. Shake and add 25.0 mL of *periodic acetic acid solution R*. Shake and allow to stand for 30 min. Add 40 mL of a 75 g/L solution of *potassium iodide R*. Allow to stand for 1 min. Add 1 mL of *starch solution R*. Titrate the iodine with *0.1 M sodium thiosulfate*. Carry out a blank titration.

1 mL of *0.1 M sodium thiosulfate* is equivalent to 2.3 mg of glycerol.

Composition of fatty acids
Gas chromatography (*2.4.22, Method A*).

Composition of the fatty-acid fraction of the substance:
— *lauric acid*: maximum 5.0 per cent;
— *myristic acid*: maximum 5.0 per cent;
— *stearic acid and palmitic acid*: different nominal amounts and minimum 90.0 per cent for the sum of $C_{18}H_{36}O_2$ and $C_{16}H_{32}O_2$.

Ethylene oxide and dioxan (*2.4.25*)
Maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

Water (*2.5.12*)
Maximum 1.0 per cent, determined on 1.00 g. Use a mixture of 30 volumes of *anhydrous methanol R* and 70 volumes of *methylene chloride R* as solvent.

Total ash (*2.4.16*)
Maximum 0.2 per cent.

LABELLING

The label states:
— the nominal hydroxyl value;
— the nominal saponification value;
— the type of the macrogol used (mean relative molecular mass) or the number of moles of ethylene oxide reacted per mole of substance (nominal value).

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for stearoyl macrogolglycerides used as self-emulsifying agents, solubilisers, modified-release agents and wetting agents.

Hydroxyl value
(see Tests).

Saponification value
(see Tests).

Composition of fatty acids
(see Tests).

Ph Eur

Stearyl Alcohol

(Ph. Eur. monograph 0753)

Action and use
Excipient.

Ph Eur

DEFINITION

Mixture of solid alcohols, mainly octadecan-1-ol ($C_{18}H_{38}O$; M_r 270.5), of animal or vegetable origin.

Content
Minimum 95.0 per cent of $C_{18}H_{38}O$.

CHARACTERS

Appearance
White or almost white, unctuous flakes, granules or mass.

Solubility
Practically insoluble in water, soluble in ethanol (96 per cent). When melted, it is miscible with fatty oils, with liquid paraffin and with melted wool fat.

IDENTIFICATION

Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (b).

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution B_6 (*2.2.2, Method II*).

Dissolve 0.50 g in 20 mL of boiling *ethanol (96 per cent) R*. Allow to cool.

Melting point (*2.2.14*)
57 °C to 60 °C.

Acid value (*2.5.1*)
Maximum 1.0.

Hydroxyl value (*2.5.3, Method A*)
197 to 217.

Iodine value (*2.5.4, Method A*)
Maximum 2.0.

Dissolve 2.00 g in *methylene chloride R*, warming if necessary and dilute to 25 mL with the same solvent.

Saponification value (*2.5.6*)
Maximum 2.0.

ASSAY

Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution Dissolve 0.100 g of the substance to be examined in *ethanol (96 per cent) R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dissolve 50 mg of *cetyl alcohol R* in *ethanol (96 per cent) R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 50 mg of *stearyl alcohol CRS* in *ethanol (96 per cent) R* and dilute to 5 mL with the same solvent.

Reference solution (c) Mix 1 mL of reference solution (a) and 1 mL of reference solution (b) and dilute to 10 mL with *ethanol (96 per cent) R*.

Column:
— *size*: l = 30 m, Ø = 0.32 mm,

— *stationary phase*: *methylpolysiloxane R* (1 µm).

Carrier gas *helium for chromatography R.*

Flow rate 1 mL/min.

Split ratio 1:100.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 20	150 → 250
	20 - 40	250
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 1 µL of the test solution and reference solutions (b) and (c).

System suitability Reference solution (c):

— *resolution*: minimum 5.0 between the peaks due to cetyl alcohol and stearyl alcohol.

Calculate the percentage content of $C_{18}H_{38}O$.

Ph Eur

Streptokinase Concentrated Solution

Streptokinase Bulk Solution

(Ph. Eur. monograph 0356)

Action and use

Plasminogen activator; fibrinolytic.

Preparation

Streptokinase Injection

Ph Eur

DEFINITION

Streptokinase concentrated solution is a preparation of a protein obtained from culture filtrates of certain strains of haemolytic *Streptococcus* group C; it has the property of combining with human plasminogen to form plasminogen activator. It may contain buffer salts and other excipients. The potency is not less than 510 IU per microgram of nitrogen.

CHARACTERS

Appearance

Clear, colourless liquid.

IDENTIFICATION

A. Place 0.5 mL of citrated human plasma in a polystyrene tube maintained in a water-bath at 37 °C. Add 0.1 mL of a dilution of the preparation to be examined containing 10 000 IU of streptokinase activity per millilitre in *phosphate buffer solution pH 7.2 R* and 0.1 mL of a solution of *human thrombin R* containing 20 IU/mL in *phosphate buffer solution pH 7.2 R*. Mix immediately. A clot forms and lyses within 30 min. Repeat the procedure using citrated bovine plasma. The clot does not lyse within 60 min.

B. Perform an immunochemical test using double immunodiffusion techniques (*2.7.1*). Place in the central cavity about 80 µL of goat or rabbit antistreptokinase serum containing about 10 000 units of antistreptokinase activity per millilitre; place in each of the surrounding cavities about 80 µL of a dilution of the preparation to be examined containing 125 000 IU of streptokinase activity per millilitre. Allow the plates to stand in a humidified tank for 24 h. Only one precipitation arc appears and it is well defined.

TESTS

pH (*2.2.3*)

6.8 to 7.5.

Dilute the preparation to be examined in *carbon dioxide-free water R* to obtain a solution containing at least 1000 000 IU of streptokinase activity per millilitre.

Streptodornase

Maximum 10 IU of streptodornase activity per 100 000 IU of streptokinase activity.

Test solution Dilute the preparation to be examined in *imidazole buffer solution pH 6.5 R* to obtain a solution containing 150 000 IU of streptokinase activity per millilitre.

Reference solution Dissolve in *imidazole buffer solution pH 6.5 R* a reference preparation of streptodornase, calibrated in International Units against the International Standard of streptodornase, to obtain a solution containing 20 IU of streptodornase activity per millilitre.

The equivalence in International Units of the International Standard is stated by the World Health Organization.

To each of 8 numbered centrifuge tubes, add 0.5 mL of a 1 g/L solution of *sodium deoxyribonucleate R* in *imidazole buffer solution pH 6.5 R*. To tube number 1 and tube number 2 add 0.25 mL of *imidazole buffer solution pH 6.5 R*, 0.25 mL of the test solution and, immediately, 3.0 mL of perchloric acid (25 g/L $HClO_4$). Mix, centrifuge at about 3000 *g* for 5 min and measure the absorbances (*2.2.25*) of the supernatant liquids at 260 nm, using as the compensation liquid a mixture of 1.0 mL of *imidazole buffer solution pH 6.5 R* and 3.0 mL of perchloric acid (25 g/L $HClO_4$) (absorbances A_1 and A_2). To the other 6 tubes (numbers 3 to 8) add 0.25 mL, 0.25 mL, 0.125 mL, 0.125 mL, 0 mL and 0 mL respectively of *imidazole buffer solution pH 6.5 R*; add to each tube 0.25 mL of the test solution and 0 mL, 0 mL, 0.125 mL, 0.125 mL, 0.25 mL and 0.25 mL respectively of the reference solution. Mix the contents of each tube and heat at 37 °C for 15 min. To each tube add 3.0 mL of perchloric acid (25 g/L $HClO_4$), mix and centrifuge. Measure the absorbances (*2.2.25*) of the supernatant liquids at 260 nm using the compensation liquid described above (absorbances A_3 to A_8). The absorbances comply with the following requirement:

$$(A_3 + A_4) - (A_1 + A_2) < \frac{(A_5 + A_6 + A_7 + A_8)}{2} - (A_3 + A_4)$$

Streptolysin

In a polystyrene tube, use a quantity of the preparation to be examined equivalent to 500 000 IU of streptokinase activity and dilute to 0.5 mL with a mixture of 1 volume of *phosphate buffer solution pH 7.2 R* and 9 volumes of a 9 g/L solution of *sodium chloride R*. Add 0.4 mL of a 23 g/L solution of *sodium thioglycollate R*. Heat in a water-bath at 37 °C for 10 min. Add 0.1 mL of a solution of a reference preparation of human antistreptolysin O containing 5 IU/mL. Heat at 37 °C for 5 min. Add 1 mL of *rabbit erythrocyte suspension R*. Heat at 37 °C for 30 min. Centrifuge at about 1000 *g*. In the same manner, prepare a polystyrene tube in which the solution of the preparation to be examined has been replaced by 0.5 mL of a mixture of 1 volume of *phosphate buffer solution pH 7.2 R* and 9 volumes of a 9 g/L solution of *sodium chloride R*. Measure the absorbances (*2.2.25*) of the supernatant liquids

at 550 nm. The absorbance of the test solution is not more than 50 per cent greater than that of the reference solution.

Related substances

Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution Dilute the preparation to be examined with *water R* to obtain a concentration of about 0.5-1 g/L, depending on the chromatographic system used.

Reference solution Dilute 1 volume of *streptokinase for system suitability CRS* with 49 volumes of *water R*.

Column:
- *size*: *l* = 0.10 m, Ø = 4.6 mm;
- *stationary phase*: *styrene-divinylbenzene copolymer R* (10 µm) with a pore size of 200 nm;
- *temperature*: 25 °C.

Mobile phase:
- *mobile phase A*: *trifluoroacetic acid R, water for chromatography R* (1:1000 *V/V*); degas;
- *mobile phase B*: *trifluoroacetic acid R, acetonitrile for chromatography R* (1:1000 *V/V*); degas;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 1	68	32
1 - 4	68 → 52	32 → 48
4 - 5	52	48
5 - 7	0	100
7 - 10	68	32

The above conditions may be modified to improve the separation efficiency of the chromatographic system.

Flow rate 5 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µL.

Retention time: streptokinase = 2.3 min to 2.8 min.

System suitability Reference solution:
- *symmetry factor*: maximum 1.9 for the peak due to streptokinase;
- *peak-to-valley ratio*: minimum 2, where H_p = height above the baseline of the 1st peak eluting after the principal peak and H_v = height above the baseline of the lowest point of the curve separating this peak from the 2nd peak eluting after the principal peak;
- the chromatogram obtained with the reference solution is similar to the chromatogram supplied with *streptokinase for system suitability CRS*.

Limit:
- *total*: maximum 5 per cent.

Bacterial endotoxins (*2.6.14*)

Less than 0.02 IU per 100 IU of streptokinase activity, if intended for use without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Nitrogen (*2.5.9*)

Potency

The potency of streptokinase is determined by comparing its capacity to activate plasminogen to form plasmin with the same capacity of a reference preparation of streptokinase calibrated in International Units; the formation of plasmin is determined using a suitable chromogenic substrate.

The International Unit is the activity of a stated amount of the International Standard for streptokinase. The equivalence in International Units of the International Standard is stated by the World Health Organization.

Reference and test solutions

Prepare 2 independent series of at least 3 dilutions of each of the preparation to be examined and of the reference preparation of streptokinase in *tris(hydroxymethyl) aminomethane sodium chloride buffer solution pH 7.4 R1*, in the linear range of the assay (a range of 0.5-4.0 IU/mL has been found suitable). Prepare and maintain all solutions at 37 °C.

Substrate solution

Mix 1.0 mL of *tris(hydroxymethyl)aminomethane buffer solution pH 7.4 R* with 1.0 mL of *chromogenic substrate R3*. Add 5 µL of a 100 g/L solution of *polysorbate 20 R*. Keep at 37 °C in a water-bath. Immediately before commencing the activation assay, add 45 µL of a 1 mg/mL solution of *human plasminogen R*.

Method

Analyse each streptokinase dilution, maintained at 37 °C, in duplicate. Initiate the activation reaction by adding 60 µL of each dilution to 40 µL of substrate solution. For blank wells, use 60 µL of *tris(hydroxymethyl)aminomethane sodium chloride buffer solution pH 7.4 R1* instead of the reference and test solutions. Allow the reaction to proceed at 37 °C for 20 min and read the absorbance (*2.2.25*) at 405 nm. If a suitable thermostatted plate reader is available, this may be used to monitor the reaction. Alternatively, it may be necessary to stop the reaction after 20 min using 50 µL of a 50 per cent *V/V* solution of *glacial acetic acid R*. Best results are obtained when the absorbance for the highest streptokinase concentration is between 0.1 and 0.2 (after blank subtraction). If necessary, adjust the time of incubation in order to reach this range of absorbances.

Calculate the regression of the absorbance on log concentrations of the solutions of the preparation to be examined and of the reference preparation of streptokinase and calculate the potency of the preparation to be examined using a suitable statistical method, for example the parallel-line assay (*5.3*).

The estimated potency is not less than 90 per cent and not more than 111 per cent of the stated potency. The confidence limits (P = 0.95) are not less than 80 per cent and not more than 125 per cent of the estimated potency.

STORAGE

In an airtight container, protected from light and at a temperature of −20 °C. If the preparation is sterile, store in a sterile, airtight, tamper-evident container.

LABELLING

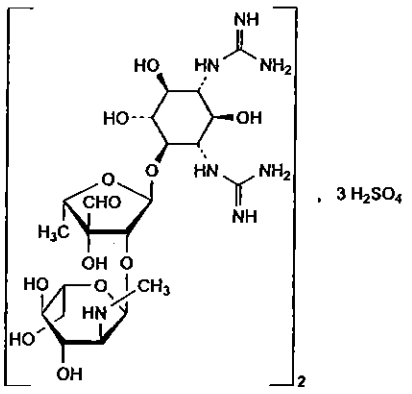
The label states:
- the number of International Units of streptokinase activity per milligram, calculated with reference to the dried preparation;
- that the preparation is suitable for use in the manufacture of parenteral preparations.

Ph Eur

Streptomycin Sulfate

Streptomycin Sulphate

(Ph. Eur. monograph 0053)



$C_{42}H_{84}N_{14}O_{36}S_3$ 1457 *3810-74-0*

Action and use
Aminoglycoside antibacterial; antituberculosis drug.

Preparation
Streptomycin Injection

Ph Eur

DEFINITION

Bis[N^1,N^3-dicarbamimidoyl-4-*O*-[5-deoxy-2-*O*-[2-deoxy-2-(methylamino)-α-L-glucopyranosyl]-3-*C*-formyl-α-L-lyxofuranosyl]-D-streptamine] trisulfate, a substance produced by the growth of certain strains of *Streptomyces griseus* or obtained by any other means. Stabilisers may be added. The potency is not less than 720 IU/mg, calculated with reference to the dried substance.

PRODUCTION

It is produced by methods of manufacture designed to eliminate or minimise substances lowering blood pressure.

CHARACTERS

A white or almost white powder, hygroscopic, very soluble in water, practically insoluble in anhydrous ethanol.

IDENTIFICATION

A. Examine by thin-layer chromatography (*2.2.27*), using a plate coated with a 0.75 mm layer of the following mixture: mix 0.3 g of *carbomer R* with 240 mL of *water R* and allow to stand, with moderate shaking, for 1 h; adjust to pH 7 by the gradual addition, with continuous shaking, of *dilute sodium hydroxide solution R* and add 30 g of *silica gel H R*.

Heat the plate at 110 °C for 1 h, allow to cool and use immediately.

Test solution Dissolve 10 mg of the substance to be examined in *water R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dissolve 10 mg of *streptomycin sulfate for identification CRS* in *water R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 10 mg of *kanamycin monosulfate CRS*, 10 mg of *neomycin sulfate CRS* and 10 mg of *streptomycin sulfate for identification CRS* in *water R* and dilute to 10 mL with the same solvent.

Apply separately to the plate 10 µL of each solution. Develop over a path of 12 cm using a 70 g/L solution of *potassium dihydrogen phosphate R*. Dry the plate in a current of warm air, and spray with a mixture of equal volumes of a 2 g/L solution of *1,3-dihydroxynaphthalene R* in *ethanol (96 per cent) R* and a 460 g/L solution of *sulfuric acid R*. Heat at 150 °C for 5 min to 10 min. The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

B. Dissolve 5 mg to 10 mg in 4 mL of *water R* and add 1 mL of *1 M sodium hydroxide*. Heat in a water-bath for 4 min. Add a slight excess of *dilute hydrochloric acid R* and 0.1 mL of *ferric chloride solution R1*. A violet colour develops.

C. Dissolve 0.1 g in 2 mL of *water R*, add 1 mL of *α-naphthol solution R* and 2 mL of a mixture of equal volumes of *strong sodium hypochlorite solution R* and *water R*. A red colour develops.

D. Dissolve about 10 mg in 5 mL of *water R* and add 1 mL of *1 M hydrochloric acid*. Heat in a water-bath for 2 min. Add 2 mL of a 5 g/L solution of *α-naphthol R* in *1 M sodium hydroxide* and heat in a water-bath for 1 min. A faint yellow colour develops.

E. It gives the reactions of sulfates (*2.3.1*).

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Appearance of solution

Solution S is not more intensely coloured than intensity 3 of the range of reference solutions of the most appropriate colour (*2.2.2, Method II*). Allow to stand protected from light, at a temperature of about 20 °C for 24 h. Solution S is not more opalescent than reference suspension II (*2.2.1*).

pH

(*2.2.3*): 4.5 to 7.0 for solution S.

Specific absorbance (*2.2.25*)

10.0 to 12.4, determined at the absorption maximum at 525 nm (dried substance).

Dissolve 0.100 g in *water R* and dilute to 100.0 mL with the same solvent. Introduce separately 5.0 mL of the solution, and 5.0 mL of *water R* (blank) into 2 volumetric flasks. To each flask add 5.0 mL of *0.2 M sodium hydroxide* and heat for exactly 10 min in a water-bath.

Cool in ice for exactly 5 min, add 3 mL of a 15 g/L solution of *ferric ammonium sulfate R* in *0.5 M sulfuric acid*, dilute to 25.0 mL with *water R* and mix. Exactly 20 min after the addition of the ferric ammonium sulfate solution, measure the absorbance of the test solution in a 2 cm cell, using the blank as compensation liquid.

Methanol

Examine by gas chromatography (*2.2.28*).

Test solution Dissolve 1.00 g of the substance to be examined in *water R* and dilute to 25.0 mL with the same solvent.

Reference solution Dilute 12.0 mg of *methanol R* to 100.0 mL with *water R*.

The chromatographic procedure may be carried out using:
— a column 1.5 m to 2.0 m long and 2 mm to 4 mm in internal diameter, packed with *ethylvinylbenzene-divinylbenzene copolymer R* (150 µm to 180 µm);
— *nitrogen for chromatography R* as the carrier gas at a constant flow rate of 30 mL to 40 mL per minute;
— a flame-ionisation detector.

Maintain the column at a constant temperature between 120 °C and 140 °C and the injection port and the detector at a temperature at least 50 °C higher than that of the column. Inject the test solution and the reference solution. The area of the peak due to methanol in the chromatogram obtained with the test solution is not greater than the area of the peak in the chromatogram obtained with the reference solution (0.3 per cent).

Streptomycin B

Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution Dissolve 0.2 g of the substance to be examined in a freshly prepared mixture of 3 volumes of *sulfuric acid R* and 97 volumes of *methanol R* and dilute to 5 mL with the same mixture of solvents. Heat under a reflux condenser for 1 h, cool, rinse the condenser with *methanol R* and dilute to 20 mL with the same solvent (10 g/L solution).

Reference solution Dissolve 36 mg of *mannose R* in a freshly prepared mixture of 3 volumes of *sulfuric acid R* and 97 volumes of *methanol R* and dilute to 5 mL with the same mixture of solvents. Heat under a reflux condenser for 1 h, cool, rinse the condenser with *methanol R* and dilute to 50 mL with the same solvent. Dilute 5 mL of the solution to 50 mL with *methanol R* (0.3 g/L solution expressed as streptomycin B; 1 mg of *mannose R* is equivalent to 4.13 mg of streptomycin B).

Apply separately to the plate 10 µL of each solution. Develop over a path of 13 cm to 15 cm using a mixture of 25 volumes of *glacial acetic acid R*, 25 volumes of *methanol R* and 50 volumes of *toluene R*. Allow the plate to dry in air and spray with a freshly prepared mixture of equal volumes of a 2 g/L solution of *1,3-dihydroxynaphthalene R* in *ethanol (96 per cent) R* and a 20 per cent *V/V* solution of *sulfuric acid R* and heat at 110 °C for 5 min. Any spot corresponding to streptomycin B in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (3.0 per cent).

Sulfate

18.0 per cent to 21.5 per cent of sulfate (dried substance).

Dissolve 0.250 g in 100 mL of *water R* and adjust the solution to pH 11 using *concentrated ammonia R*. Add 10.0 mL of *0.1 M barium chloride* and about 0.5 mg of *phthalein purple R*. Titrate with *0.1 M sodium edetate*, adding 50 mL of *ethanol (96 per cent) R* when the colour of the solution begins to change and continuing the titration until the violet-blue colour disappears.

1 mL of *0.1 M barium chloride* is equivalent to 9.606 mg of sulfate (SO_4).

Loss on drying

(*2.2.32*): maximum 7.0 per cent, determined on 1.000 g by drying at 60 °C at a pressure not exceeding 0.1 kPa for 24 h.

Sulfated ash

(*2.4.14*): maximum 1.0 per cent, determined on 1.000 g.

Bacterial endotoxins (*2.6.14*)

Less than 0.25 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for removal of bacterial endotoxins.

ASSAY

Carry out the microbiological assay of antibiotics (*2.7.2*). Use *streptomycin sulfate CRS* as the chemical reference substance.

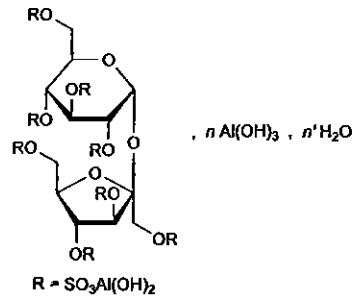
STORAGE

In an airtight container. If the substance is sterile, the container is also sterile and tamper-evident.

Ph Eur

Sucralfate

(Ph. Eur. monograph 1796)



$C_{12}H_{30}Al_8O_{51}S_8[Al(OH)_3]_n[H_2O]_{n'}$
in which n = 8 to 10 and n' = 22 to 31

Action and use

Treatment of gastric and duodenal ulcers.

Ph Eur

DEFINITION

β-D-Fructofuranosyl-α-D-glucopyranoside octakis (dihydroxyaluminium sulfate) with 8-10 molecules of aluminium hydroxide and 22-31 molecules of water.

Content

— *β-D-fructofuranosyl-α-D-glucopyranoside octakis(sulfate)* (*sucrose octasulfate*) ($C_{12}H_{14}O_{35}S_8^{8-}$; M_r 975): 30.0 per cent to 38.0 per cent;
— *aluminium* (Al; A_r 26.98): 15.5 per cent to 18.5 per cent.

CHARACTERS

Appearance

White or almost white, amorphous powder.

Solubility

Practically insoluble in water, in ethanol (96 per cent) and in methylene chloride. It dissolves in dilute solutions of mineral acids and alkali hydroxides.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison sucralfate CRS.

B. To 2 g add 10 mL of a 10.3 g/L solution of *hydrochloric acid R* and boil. Cool and neutralise with a 4 g/L solution of *sodium hydroxide R*. To 5 mL of the solution add 0.15 mL of freshly prepared *copper sulfate solution R* and 2 mL of freshly prepared *dilute sodium hydroxide solution R*. The solution is blue and clear and remains so after boiling. To the hot solution add 4 mL of *dilute hydrochloric acid R* and boil for 1 min. Add 4 mL of *dilute sodium hydroxide solution R*; an orange precipitate is formed immediately.

C. Dissolve about 15 mg in a mixture of 0.5 mL of *dilute hydrochloric acid R* and 2 mL of *water R*. The solution gives the reaction of aluminium (*2.3.1*).

TESTS

Impurity A

Liquid chromatography (*2.2.29*).

Test solution Dissolve 450.0 mg of the substance to be examined in a mixture of equal volumes of an 88 g/L solution of *sodium hydroxide R* and a 196.2 g/L solution of *sulfuric acid R* and dilute to 10.0 mL with the same mixture of solvents. Without delay, while shaking at a moderate rate, add a volume (*V*), accurately measured in millilitres, of a 4 g/L solution of *sodium hydroxide R* to adjust the solution to approximately pH 2.3. Dilute the solution with (15.0 −*V*) mL of *water R*. Shake for 1 min. If the pH is not between 2.3 and 3.5, repeat the test using a different volume of a 4 g/L solution of *sodium hydroxide R*.

Reference solution (a) Dissolve 40.0 mg of *potassium sucrose octasulfate CRS* in the mobile phase and dilute to 5.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 10.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.0 mm;
— *stationary phase*: *aminopropylsilyl silica gel for chromatography R* (5 µm).

Mobile phase 70 g/L solution of *ammonium sulfate R*, adjusted to pH 3.5 with *phosphoric acid R*.

Flow rate 1 mL/min.

Detection Differential refractometer.

Injection 50 µL of the test solution and reference solution (b).

Relative retention With reference to sucrose octasulfate (retention time = about 6 min): impurity A = about 0.6.

System suitability Reference solution (b):
— *number of theoretical plates*: minimum 400;
— *symmetry factor*: maximum 4.0.

Limit:
— *impurity A*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (5.0 per cent).

Neutralising capacity

Disperse 0.25 g in 100.0 mL of *0.1 M hydrochloric acid*, previously heated at 37 °C, stir continuously for 1 h in a water-bath at 37 °C and cool. Titrate 20.0 mL of this solution with *0.1 M sodium hydroxide* to pH 3.5; not more than 14.0 mL of *0.1 M sodium hydroxide* is required.

Chlorides (*2.4.4*)

Maximum 0.50 per cent.

Dissolve 0.10 g in 5 mL of *dilute nitric acid R* and dilute to 50 mL with *water R*. Dilute 5 mL of this solution to 15 mL with *water R*.

ASSAY

Aluminium

Disperse 1.0 g in 10 mL of *6 M hydrochloric acid R*. Heat with continuous stirring in a water-bath at 70 °C for 5 min. Cool to room temperature, transfer quantitatively to a volumetric flask, dilute to 250.0 mL with *water R*, and mix. Filter the solution, discarding the 1st portion of the filtrate. To 10.0 mL of the solution, add 10.0 mL of *0.1 M sodium edetate* and 30 mL of a mixture of equal volumes of *ammonium acetate solution R* and *dilute acetic acid R*. Heat in a water-bath at 70 °C for 5 min, then cool. Add 25 mL of *ethanol (96 per cent) R* and 1 mL of a freshly prepared 0.25 g/L solution of *dithizone R* in *ethanol (96 per cent) R*.

Titrate the excess of sodium edetate with *0.1 M zinc sulfate* until the colour changes to pink.

1 mL of *0.1 M sodium edetate* is equivalent to 2.698 mg of Al.

Sucrose octasulfate

Liquid chromatography (*2.2.29*) as described in the test for impurity A with the following modifications.

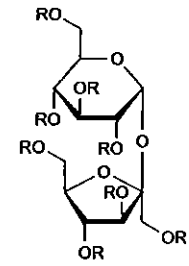
Mobile phase 132 g/L solution of *ammonium sulfate R*, adjusted to pH 3.5 with *phosphoric acid R*.

Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{12}H_{14}O_{35}S_8$ taking into account the assigned content of *potassium sucrose octasulfate CRS* and by multiplying the potassium sucrose octasulfate content by 0.757.

IMPURITIES

Specified impurities *A*.



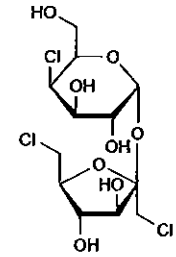
R = SO_3H
and H in a 7 to 1 ratio

A. β-D-fructofuranosyl-α-D-glucopyranoside heptakis (hydrogen sulfate).

Ph Eur

Sucralose

(Ph. Eur. monograph 2368)



$C_{12}H_{19}Cl_3O_8$ 397.6 *56038-13-2*

Action and use

Sweetening agent.

Ph Eur

DEFINITION

1,6-Dichloro-1,6-dideoxy-β-D-fructofuranosyl 4-chloro-4-deoxy-α-D-galactopyranoside.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility
Freely soluble in water, soluble in anhydrous ethanol, slightly soluble in ethyl acetate.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *sucralose CRS.*

TESTS

Specific optical rotation (*2.2.7*)
+ 84.0 to + 87.5 (anhydrous substance).

Dissolve 2.50 g in *water R* and dilute to 25.0 mL with the same solvent.

Impurities H and I

Test solution Dissolve 2.5 g of the substance to be examined in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dissolve 1.0 g of *mannitol R* in *water R* and dilute to 10.0 mL with the same solvent.

Reference solution (b) Dissolve 1.0 g of *mannitol R* and 4.0 mg of *fructose R* in *water R* and dilute to 10.0 mL with the same solvent.

Plate *TLC silica gel plate R.*

Application 5 µL by applying the solution slowly in 1 µL aliquots and allowing the plate to dry between applications; the 3 spots must be of a similar size.

Detection Spray with a solution prepared as follows: dissolve 1.23 g of *p-anisidine R* and 1.66 g of *phthalic acid R* in 100 mL of *methanol R*; store the solution in darkness and in a refrigerator to prevent it becoming discoloured; discard if the solution becomes discoloured; heat the plate at 100 ± 2 °C for 15 min and examine immediately against a dark background.

System suitability The spot due to mannitol obtained with reference solution (a) is colourless; darkening of the mannitol spot indicates that the plate has been held for too long in the oven and a 2nd plate has to be prepared.

Limit:
— *sum of impurities H and I*: any spot is not more intense than the spot due to fructose obtained with reference solution (b) (0.1 per cent).

Related substances
Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 1.0 g of the substance to be examined in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dilute 0.5 mL of the test solution to 100.0 mL with *methanol R.*

Reference solution (b) Dissolve the contents of a vial of *sucralose impurity B CRS* in 1.0 mL of the test solution.

Plate *TLC octadecylsilyl silica gel plate R.*

Mobile phase *acetonitrile R*, 50 g/L solution of *sodium chloride R* (30:70 *V/V*).

Application 5 µL.

Development Over 3/4 of the plate.

Drying In air.

Detection Spray with a 15 per cent *V/V* solution of *sulfuric acid R* in *methanol R* and heat at 125 °C for 10 min.

Retardation factors Impurity A = about 0.3; impurity B = about 0.35; sucralose = about 0.45; impurity F = about 0.67; impurity G = about 0.70; impurity E = about 0.72; impurity D = about 0.8.

System suitability Reference solution (b):
— the chromatogram shows 2 clearly separated spots due to impurity B and sucralose.

Limits:
— *impurities A, B, D, E, F, G*: any spot, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

Water (*2.5.12*)
Maximum 2.0 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*)
Maximum 0.7 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.25 g of the substance to be examined in the mobile phase and dilute to 25.0 mL with the mobile phase.

Reference solution Dissolve 0.25 g of *sucralose CRS* in the mobile phase and dilute to 25.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.10 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R*, *water R* (15:85 *V/V*).

Flow rate 1.5 mL/min.

Detection Refractometer maintained at a constant temperature.

Injection 20 µL.

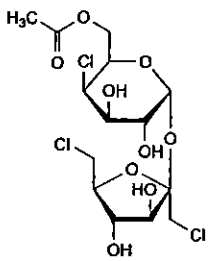
Retention time Sucralose = about 3 min.

System suitability Reference solution:
— *symmetry factor*: maximum 2.0 for the peak due to sucralose.

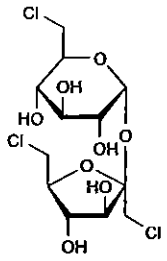
Calculate the percentage content of $C_{12}H_{19}Cl_3O_8$ taking into account the assigned content of *sucralose CRS.*

IMPURITIES

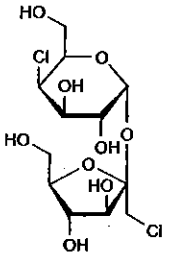
Specified impurities *A, B, D, E, F, G, H, I.*



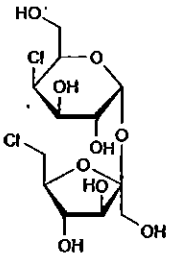
A. 1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl 6-*O*-acetyl-4-chloro-4-deoxy-α-D-galactopyranoside (6-*O*-acetylsucralose),



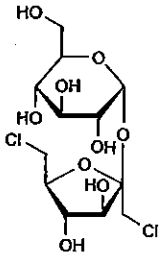
B. 1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl 6-chloro-6-deoxy-α-D-glucopyranoside (1′,6,6′-trichloro-1′,6,6′-trideoxysucrose),



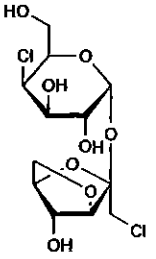
D. 1-chloro-1-deoxy-β-D-fructofuranosyl 4-chloro-4-deoxy-α-D-galactopyranoside (1′,4-dichloro-1′,4-dideoxygalactosucrose),



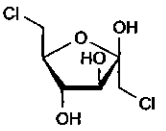
E. 6-chloro-6-deoxy-β-D-fructofuranosyl 4-chloro-4-deoxy-α-D-galactopyranoside (4,6′-dichloro-4,6′-dideoxygalactosucrose),



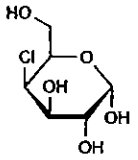
F. 1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl α-D-glucopyranoside (1′,6′-dichloro-1′,6′-dideoxysucrose),



G. 3,6-anhydro-1-chloro-1-deoxy-β-D-fructofuranosyl 4-chloro-4-deoxy-α-D-galactopyranoside (3′,6′-anhydro-1′,4-dichloro-1′,4-dideoxygalactosucrose),



H. 1,6-dichloro-1,6-dideoxy-β-D-fructofuranose,



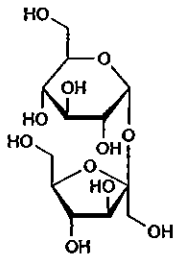
I. 4-chloro-4-deoxy-α-D-galactopyranose.

Ph Eur

Sucrose[1]

Refined Sugar

(Ph. Eur. monograph 0204)



$C_{12}H_{22}O_{11}$ 342.3 *57-50-1*

Action and use
Sweetening agent.

Ph Eur

DEFINITION

β-D-Fructofuranosyl α-D-glucopyranoside.

It contains no additives.

♦CHARACTERS

Appearance
White or almost white, crystalline powder, or lustrous, colourless or white or almost white crystals.

Solubility
Very soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in anhydrous ethanol.

IDENTIFICATION

First identification: A.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *sucrose CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in a mixture of 2 volumes of *water R* and 3 volumes of *methanol R* and dilute to 20 mL with the same mixture of solvents.

[1] *This monograph has undergone pharmacopoeial harmonisation. See chapter 5.8 Pharmacopoeial harmonisation.*

Reference solution (a) Dissolve 10 mg of *sucrose CRS* in a mixture of 2 volumes of *water R* and 3 volumes of *methanol R* and dilute to 20 mL with the same mixture of solvents.

Reference solution (b) Dissolve 10 mg of *fructose R*, 10 mg of *glucose R*, 10 mg of *lactose monohydrate R* and 10 mg of *sucrose R* in a mixture of 2 volumes of *water R* and 3 volumes of *methanol R* and dilute to 20 mL with the same mixture of solvents.

Plate *TLC silica gel G plate R.*

Mobile phase cold saturated boric acid solution R, 60 per cent *V/V* solution of *glacial acetic acid R, anhydrous ethanol R, acetone R, ethyl acetate R* (10:15:20:60:60 *V/V/V/V/V*).

Application 2 µL.

Development In an unsaturated tank, over 3/4 of the plate.

Drying In a current of warm air.

Detection Spray with a solution of 0.5 g of *thymol R* in a mixture of 5 mL of *sulfuric acid R* and 95 mL of *ethanol (96 per cent) R*. Heat the plate at 130 °C for 10 min.

System suitability The chromatogram obtained with reference solution (b) shows 4 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dilute 1 mL of solution S (see Tests) to 100 mL with *water R*. To 5 mL of the solution add 0.15 mL of freshly prepared *copper sulfate solution R* and 2 mL of freshly prepared *dilute sodium hydroxide solution R*. The solution is blue and clear and remains so after boiling. To the hot solution add 4 mL of *dilute hydrochloric acid R* and boil for 1 min. Add 4 mL of *dilute sodium hydroxide solution R*. An orange precipitate is formed immediately.♦

TESTS

Solution S

Dissolve 50.0 g in *water R* and dilute to 100 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*).

Conductivity (*2.2.38*)

Maximum 35 µS·cm^{-1} at 20 °C.

Dissolve 31.3 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 mL with the same solvent. Measure the conductivity of the solution (C_1), while gently stirring with a magnetic stirrer, and that of the water used for preparing the solution (C_2). The readings must be stable within 1 per cent over a period of 30 s. Calculate the conductivity of the solution of the substance to be examined using the following expression:

$$C_1 - 0.35\ C_2$$

Specific optical rotation (*2.2.7*)

+ 66.3 to + 67.0.

Dissolve 26.0 g in *water R* and dilute to 100.0 mL with the same solvent.

♦Colour value

Maximum 45.

Dissolve 50.0 g in 50.0 mL of *water R*. Mix, filter (diameter of pores 0.45 µm) and degas. Measure the absorbance (*2.2.25*) at 420 nm, using a minimum path length of 4 cm (a path length of 10 cm or more is preferred).

Calculate the colour value using the following expression:

$$\frac{A \times 1000}{b \times c}$$

A = absorbance measured at 420 nm;
b = path length in centimetres;
c = concentration of the solution, in grams per millilitre, calculated from the refractive index (*2.2.6*) of the solution; use Table 0204.-1 and interpolate the values if necessary.

Table 0204.-1

n_D^{20}	c (g/mL)
1.4138	0.570
1.4159	0.585
1.4179	0.600
1.4200	0.615
1.4221	0.630
1.4243	0.645
1.4264	0.661

System suitability:

— *repeatability*: the absolute difference between 2 results is not greater than 3.♦

Dextrins

If intended for use in the manufacture of large-volume parenteral preparations, it complies with the test for dextrins. To 2 mL of solution S add 8 mL of *water R*, 0.05 mL of *dilute hydrochloric acid R* and 0.05 mL of *0.05 M iodine*. The solution remains yellow.

Reducing sugars

To 5 mL of solution S in a test-tube about 150 mm long and 16 mm in diameter add 5 mL of *water R*, 1.0 mL of *1 M sodium hydroxide* and 1.0 mL of a 1 g/L solution of *methylene blue R*. Mix and place in a water-bath. After exactly 2 min, take the tube out of the bath and examine the solution immediately. The blue colour does not disappear completely. Ignore any blue colour at the air/solution interface.

Sulfites

Maximum 10 ppm, calculated as SO_2.

Determine the sulfites content by a suitable enzymatic method based on the following reactions. Sulfite is oxidised by sulfite oxidase to sulfate and hydrogen peroxide which in turn is reduced by nicotinamide-adenine dinucleotide-peroxidase in the presence of reduced nicotinamide-adenine dinucleotide (NADH). The amount of NADH oxidised is proportional to the amount of sulfite.

Test solution Dissolve 4.0 g of the substance to be examined in freshly prepared *distilled water R* and dilute to 10.0 mL with the same solvent.

Reference solution Dissolve 4.0 g of the substance to be examined in freshly prepared *distilled water R*, add 0.5 mL of *sulfite standard solution (80 ppm SO_2) R* and dilute to 10.0 mL with freshly prepared *distilled water R*.

Blank solution Freshly prepared *distilled water R*.

Separately introduce 2.0 mL each of the test solution, the reference solution and the blank in 10 mm cuvettes and add the reagents as described in the instructions in the kit for sulfite determination. Measure the absorbance (*2.2.25*) at the absorption maximum at about 340 nm before and at the end of the reaction time and subtract the value obtained with the blank.

The absorbance difference of the test solution is not greater than half the absorbance difference of the reference solution.

Loss on drying (*2.2.32*)
Maximum 0.1 per cent, determined on 2.000 g by drying in an oven at 105 °C for 3 h.

Bacterial endotoxins (*2.6.14*)
Less than 0.25 IU/mg, if intended for use in the manufacture of large-volume parenteral preparations.

LABELLING
The label states, where applicable, that the substance is suitable for use in the manufacture of large-volume parenteral preparations.

Ph Eur

Syrup

Syrup

(Liquid Sucrose, Ph. Eur. monograph 2797)

Ph Eur

DEFINITION
Aqueous solution of sucrose.

Content
— *dry matter*: 66.0 per cent *m/m* to 67.5 per cent *m/m*.

CHARACTERS
Appearance
Clear, colourless or pale yellow, viscous liquid.

Solubility
Miscible with glycerol.

IDENTIFICATION
A. Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (c).

Results The principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (c).

B. Dilute 75 g to 100 mL with *water R*. Dilute 1 mL of the solution to 100 mL with *water R*. To 5 mL of this solution add 0.15 mL of freshly prepared *copper sulfate solution R* and 2 mL of freshly prepared *dilute sodium hydroxide solution R*. The solution is blue and clear and remains so after boiling. To the hot solution add 4 mL of *dilute hydrochloric acid R* and boil for 1 min. Add 4 mL of *dilute sodium hydroxide solution R*. An orange precipitate is formed immediately.

TESTS
Colour value
Maximum 30.

Dilute a quantity of the substance to be examined corresponding to 50.0 g of dry matter (see Tests) to 100.0 g with *water R*. Filter through a membrane filter (nominal pore size 0.45 µm) and allow to stand for about 15 min. Measure the absorbance (*2.2.25*) at 420 nm, using a minimum path length of 5 cm (a path length of 10 cm or more is preferred).

Calculate the colour value using the following expression:

$$\frac{A \times 1000}{b \times c}$$

A = absorbance measured at 420 nm;
b = path length, in centimetres;
c = concentration of the solution obtained, in grams per millilitre, calculated from the refractive index (*2.2.6*) of the solution; use Table 2797.-1 and interpolate the values if necessary.

Table 2797.-1

n_D^{20}	*c* (g/mL)
1.4138	0.570
1.4159	0.585
1.4179	0.600
1.4200	0.615
1.4221	0.630
1.4243	0.645
1.4264	0.661

System suitability:
— *repeatability*: the absolute difference between 2 results is not greater than 3.

Conductivity (*2.2.38*)
Maximum 35 µS·cm^{-1} at 20 °C.

Dilute a quantity of the substance to be examined corresponding to 31.3 ± 0.1 g of dry matter (see Tests) to 100.0 mL with *carbon dioxide-free water R*. Measure the conductivity of the solution (C_1), while gently stirring with a magnetic stirrer, and that of the water used for preparing the solution (C_2). The readings must be stable within 1 per cent over a period of 30 s. Calculate the conductivity of the solution of the substance to be examined using the following expression:

$$C_1 - 0.35\ C_2$$

Related substances
Liquid chromatography (*2.2.29*).

Test solution (a) Dilute 0.200 g of the substance to be examined to 10.0 mL with *water R*.

Test solution (b) Dilute 1.0 mL of test solution (a) to 20.0 mL with *water R*.

Reference solution (a) Dilute 1.0 mL of test solution (a) to 200.0 mL with *water R*.

Reference solution (b) Dissolve 10.0 mg of *raffinose pentahydrate R* (impurity A) and 14.0 mg of *glucose R* (impurity C) in *water R* and dilute to 50.0 mL with the same solvent. To 1.0 mL of the solution add 26.0 mg of *sucrose CRS* and dilute to 2.0 mL with *water R*.

Reference solution (c) Dissolve 33.0 mg of *sucrose CRS* in *water R* and dilute to 5.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with *water R*.

Precolumn:
— *size*: *l* = 0.03 m, Ø = 8.0 mm;
— *stationary phase*: *strong cation-exchange resin (calcium form) R* (9 µm).

Column:
— *size*: *l* = 0.30 m, Ø = 7.8 mm;
— *stationary phase*: *strong cation-exchange resin (calcium form) R* (9 µm);
— *temperature*: 80 ± 1 °C.

Mobile phase *water for chromatography R*.

Flow rate 0.5 mL/min.

Detection Differential refractometer maintained at a constant temperature (about 40 °C).

Injection 10 µL of test solution (a) and reference solutions (a) and (b).

Run time 3 times the retention time of sucrose.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A (or B) and C.

Impurity A is present in sucrose obtained from sugar beet and impurity B is present in sucrose obtained from sugar cane.

Relative retention With reference to sucrose (retention time = about 10 min): impurities A and B = about 0.9; impurity C = about 1.2.

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to sucrose; minimum 2.5, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to sucrose.

Calculation of percentage contents:

— for each impurity, use the concentration of sucrose in reference solution (a).

Limits:

— *impurity C*: maximum 0.7 per cent;

— *unspecified impurities*: for each impurity, maximum 0.5 per cent;

— *total*: maximum 2.0 per cent;

— *reporting threshold*: 0.05 per cent.

Sulfites

Maximum 10 ppm, calculated as SO_2.

Determine the sulfites content by a suitable enzymatic method based on the following reactions. Sulfite is oxidised by sulfite oxidase to sulfate and hydrogen peroxide which in turn is reduced by nicotinamide-adenine dinucleotide-peroxidase in the presence of reduced nicotinamide-adenine dinucleotide (NADH). The amount of NADH oxidised is proportional to the amount of sulfite.

Test solution Dilute 4.0 g of the substance to be examined to 10.0 mL with freshly prepared *distilled water R*.

Reference solution To 4.0 g of the substance to be examined add 0.5 mL of *sulfite standard solution (80 ppm SO_2) R* and dilute to 10.0 mL with freshly prepared *distilled water R*.

Blank solution Freshly prepared *distilled water R*.

Separately introduce 2.0 mL each of the test solution, the reference solution and the blank solution into 10 mm cuvettes and add the reagents as described in the instructions in the kit for sulfite determination. Measure the absorbance (*2.2.25*) at the absorption maximum at about 340 nm before and at the end of the reaction time and subtract the value obtained with the blank.

The absorbance difference of the test solution is not greater than half the absorbance difference of the reference solution.

Dry matter

Determine the refractive index (*2.2.6*). Use Table 2797.-2 and interpolate the values if necessary.

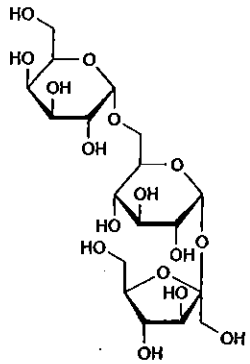
Table 2797.-2

Dry matter (per cent *m/m*)	n_D^{20}
65	1.453478
66	1.455839
67	1.458217
68	1.460613
69	1.463026

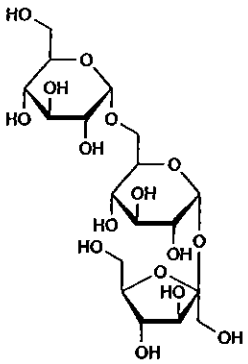
IMPURITIES

Specified impurities *C*.

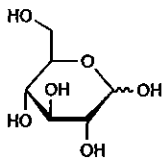
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, D*.



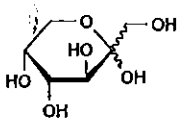
A. β-D-fructofuranosyl α-D-galactopyranosyl-(1→6)-α-D-glucopyranoside (raffinose),



B. β-D-fructofuranosyl α-D-glucopyranosyl-(1→6)-α-D-glucopyranoside (theanderose),



C. D-glucopyranose (glucose),



D. D-*arabino*-hex-2-ulopyranose (fructose).

Sucrose Monopalmitate

(Ph. Eur. monograph 2319)

Ph Eur

DEFINITION

Mixture of sucrose monoesters, mainly sucrose monopalmitate, obtained by transesterification of palmitic (hexadecanoic) acid methyl esters of vegetable origin with *Sucrose (0204)*. The manufacture of the fatty acid methyl esters includes a distillation step.

It contains variable quantities of mono-, di-, tri- and polyesters.

Content

— *monoesters*: minimum 55.0 per cent;
— *diesters*: maximum 40.0 per cent;
— *sum of triesters and polyesters*: maximum 20.0 per cent.

CHARACTERS

Appearance

White or almost white, unctuous powder.

Solubility

Very slightly soluble in water, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Composition of fatty acids (see Tests).

B. It complies with the limits of the assay.

TESTS

Acid value (*2.5.1*)

Maximum 6.0, determined on 3.00 g.

Use a freshly neutralised mixture of 1 volume of *water R* and 2 volumes of *2-propanol R* as solvent and heat gently.

Composition of fatty acids (*2.4.22, Method C*)

Use the mixture of calibrating substances in Table 2.4.22.-1.

Composition of the fatty-acid fraction of the substance:
— *lauric acid*: maximum 3.0 per cent;
— *myristic acid*: maximum 3.0 per cent;
— *palmitic acid*: 70.0 per cent to 85.0 per cent;
— *stearic acid*: 10.0 per cent to 25.0 per cent;
— *sum of the contents of palmitic acid and stearic acid*: minimum 90.0 per cent.

Free sucrose

Liquid chromatography (*2.2.29*).

Solvent mixture *water for chromatography R, tetrahydrofuran for chromatography R* (12.5:87.5 *V/V*).

Test solution Dissolve 0.200 g of the substance to be examined in the solvent mixture and dilute to 4.0 mL with the solvent mixture.

Reference solution (a) Dissolve 20.0 mg of *sucrose CRS* in the solvent mixture and dilute to 10.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) In 4 volumetric flasks, introduce respectively 5.0 mg, 10.0 mg, 20.0 mg and 25.0 mg of *sucrose CRS*, dissolve in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Column:
— *size*: l = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: spherical *aminopropylsilyl silica gel for chromatography R* (4 µm).

Mobile phase:
— *mobile phase A*: 0.01 g/L solution of *ammonium acetate R* in *acetonitrile for chromatography R*;
— *mobile phase B*: 0.01 g/L solution of *ammonium acetate R* in a mixture of 10 volumes of *water for chromatography R* and 90 volumes of *tetrahydrofuran for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Flow rate (mL/min)
0 - 1	100	0	1.0
1 - 9	100 → 0	0 → 100	1.0
9 - 16	0	100	1.0
16 - 16.01	0	100	1.0 → 2.5
16.01 - 32	0	100	2.5

Detection Evaporative light-scattering detector; the following settings have been found to be suitable; if the detector has different setting parameters, adjust the detector settings so as to comply with the system suitability criterion:
— *carrier gas*: *nitrogen R*;
— *flow rate*: 1.0 mL/min;
— *evaporator temperature*: 45 °C;
— *nebuliser temperature*: 40 °C.

Injection 20 µL.

Retention time About 26 min.

System suitability Reference solution (a):
— *signal-to-noise ratio*: minimum 10.

Limit Maximum 4.0 per cent.

Water (*2.5.12*)

Maximum 4.0 per cent, determined on 0.20 g.

Total ash (*2.4.16*)

Maximum 1.5 per cent.

ASSAY

Size-exclusion chromatography (*2.2.30*): use the normalisation procedure.

Test solution Dissolve 60.0 mg of the substance to be examined in *tetrahydrofuran R* and dilute to 4.0 mL with the same solvent.

Column:
— *size*: l = 0.6 m, Ø = 7 mm;
— *stationary phase*: *styrene-divinylbenzene copolymer R* (5 µm) with a pore size of 10 nm.

Mobile phase: *tetrahydrofuran R*.

Flow rate 1.2 mL/min.

Detection Differential refractometer.

Injection 20 µL.

Relative retention With reference to monoesters (retention time = about 13 min): diesters = about 0.93; triesters and polyesters = about 0.91.

Calculations:
— *disregard limit*: disregard the peaks having a signal-to-noise ratio less than 10;
— *free fatty acids*: calculate the percentage content of free fatty acids (*C*), using the following expression:

$$\frac{I_A \times 256}{56.11 \times 10}$$

I_A = acid value (see Tests);
256 = rounded molar mass of palmitic acid, in grams per mole;
56.11 = molar mass of potassium hydroxide, in grams per mole.

— *monoesters*: calculate the percentage content of monoesters using the following expression:

$$\left[\frac{X}{X+Y+Z}\ (100-A-B)\right]-C$$

A = percentage content of free sucrose (see Tests);
B = percentage content of water (see Tests);
C = percentage content of free fatty acids;
X = area of the peak due to monoesters and free fatty acids;
Y = area of the peak due to diesters;
Z = area of the peak due to triesters and polyesters.

— *diesters*: calculate the percentage content of diesters using the following expression:

$$\frac{Y}{X+Y+Z}\ (100-A-B)$$

— *sum of triesters and polyesters*: calculate the sum of the percentage contents of triesters and polyesters using the following expression:

$$\frac{Z}{X+Y+Z}\ (100-A-B)$$

STORAGE

Protected from humidity.

Ph Eur

Sucrose Stearate

(Ph. Eur. monograph 2318)

Ph Eur

DEFINITION

Mixture of sucrose esters, mainly sucrose stearate, obtained by transesterification of stearic (octadecanoic) acid methyl esters of vegetable origin with *sucrose (0204)*.

The manufacture of the fatty acid methyl esters includes a distillation step.

It contains variable quantities of mono-, di-, tri- and polyesters.

Content

Sucrose stearate type I:
— *monoesters*: minimum 50.0 per cent;
— *diesters*: maximum 40.0 per cent;
— *sum of triesters and polyesters*: maximum 25.0 per cent;

Sucrose stearate type II:
— *monoesters*: 20.0 per cent to 45.0 per cent;
— *diesters*: 30.0 per cent to 40.0 per cent;
— *sum of triesters and polyesters*: maximum 30.0 per cent;

Sucrose stearate type III:
— *monoesters*: 15.0 per cent to 25.0 per cent;
— *diesters*: 30.0 per cent to 45.0 per cent;
— *sum of triesters and polyesters*: 35.0 per cent to 50.0 per cent.

CHARACTERS

Appearance

White or almost white, unctuous powder.

Solubility

Very slightly soluble in water, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Composition of fatty acids (see Tests).

B. It complies with the limits of the assay.

TESTS

Acid value *(2.5.1)*

Maximum 6.0, determined on 3.00 g.

Use a freshly neutralised mixture of 1 volume of *water R* and 2 volumes of *2-propanol R* as solvent and heat gently.

Composition of fatty acids *(2.4.22, Method C)*

Use the mixture of calibrating substances in Table 2.4.22.-1.

Composition of the fatty-acid fraction of the substance:
— *lauric acid*: maximum 3.0 per cent;
— *myristic acid*: maximum 3.0 per cent;
— *palmitic acid*: 25.0 per cent to 40.0 per cent;
— *stearic acid*: 55.0 per cent to 75.0 per cent;
— *sum of the contents of palmitic acid and stearic acid*: minimum 90.0 per cent.

Free sucrose

Liquid chromatography *(2.2.29)*.

Solvent mixture *water for chromatography R, tetrahydrofuran for chromatography R* (12.5:87.5 *V/V*).

Test solution Dissolve 0.200 g of the substance to be examined in the solvent mixture and dilute to 4.0 mL with the solvent mixture.

Reference solution (a) Dissolve 20.0 mg of *sucrose CRS* in the solvent mixture and dilute to 10.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) In 4 volumetric flasks, introduce respectively 5.0 mg, 10.0 mg, 20.0 mg and 25.0 mg of *sucrose CRS*, dissolve in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Column:
— *size*: l = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: spherical *aminopropylsilyl silica gel for chromatography R* (4 µm).

Mobile phase:
— *mobile phase A*: 0.01 g/L solution of *ammonium acetate R* in *acetonitrile for chromatography R*;
— *mobile phase B*: 0.01 g/L solution of *ammonium acetate R* in a mixture of 10 volumes of *water for chromatography R* and 90 volumes of *tetrahydrofuran for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Flow rate (mL/min)
0 - 1	100	0	1.0
1 - 9	100 → 0	0 → 100	1.0
9 - 16	0	100	1.0
16 - 16.01	0	100	1.0 → 2.5
16.01 - 32	0	100	2.5

Detection Evaporative light-scattering detector; the following settings have been found to be suitable; if the detector has different setting parameters, adjust the detector settings so as to comply with the system suitability criterion:
— *carrier gas*: *nitrogen R*;
— *flow rate*: 1.0 mL/min;
— *evaporator temperature*: 45 °C;
— *nebuliser temperature*: 40 °C.

Injection 20 µL.

Retention time About 26 min.

System suitability Reference solution (a):
— *signal-to-noise ratio*: minimum 10.

Limit:
— *sucrose*: maximum 4.0 per cent.

Water (*2.5.12*)
Maximum 4.0 per cent, determined on 0.20 g.

Total ash (*2.4.16*)
Maximum 1.5 per cent.

ASSAY
Size-exclusion chromatography (*2.2.30*): use the normalisation procedure.

Test solution Dissolve 60.0 mg of the substance to be examined in *tetrahydrofuran R* and dilute to 4.0 mL with the same solvent.

Column:
— *size*: *l* = 0.6 m, Ø = 7 mm;
— *stationary phase*: *styrene-divinylbenzene copolymer R* (5 µm) with a pore size of 10 nm.

Mobile phase *tetrahydrofuran R*.

Flow rate 1.2 mL/min.

Detection Differential refractometer.

Injection 20 µL.

Relative retention With reference to monoesters (retention time = about 13 min): diesters = about 0.94; triesters and polyesters = about 0.92.

Calculations:
— *disregard limit*: disregard the peaks having a signal-to-noise ratio less than 10;
— *free fatty acids*: calculate the percentage content of free fatty acids (*C*) using the following expression:

$$\frac{I_A \times 284}{56.11 \times 10}$$

I_A = acid value (see Tests);
284 = rounded molar mass of stearic acid, in grams per mole;
56.11 = molar mass of potassium hydroxide, in grams per mole.

— *monoesters*: calculate the percentage content of monoesters using the following expression:

$$\left[\frac{X}{X+Y+Z}\ (100-A-B)\right]-C$$

A = percentage content of free sucrose (see Tests);
B = percentage content of water (see Tests);
C = percentage content of free fatty acids;
X = area of the peak due to monoesters and free fatty acids;
Y = area of the peak due to diesters;
Z = area of the peak due to triesters and polyesters.

— *diesters*: calculate the percentage content of diesters using the following expression:

$$\frac{Y}{X+Y+Z}\ (100-A-B)$$

— *sum of triesters and polyesters*: calculate the sum of the percentage contents of triesters and polyesters using the following expression:

$$\frac{Z}{X+Y+Z}\ (100-A-B)$$

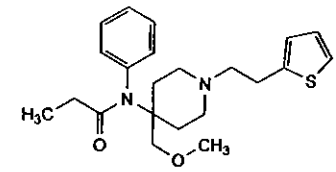
LABELLING
The label states the type of sucrose stearate (type I, II or III).

STORAGE
Protected from humidity.

Ph Eur

Sufentanil

(Ph. Eur. monograph 1569)



$C_{22}H_{30}N_2O_2S$ 386.6 *56030-54-7*

Action and use
Opioid receptor agonist; analgesic.

Ph Eur

DEFINITION
N-[4-(Methoxymethyl)-1-[2-(thiophen-2-yl)ethyl]piperidin-4-yl]-*N*-phenylpropanamide.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white powder.

Solubility
Practically insoluble in water, freely soluble in ethanol (96 per cent) and in methanol.

mp
About 98 °C.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of sufentanil.*

TESTS
Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 0.10 g in *methanol R* and dilute to 20 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) In order to produce impurity E *in situ*, dissolve 10 mg of the substance to be examined in 10.0 mL of *dilute hydrochloric acid R*. Heat on a water-bath under a reflux condenser for 4 h. Add 10.0 mL of *dilute sodium hydroxide solution R*. Evaporate to dryness on a water-bath. Cool and take up the residue in 10 mL of *methanol R*. Filter.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *methanol R*. Dilute 5.0 mL of this solution to 20.0 mL with *methanol R*.

Column:
— *size*: *l* = 0.1 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
— *mobile phase A*: 5 g/L solution of *ammonium carbonate R* in a mixture of 10 volumes of *tetrahydrofuran R* and 90 volumes of *water R*;
— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	90 → 40	10 → 60
15 - 20	40	60

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µL.

Relative retention With reference to sufentanil (retention time = about 13 min): impurity D = about 0.85; impurity E = about 0.9; impurity F = about 0.95; impurity H = about 1.1.

System suitability Reference solution (a):
— *resolution*: minimum 4.0 between the peaks due to impurity E and sufentanil.

Limits:
— *impurities D, F, H*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);
— *unspecified impurities*: for each impurity, not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 2 h.

ASSAY

Dissolve 0.300 g in 50 mL of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R* and titrate with *0.1 M perchloric acid*, using 0.2 mL of *naphtholbenzein solution R* as indicator.

1 mL of *0.1 M perchloric acid* is equivalent to 38.66 mg of $C_{22}H_{30}N_2O_2S$.

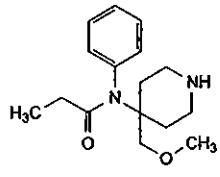
STORAGE

Protected from light.

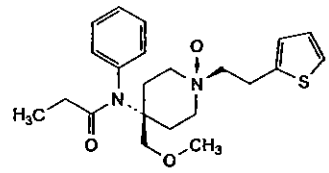
IMPURITIES

Specified impurities *D, F, H.*

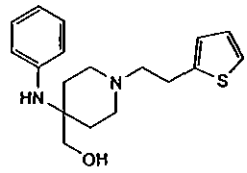
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, E, G, I.*



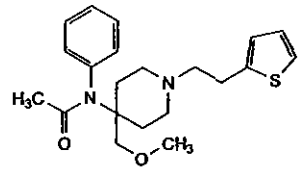
A. *N*-[4-(methoxymethyl)piperidin-4-yl]-*N*-phenylpropanamide,



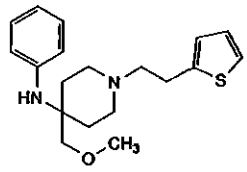
B. *cis*-4-(methoxymethyl)-4-(phenylpropanoylamino)-1-[2-(thiophen-2-yl)ethyl]piperidine 1-oxide,



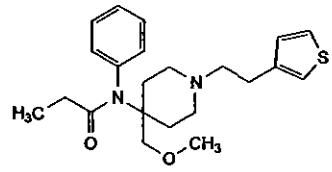
C. [4-(phenylamino)-1-[2-(thiophen-2-yl)ethyl]piperidin-4-yl]methanol,



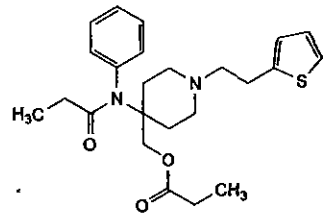
D. *N*-[4-(methoxymethyl)-1-[2-(thiophen-2-yl)ethyl]piperidin-4-yl]-*N*-phenylacetamide,



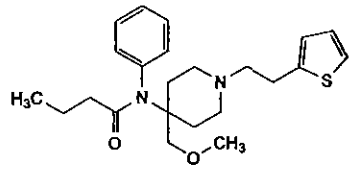
E. 4-(methoxymethyl)-*N*-phenyl-1-[2-(thiophen-2-yl)ethyl]piperidin-4-amine,



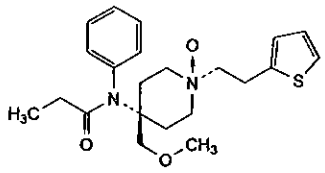
F. *N*-[4-(methoxymethyl)-1-[2-(thiophen-3-yl)ethyl]piperidin-4-yl]-*N*-phenylpropanamide,



G. [4-(phenylpropanoylamino)-1-[2-(thiophen-2-yl)ethyl]piperidin-4-yl]methyl propanoate,



H. *N*-[4-(methoxymethyl)-1-[2-(thiophen-2-yl)ethyl]piperidin-4-yl]-*N*-phenylbutanamide,

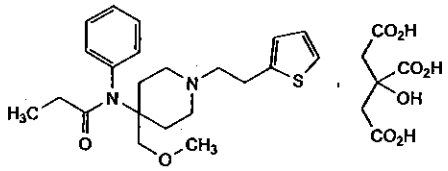


I. *trans*-4-(methoxymethyl)-4-(phenylpropanoylamino)-1-[2-(thiophen-2-yl)ethyl]piperidine 1-oxide.

Ph Eur

Sufentanil Citrate

(Ph. Eur. monograph 1269)



$C_{28}H_{38}N_2O_9S$ 578.7 *60561-17-3*

Action and use
Opioid receptor agonist; analgesic.

Ph Eur

DEFINITION
N-[4-(Methoxymethyl)-1-[2-(thiophen-2-yl)ethyl]piperidin-4-yl]-*N*-phenylpropanamide citrate.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Soluble in water, freely soluble in methanol, soluble in ethanol (96 per cent).

mp
About 140 °C, with decomposition.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of sufentanil citrate.*

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 0.2 g in *water R* and dilute to 20 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) In order to produce impurity E *in situ*, dissolve 10 mg of the substance to be examined in 10.0 mL of *dilute hydrochloric acid R*. Heat on a water-bath under a reflux condenser for 4 h. Add 10.0 mL of *dilute sodium hydroxide solution R*. Evaporate to dryness on a water-bath. Cool and take up the residue in 10 mL of *methanol R*. Filter.

Reference solution (b) Dilute 5.0 mL of the test solution to 100.0 mL with *methanol R*. Dilute 1.0 mL of this solution to 10.0 mL with *methanol R*.

Column:
— *size*: *l* = 0.1 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
— *mobile phase A*: 5 g/L solution of *ammonium carbonate R* in a mixture of 10 volumes of *tetrahydrofuran R* and 90 volumes of *water R*;
— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	90 → 40	10 → 60
15 - 20	40	60

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µL.

Relative retention With reference to sufentanil (retention time = about 13 min): impurity A = about 0.3; impurity B = about 0.4; impurity I = about 0.45; impurity C = about 0.7; impurity D = about 0.85; impurity E = about 0.9; impurity F = about 0.95; impurity G = about 1.05; impurity H = about 1.1.

System suitability Reference solution (a):
— *resolution*: minimum 4.0 between the peaks due to impurity E and sufentanil.

Limits:
— *impurities A, B, C, D, E, F, G, H, I*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
— *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);
— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak with a relative retention with reference to sufentanil of 0.05 or less.

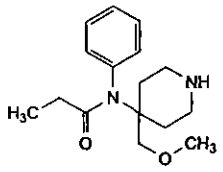
Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C.

ASSAY
Dissolve 0.400 g in 50 mL of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R* and titrate with *0.1 M perchloric acid*, using 0.2 mL of *naphtholbenzein solution R* as indicator.

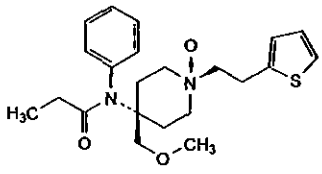
1 mL of *0.1 M perchloric acid* is equivalent to 57.87 mg of $C_{28}H_{38}N_2O_9S$.

STORAGE
Protected from light.

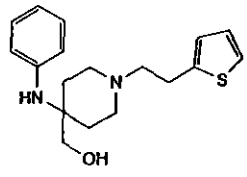
IMPURITIES
Specified impurities *A, B, C, D, E, F, G, H, I.*



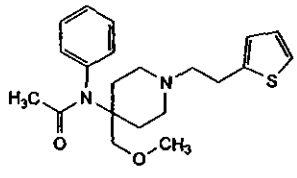
A. *N*-[4-(methoxymethyl)piperidin-4-yl]-*N*-phenylpropanamide,



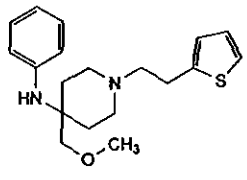
B. *cis*-4-(methoxymethyl)-4-(phenylpropanoylamino)-1-[2-(thiophen-2-yl)ethyl]piperidine 1-oxide,



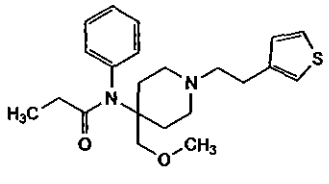
C. [4-(phenylamino)-1-[2-(thiophen-2-yl)ethyl]piperidin-4-yl]methanol,



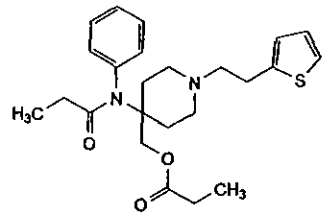
D. *N*-[4-(methoxymethyl)-1-[2-(thiophen-2-yl)ethyl]piperidin-4-yl]-*N*-phenylacetamide,



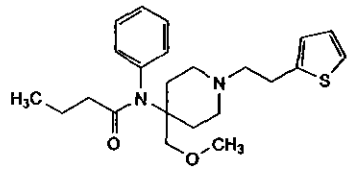
E. 4-(methoxymethyl)-*N*-phenyl-1-[2-(thiophen-2-yl)ethyl]piperidin-4-amine,



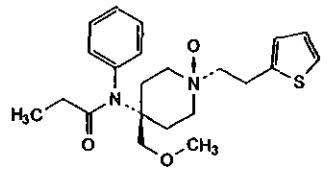
F. *N*-[4-(methoxymethyl)-1-[2-(thiophen-3-yl)ethyl]piperidin-4-yl]-*N*-phenylpropanamide,



G. [4-(phenylpropanoylamino)-1-[2-(thiophen-2-yl)ethyl]piperidin-4-yl]methyl propanoate,



H. *N*-[4-(methoxymethyl)-1-[2-(thiophen-2-yl)ethyl]piperidin-4-yl]-*N*-phenylbutanamide,



I. *trans*-4-(methoxymethyl)-4-(phenylpropanoylamino)-1-[2-(thiophen-2-yl)ethyl]piperidine 1-oxide.

Ph Eur

Compressible Sugar

Action and use
Excipient.

DEFINITION
Compressible Sugar contains Sucrose and maltodextrin or dried glucose syrup. It contains 95.0 to 98.0% of Sucrose and 2 to 5% of dried glucose syrup or maltodextrin. It may contain a suitable lubricant, invert sugar or suitable colouring matter.

CHARACTERISTICS
Dry free-flowing powder or microcrystalline agglomerates.

Very soluble in *water*.

Dissolve 20 g in 80 mL of *water*, dilute to 100 mL with *water* and filter (solution S).

IDENTIFICATION
A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of compressible sugar *(RS 401)*.

B. The specific optical rotation of the uninverted solution obtained in the Assay is not less than 62.6 and the acid-inverted solution is laevorotatory.

TESTS
Conductivity
Not more than 35 $\mu S \cdot cm^{-1}$, Appendix V O. Dissolve 31.3 g in *carbon dioxide-free water* prepared from *distilled water* and dilute to 100 mL with the same solvent. Measure the conductivity of the solution (C_1), while gently stirring with a magnetic stirrer, and that of the water used for preparing the solution (C_2). The readings must be stable within 1% over a period of 30 seconds. Calculate the conductivity of the

solution of the substance being examined from the expression C_1-0.35C_2.

Calcium
Add 1 mL of *ammonium oxalate* to 5 mL of solution S. The solution remains clear for not less than 1 minute.

Lead
Not more than 0.5 ppm of Pb when determined by *atomic absorption spectrometry*, Appendix II D, Method II, using an apparatus equipped with a graphite furnace and the following solution. Dissolve 50 mg in 0.5 mL of *lead-free nitric acid* in a polyfluorocarbon-lined digestion bomb and heat at 150° for 5 hours. Allow to cool and dilute to 5 mL with *water*. Measure the absorbance at 283.3 nm, maintaining the drying temperature of the furnace at 110°, the ashing temperature at 600° and the atomising temperature at 2100°.

Chloride
2 mL of solution S diluted to 15 mL with *water* complies with the *limit test for chlorides*, Appendix VII (125 ppm).

Sulfate
7.5 mL of solution S diluted to 15 mL with *water* complies with the *limit test for sulfates*, Appendix VII (100 ppm).

Loss on drying
When dried at 105° for 4 hours, loses 0.25 to 1.0% of its weight. Use 1 g.

ASSAY
To 26 g of the substance being examined, previously dried, add 0.3 mL of a saturated aqueous solution of *lead(II) acetate* and 90 mL of *water*, shake, dilute to 100 mL with *water* and mix. Distribute evenly on the surface of a sheet of medium-fast filter paper 8 g of chromatographic siliceous earth and filter the solution with the aid of vacuum, discarding the first 20 mL of the filtrate. Transfer 25 mL of the filtrate into each of two 50 mL flasks. Slowly add 6 mL of *2M hydrochloric acid* to one flask while rotating it, add 10 mL of *water*, mix, place the flask in a water bath at 60°, continuously shake for 3 minutes and allow the flask to stand in the water bath for a further 7 minutes. Immediately cool to 20° and dilute the solution with *water* to 50 mL and mix. Cool the contents of the second flask to 20°, dilute with *water* to volume and mix. Maintain both flasks at 20° for 30 minutes. Determine the *specific optical rotation*, Appendix V F, of each solution at 20°. Calculate the percentage of $C_{12}H_{22}O_{11}$ using the expression $100(a_0 - a_i)/88.3$ where a_0 and a_i are the specific optical rotations of the uninverted and acid-inverted solutions respectively.

Sugar Spheres

(Ph. Eur. monograph 1570)

Action and use
Excipient.

Ph Eur

DEFINITION
Sugar spheres contain not more than 92 per cent of sucrose, calculated on the dried basis. The remainder consists of maize starch and may also contain starch hydrolysates and colour additives. The diameter of sugar spheres varies usually from 200 µm to 2000 µm and the upper and lower limits of the size of the sugar spheres are stated on the label.

IDENTIFICATION
A. Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel G plate R*.

Solvent mixture *water R, methanol R* (2:3 *V/V*).

Test solution Mix 2 mL of solution S (see Tests) with 3 mL of *methanol R* and dilute to 20 mL with the solvent mixture.

Reference solution (a) Dissolve 10 mg of *sucrose GRS* in the solvent mixture and dilute to 20 mL with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *fructose R*, 10 mg of *glucose R*, 10 mg of *lactose monohydrate R* and 10 mg of *sucrose R* in the solvent mixture and dilute to 20 mL with the solvent mixture.

Apply to the plate 2 µL of each solution and thoroughly dry the points of application. Develop over a path of 15 cm using a mixture of 10 volumes of *water R*, 15 volumes of *methanol R*, 25 volumes of *anhydrous acetic acid R* and 50 volumes of *ethylene chloride R*, measured accurately as a slight excess of water causes cloudiness of the solution. Dry the plate in a current of warm air. Repeat the development immediately after renewing the mobile phase. Dry the plate in a current of warm air and spray evenly with a 5 g/L solution of *thymol R* in a mixture of 5 volumes of *sulfuric acid R* and 95 volumes of *ethanol (96 per cent) R*. Heat at 130 °C for 10 min. The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows 4 clearly separated spots.

B. To a water slurry of the insoluble portion obtained in the assay, add 0.05 mL of *iodine solution R1*. A dark-blue colour is produced, which disappears on heating.

C. To 5 mL of solution S add 0.15 mL of freshly prepared *copper sulfate solution R* and 2 mL of freshly prepared *dilute sodium hydroxide solution R*. The solution is blue and clear and remains so after boiling. To the hot solution add 4 mL of *dilute hydrochloric acid R* and boil for 1 min. Add 4 mL of *dilute sodium hydroxide solution R*. An orange precipitate is formed immediately.

TESTS
Solution S
To 0.5 g in a 100 mL volumetric flask add 80 mL of *water R* and shake until the sucrose is dissolved. Dilute to 100.0 mL with *water R*. Filter under vacuum to obtain a clear solution.

Fineness (*2.9.35*)
Not less than 90 per cent *m/m* of the sugar spheres are between the lower and the upper limits of the size of the sugar spheres stated on the label.

Loss on drying (*2.2.32*)
Maximum 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulfated ash (*2.4.14*)
Maximum 0.2 per cent, determined on 2 g.

Microbial contamination
TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*).

TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

Absence of *Escherichia coli* (*2.6.13*).

Absence of *Salmonella* (*2.6.13*).

ASSAY

Sucrose content

Weigh 10.000 g of ground sugar spheres in a 100 mL flask and make up to 100.0 mL with *water R*. Stir and decant. Filter under vacuum to obtain a clear solution (the insoluble portion is used for identification test B). Measure the angle of optical rotation (*2.2.7*) and calculate the sucrose percentage content using the following expression:

$$\frac{10^6 \times \alpha}{66.5 \times l \times m \times (100 - H)}$$

α = angle of rotation;
l = length of the polarimeter tube, in decimetres;
m = exact mass of the sample, in grams;
H = loss on drying.

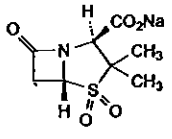
LABELLING

The label states the upper and the lower limits of the size of the sugar spheres.

Ph Eur

Sulbactam Sodium

(Ph. Eur. monograph 2209)



$C_8H_{10}NNaO_5S$ 255.2 *69388-84-7*

Action and use

Beta-lactam antibacterial.

Ph Eur

DEFINITION

Sodium (2*S*,5*R*)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylate 4,4-dioxide.

Semi-synthetic product derived from a fermentation product.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, hygroscopic, crystalline powder.

Solubility

Freely soluble in water, sparingly soluble in ethyl acetate, very slightly soluble in ethanol (96 per cent). It is freely soluble in dilute acids.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *sulbactam sodium CRS*.

B. It gives reaction (a) of sodium (*2.3.1*).

TESTS

Appearance of solution

The solution is clear (*2.2.1*).

Dissolve 2.0 g in *water R* and dilute to 20 mL with the same solvent.

Absorbance (*2.2.25*)

Maximum 0.10 at 430 nm.

Dissolve 1.0 g in *water R* and dilute to 100.0 mL with the same solvent.

pH (*2.2.3*)

4.5 to 7.2; if the substance is sterile: 5.2 to 7.2.

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 mL with the same solvent.

Specific optical rotation (*2.2.7*)

+ 219 to + 233 (anhydrous substance).

Dissolve 0.500 g in *water R* and dilute to 50.0 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Solution A 2.72 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 4.0 with *dilute phosphoric acid R*.

Solution B Dilute 2 mL of *acetonitrile R1* to 100.0 mL with solution A.

Test solution Suspend 77.0 mg of the substance to be examined in 2 mL of *acetonitrile R1*, sonicate for about 5 min and dilute to 100.0 mL with solution A.

Reference solution (a) Suspend 70.0 mg of *sulbactam CRS* in 2 mL of *acetonitrile R1*, sonicate for about 5 min and dilute to 100.0 mL with solution A.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 100.0 mL with solution B. Dilute 1.0 mL of this solution to 10.0 mL with solution B.

Reference solution (c) Dissolve 15.0 mg of *6-aminopenicillanic acid R* (impurity B) in solution A and dilute to 50.0 mL with solution A.

Reference solution (d) Mix 1 mL of reference solution (a) and 1 mL of reference solution (c) and dilute to 25.0 mL with solution B.

Reference solution (e) Dissolve 8 mg of *sulbactam for peak identification CRS* (containing impurities A, C, D, E and F) in 1 mL of *acetonitrile R1*, sonicate for about 5 min and dilute to 10 mL with solution B.

Column:
- *size*: l = 0.10 m, Ø = 4.0 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3.0 µm);
- *temperature*: 40 °C.

Mobile phase:
- *mobile phase A*: 5.44 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 4.0 with *dilute phosphoric acid R*;
- *mobile phase B*: mobile phase A, *acetonitrile R1* (40:60 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2.0	98	2
2.0 - 9.5	98 → 50	2 → 50
9.5 - 12.0	50	50

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µL of the test solution, solution B and reference solutions (b), (d) and (e).

Identification of impurities Use the chromatogram supplied with *sulbactam for peak identification CRS* and the chromatogram obtained with reference solution (e) to identify the peaks due to impurities A, C, D, E and F; use the

chromatogram obtained with reference solution (d) to identify the peak due to impurity B.

Relative retention With reference to sulbactam (retention time = about 3 min): impurity A = about 0.4; impurity B = about 0.7; impurity C = about 2.3; impurity D = about 3.1; impurity E = about 3.3; impurity F = about 3.9.

System suitability Reference solution (d):
— *resolution*: minimum 5.0 between the peaks due to impurity B and sulbactam.

Calculation of percentage contents:
— *correction factors*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.6; impurity B = 0.5; impurity D = 0.5; impurity F = 0.6;
— for each impurity, use the concentration of sulbactam in reference solution (b).

Limits:
— *impurity A*: maximum 0.5 per cent;
— *impurities C, E*: for each impurity, maximum 0.2 per cent;
— *impurities B, D, F*: for each impurity, maximum 0.1 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 1.0 per cent;
— *reporting threshold*: 0.05 per cent.

2-Ethylhexanoic acid (*2.4.28*)
Maximum 0.5 per cent *m/m*.

Water (*2.5.12*)
Maximum 1.0 per cent, determined on 1.00 g.

Bacterial endotoxins (*2.6.14, Method A*)
Less than 0.17 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

Calculate the percentage content of $C_8H_{10}NNaO_5S$ taking into account the assigned content of *sulbactam CRS* and a conversion factor of 1.094.

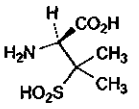
STORAGE

In an airtight container. If the substance is sterile, store in a sterile, the container is also sterile and tamper-evident.

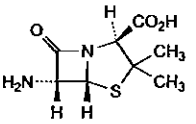
IMPURITIES

Specified impurities *A, B, C, D, E, F.*

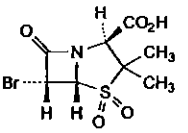
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) G.



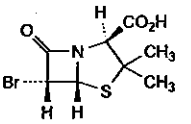
A. (2*S*)-2-amino-3-methyl-3-sulfinobutanoic acid,



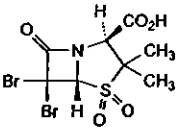
B. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),



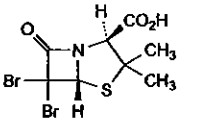
C. (2*S*,5*R*,6*R*)-6-bromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide (6-bromopenicillanic acid sulfone),



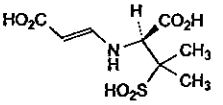
D. (2*S*,5*R*,6*R*)-6-bromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-bromopenicillanic acid),



E. (2*S*,5*R*)-6,6-dibromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide (6,6-dibromopenicillanic acid sulfone),



F. (2*S*,5*R*)-6,6-dibromo-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6,6-dibromopenicillanic acid),



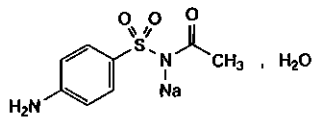
G. (2*S*)-2-[[(2*E*)-2-carboxyethenyl]amino]-3-methyl-3-sulfinobutanoic acid.

Ph Eur

Sulfacetamide Sodium

Soluble Sulfacetamide

(Ph. Eur. monograph 0107)



$C_8H_9N_2NaO_3S,H_2O$ 254.2

Action and use
Sulfonamide antibacterial.

Ph Eur

DEFINITION
Sodium acetyl[(4-aminophenyl)sulfonyl]azanide monohydrate.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or yellowish-white, crystalline powder.

Solubility
Freely soluble in water, slightly soluble in anhydrous ethanol.

IDENTIFICATION
First identification: B, F.

Second identification: A, C, E, F.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 0.1 g in *phosphate buffer solution pH 7.0 R* and dilute to 100.0 mL with the same buffer solution. Dilute 1.0 mL of this solution to 100.0 mL with *phosphate buffer solution pH 7.0 R.*

Spectral range 230-350 nm.

Absorption maximum At 255 nm.

Specific absorbance at the absorption maximum 660 to 720 (anhydrous substance).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *sulfacetamide sodium CRS.*

C. Melting point (*2.2.14*): 181 °C to 185 °C.

Dissolve 1 g in 10 mL of *water R*, add 6 mL of *dilute acetic acid R* and filter. Wash the precipitate with a small quantity of *water R* and dry at 100-105 °C for 4 h.

E. Dissolve about 1 mg of the precipitate obtained in identification C, with heating, in 1 mL of *water R*. The solution gives the reaction of primary aromatic amines (*2.3.1*) with formation of an orange-red precipitate.

F. Solution S (see Tests) gives the reactions of sodium (*2.3.1*).

TESTS
Solution S
Dissolve 1.25 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution GY_4 (*2.2.2, Method II*).

pH (*2.2.3*)
8.0 to 9.5 for solution S.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use and carry out the test protected from light.*

Test solution Dissolve 0.200 g of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dissolve 5 mg of *sulfacetamide sodium CRS* and 5 mg of *sulfanilamide R* (impurity A) in 1.0 mL of the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:
- *size: l* = 0.125 m, Ø = 4 mm;
- *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *glacial acetic acid R, methanol R, water for chromatography R* (1:10:89 *V/V/V*).

Flow rate 0.8 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µL.

Run time 7 times the retention time of sulfacetamide.

Relative retention With reference to sulfacetamide (retention time = about 5 min): impurity A = about 0.5.

System suitability Reference solution (a):
- *resolution*: minimum 5.0 between the peaks due to impurity A and sulfacetamide.

Limits:
- *correction factor*: for the calculation of the content, multiply the peak area of impurity A by 0.5;
- *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulfates (*2.4.13*)
Maximum 200 ppm.

Dissolve 2.5 g in *distilled water R* and dilute to 25 mL with the same solvent. Add 25 mL of *dilute acetic acid R*, shake for 30 min and filter. 15 mL of the filtrate complies with the limit test for sulfates.

Water (*2.5.12*)
6.0 per cent to 8.0 per cent, determined on 0.200 g.

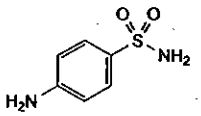
ASSAY
Dissolve 0.500 g in a mixture of 50 mL of *water R* and 20 mL of *dilute hydrochloric acid R*. Cool the solution in a bath of iced water and carry out the determination of primary aromatic amino-nitrogen (*2.5.8*), determining the end-point electrometrically.

1 mL of *0.1 M sodium nitrite* is equivalent to 23.62 mg of $C_8H_9N_2NaO_3S$.

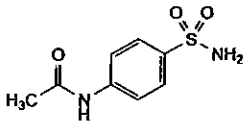
STORAGE
Protected from light.

IMPURITIES
Specified impurities *A.*

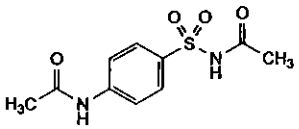
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) B, C, D.



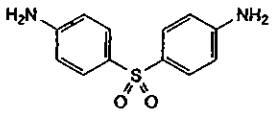
A. 4-aminobenzenesulfonamide (sulfanilamide),



B. *N*-(4-sulfamoylphenyl)acetamide,



C. *N*-[[4-(acetylamino)phenyl]sulfonyl]acetamide,

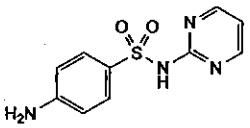


D. 4,4′-sulfonyldianiline (dapsone).

Ph Eur

Sulfadiazine

(Ph. Eur. monograph 0294)



$C_{10}H_{10}N_4O_2S$ 250.3 *68-35-9*

Action and use
Sulfonamide antibacterial.

Preparation
Sulfadiazine Injection

Ph Eur

DEFINITION
4-Amino-*N*-(pyrimidin-2-yl)benzenesulfonamide.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White, yellowish-white or pinkish-white, crystalline powder or crystals.

Solubility
Practically insoluble in water, slightly soluble in acetone, very slightly soluble in ethanol (96 per cent). It dissolves in solutions of alkali hydroxides and in dilute mineral acids.

IDENTIFICATION
First identification: A.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *sulfadiazine CRS.*

B. Thin-layer chromatography (*2.2.27*).

Solvent mixture *concentrated ammonia R, methanol R* (4:96 *V/V*)

Test solution Dissolve 20 mg of the substance to be examined in 3 mL of the solvent mixture and dilute to 5.0 mL with the solvent mixture.

Reference solution Dissolve 20 mg of *sulfadiazine CRS* in 3 mL of the solvent mixture and dilute to 5.0 mL with the solvent mixture.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *dilute ammonia R1, water R, nitromethane R, dioxan R* (3:5:40:50 *V/V/V/V*).

Application 5 µL.

Development Over 3/4 of the plate.

Drying At 105 °C.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. Place 3 g in a dry tube. Immerse the lower part of the tube, inclined at 45°, in a silicone oil bath and heat to about 270 °C. The substance to be examined decomposes and a white or yellowish-white sublimate is formed, which, after recrystallisation from *toluene R* and drying at 100 °C, melts (*2.2.14*) at 123 °C to 127 °C.

D. Dissolve about 5 mg in 10 mL of a 103 g/L solution of *hydrochloric acid R*. Dilute 1 mL of the solution to 10 mL with *water R*. The solution, without further acidification, gives the reaction of primary aromatic amines (*2.3.1*).

TESTS
Appearance of solution
The solution is not more intensely coloured than reference solution Y_5, BY_5 or GY_5 (*2.2.2, Method II*).

Dissolve 0.8 g in a mixture of 5 mL of *dilute sodium hydroxide solution R* and 5 mL of *water R*.

Acidity
To 1.25 g, finely powdered, add 25 mL of *carbon dioxide-free water R*. Heat at about 70 °C for 5 min. Cool in iced water for about 15 min and filter. To 20 mL of the filtrate add 0.1 mL of *bromothymol blue solution R1*. Not more than 0.2 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture 40 g/L solution of *sodium hydroxide R*, *acetonitrile R*, *water R* (2:20:60 *V/V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 100.0 mL with *water R*.

Reference solution (a) Dissolve 5.0 mg of *sulfadiazine impurity A CRS* and 5.0 mg of *sulfanilic acid RV* (impurity B)

in the solvent mixture and dilute to 10.0 mL with *water R*. Dilute 1.0 mL of the solution to 100.0 mL with the mobile phase. Dilute 3.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (c) Dissolve the contents of a vial of *acetylsulfadiazine CRS* (impurity E) in 1 mL of the mobile phase.

Reference solution (d) Dissolve 5 mg of *sulfadiazine for identification of impurity F CRS* in the solvent mixture and dilute to 10.0 mL with *water R*.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R*, 2.8 g/L solution of *phosphoric acid R* (10:90 *V/V*).

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 260 nm.

Injection 20 µL.

Run time 7 times the retention time of sulfadiazine.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A and B; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity E; use the chromatogram obtained with reference solution (d) to identify the peak due to impurity F.

Relative retention With reference to sulfadiazine (retention time = about 8.5 min): impurity A = about 0.26; impurity B = about 0.30; impurity E = about 2.1; impurity F = about 6.0.

System suitability Reference solution (a):
- *resolution*: minimum 2.0 between the peaks due to impurities A and B.

Limits:
- *correction factor*: for the calculation of content, multiply the peak area of impurity E by 0.7;
- *impurities A, B*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
- *impurity E*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *impurity F*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent);
- *total*: maximum 0.5 per cent;
- *disregard limit*: 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.03 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in a mixture of 20 mL of *dilute hydrochloric acid R* and 50 mL of *water R*. Cool the solution in iced water. Carry out the determination of primary aromatic amino-nitrogen (*2.5.8*), determining the end-point electrometrically.

1 mL of *0.1 M sodium nitrite* is equivalent to 25.03 mg of $C_{10}H_{10}N_4O_2S$.

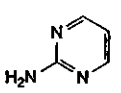
STORAGE

Protected from light.

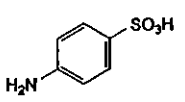
IMPURITIES

Specified impurities *A, B, E, F.*

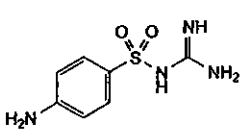
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C, D.*



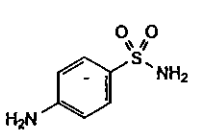
A. pyrimidin-2-amine,



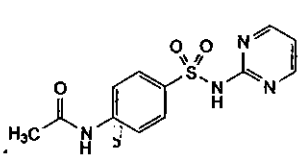
B. 4-aminobenzenesulfonic acid (sulfanilic acid),



C. [(4-aminophenyl)sulfonyl]guanidine (sulfaguanidine),



D. 4-aminobenzenesulfonamide (sulfanilamide),



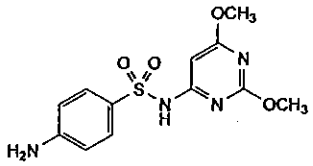
E. *N*-[4-(pyrimidin-2-ylsulfamoyl)phenyl]acetamide (acetylsulfadiazine),

F. unknown structure.

Ph Eur

Sulfadimethoxin

(Ph. Eur. monograph 2741)



$C_{12}H_{14}N_4O_4S$ 310.3 *122-11-2*

Action and use
Sulfonamide antibacterial.

Ph Eur

DEFINITION
4-Amino-*N*-(2,6-dimethoxypyrimidin-4-yl)benzene-1-sulfonamide.

Content
97.5 per cent to 102.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, slightly soluble in methylene chloride, very slightly soluble in ethanol (96 per cent).

It is freely soluble in dilute sodium hydroxide and sparingly soluble in dilute hydrochloric acid.

mp
197 °C to 202 °C.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *sulfadimethoxine CRS.*

TESTS
Acidity
Suspend 0.5 g in 25 mL of *carbon dioxide-free water R.* Heat the suspension at 70 °C for 5 min, cool quickly to room temperature and filter. Not more than 0.1 mL of *0.1 M sodium hydroxide* is required to reach pH 7.0.

Related substances
Liquid chromatography (*2.2.29*).

Solution A Dissolve 6.0 g of *sodium dihydrogen phosphate R* in 950 mL of *water for chromatography R*, adjust to pH 7.0 with *dilute sodium hydroxide solution R* and dilute to 1000 mL with *water for chromatography R.*

Test solution Dissolve 20.0 mg of the substance to be examined in 25 mL of *methanol R* and dilute to 100.0 mL with solution A.

Reference solution (a) Dissolve 20.0 mg of *sulfadimethoxine CRS* in 25 mL of *methanol R* and dilute to 100.0 mL with solution A.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

Reference solution (c) Dissolve 4 mg of *sulfadimethoxine for peak identification CRS* (containing impurities A and F) in 5 mL of *methanol R* and dilute to 20 mL with solution A.

Column:
— *size:* l = 0.25 m, Ø = 4.6 mm;
— *stationary phase: end-capped ethylene-bridged octadecylsilyl silica gel for chromatography (hybrid material) R* (5 µm);
— *temperature:* 25 °C.

Mobile phase:
— *mobile phase A: methanol R*, solution A (25:75 *V/V*);
— *mobile phase B: methanol R, acetonitrile R*, solution A (25:35:40 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	100	0
10 - 30	100 → 0	0 → 100
30 - 35	0	100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µL of the test solution and reference solutions (b) and (c).

Identification of impurities Use the chromatogram supplied with *sulfadimethoxine for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A and F.

Relative retention With reference to sulfadimethoxine (retention time = about 11 min): impurity F = about 0.4; impurity A = about 1.2.

System suitability:
— *resolution:* minimum 2.5 between the peaks due to sulfadimethoxine and impurity A in the chromatogram obtained with reference solution (c);
— *signal-to-noise ratio:* minimum 40 for the principal peak in the chromatogram obtained with reference solution (b).

Calculation of percentage contents:
— *correction factors:* multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.4; impurity F = 1.7;
— for each impurity, use the concentration of sulfadimethoxine in reference solution (b).

Limits:
— *impurities A, F:* for each impurity, maximum 0.15 per cent;
— *unspecified impurities:* for each impurity, maximum 0.10 per cent;
— *total:* maximum 0.5 per cent;
— *reporting threshold:* 0.05 per cent.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

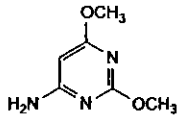
Calculate the percentage content of $C_{12}H_{14}N_4O_4S$ taking into account the assigned content of *sulfadimethoxine CRS.*

STORAGE
Protected from light.

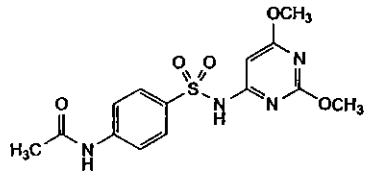
IMPURITIES
Specified impurities *A, F.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is

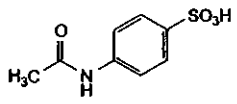
therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) B, C, D, E.



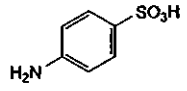
A. 2,6-dimethoxypyrimidin-4-amine,



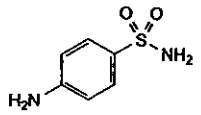
B. *N*-[4-[(2,6-dimethoxypyrimidin-4-yl)sulfamoyl] phenyl]acetamide,



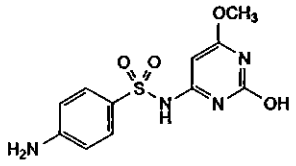
C. 4-(acetylamino)benzene-1-sulfonic acid,



D. 4-aminobenzene-1-sulfonic acid (sulfanilic acid),



E. 4-aminobenzene-1-sulfonamide (sulfanilamide),

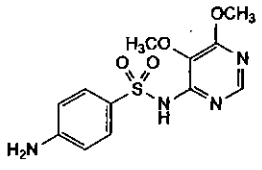


F. 4-amino-*N*-(2-hydroxy-6-methoxypyrimidin-4-yl)benzene-1-sulfonamide.

Ph Eur

Sulfadoxine

(Ph. Eur. monograph 0740)



$C_{12}H_{14}N_4O_4S$ 310.3 *2447-57-6*

Action and use
Sulfonamide antibacterial.

Ph Eur

DEFINITION
Sulfadoxine contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 4-amino-*N*-(5,6-dimethoxypyrimidin-4-yl)benzenesulfonamide, calculated with reference to the dried substance.

CHARACTERS
White or yellowish-white crystalline powder or crystals, very slightly soluble in water, slightly soluble in alcohol and in methanol. It dissolves in solutions of alkali hydroxides and in dilute mineral acids.

It melts at about 198 °C, with decomposition.

IDENTIFICATION
First identification: A, C.

Second identification: B, C, D.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *sulfadoxine CRS*. Examine the substances prepared as discs.

B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 0.5 g in 1 mL of a 40 per cent *V/V* solution of *sulfuric acid R*, heating gently. Continue heating until a crystalline precipitate appears (about 2 min). Allow to cool and add 10 mL of *dilute sodium hydroxide solution R*. Cool again. Add 25 mL of *ether R* and shake for 5 min. Separate the ether layer, dry over *anhydrous sodium sulfate R* and filter. Evaporate the solvent by heating in a water-bath. The residue melts (*2.2.14*) at 80 °C to 82 °C or at 90 °C to 92 °C.

D. Dissolve about 5 mg in 10 mL of *1 M hydrochloric acid*. Dilute 1 mL of the solution to 10 mL with *water R*. The solution, without further acidification, gives the reaction of primary aromatic amines (*2.3.1*).

TESTS
Appearance of solution
Dissolve 1.0 g in a mixture of 5 mL of *dilute sodium hydroxide solution R* and 5 mL of *water R*. The solution is not more intensely coloured than reference solution Y_5, BY_5 or GY_5 (*2.2.2, Method II*).

Acidity
To 1.25 g, finely powdered, add 25 mL of *carbon dioxide-free water R*. Heat at 70 °C for 5 min. Cool in a bath of iced water for about 15 min and filter. To 20 mL of the filtrate add 0.1 mL of *bromothymol blue solution R1*. Not more than 0.2 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Related substances
Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance.

Test solution (a) Dissolve 0.10 g of the substance to be examined in 3 mL of a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R* and dilute to 5 mL with the same mixture of solvents.

Test solution (b) Dilute 1 mL of test solution (a) to 5 mL with a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R*.

Reference solution (a) Dissolve 20 mg of *sulfadoxine CRS* in 3 mL of a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R* and dilute to 5 mL with the same mixture of solvents.

Reference solution (b) Dilute 2.5 mL of test solution (b) to 100 mL with a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R*.

Apply to the plate 5 µL of each solution. Develop over a path of 15 cm using a mixture of 3 volumes of *dilute ammonia R1*, 5 volumes of *water R*, 40 volumes of *nitromethane R* and 50 volumes of *dioxan R*. Dry the plate at 100 °C to 105 °C and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Loss on drying (*2.2.32*)
Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Not more than 0.1 per cent, determined on 1.0 g.

ASSAY
Carry out the determination of primary aromatic amino-nitrogen (*2.5.8*), using 0.250 g and determining the end-point electrometrically.

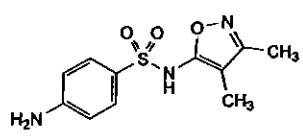
1 mL of *0.1 M sodium nitrite* is equivalent to 31.03 mg of $C_{12}H_{14}N_4O_4S$.

STORAGE
Store protected from light.

Ph Eur

Sulfafurazole

(Ph. Eur. monograph 0741)



$C_{11}H_{13}N_3O_3S$ 267.3 *127-69-5*

Action and use
Sulfonamide antibacterial.

Ph Eur

DEFINITION
Sulfafurazole contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 4-amino-*N*-(3,4-dimethylisoxazol-5-yl)benzenesulfonamide, calculated with reference to the dried substance.

CHARACTERS
White or yellowish-white, crystalline powder or crystals, practically insoluble in water, sparingly soluble in alcohol, slightly soluble in methylene chloride. It dissolves in solutions of alkali hydroxides and in dilute mineral acids.

It melts at about 197 °C, with decomposition.

IDENTIFICATION
First identification: A, C.

Second identification: B, C, D.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *sulfafurazole CRS*. Examine the substances prepared as discs.

B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

C. To 0.5 g add 1 mL of a 40 per cent *V/V* solution of *sulfuric acid R* and heat over a low flame to dissolve. Continue heating until a crystalline precipitate appears (about 2 min). Allow to cool and add 10 mL of *dilute sodium hydroxide solution R*. Cool. Shake the solution for 5 min with 25 mL of *ether R*. Separate the ether layer, dry over *anhydrous sodium sulfate R* and filter. Evaporate the solvent by heating on a water-bath. The residue melts (*2.2.14*) at 119 °C to 123 °C.

D. Dissolve about 5 mg in 10 mL of *1 M hydrochloric acid*. Dilute 1 mL of the solution to 10 mL with *water R*. The solution, without further acidification, gives the reaction of primary aromatic amines (*2.3.1*).

TESTS

Appearance of solution
Dissolve 0.4 g in a mixture of 5 mL of *dilute sodium hydroxide solution R* and 5 mL of *water R*, with gently warming if necessary. The solution is not more intensely coloured than reference solution Y_6, BY_6 or GY_6 (*2.2.2, Method II*).

Acidity
To 1.25 g, finely powdered, add 25 mL of *carbon dioxide-free water R*. Heat at 70 °C for 5 min. Cool in iced water for about 15 min and filter. To 20 mL of the filtrate add 0.1 mL of *bromothymol blue solution R1*. Not more than 0.2 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Related substances
Examine by thin-layer chromatography (*2.2.27*), using *silica gel GF_{254} R* as the coating substance.

Test solution (a) Dissolve 0.10 g of the substance to be examined in 3 mL of a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R* and dilute to 5 mL with the same mixture of solvents.

Test solution (b) Dilute 1 mL of test solution (a) to 5 mL with a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R*.

Reference solution (a) Dissolve 20 mg of *sulfafurazole CRS* in 3 mL of a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R* and dilute to 5 mL with the same mixture of solvents.

Reference solution (b) Dilute 1.25 mL of test solution (b) to 50 mL with a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R*.

Apply to the plate 5 µL of each solution. Develop over a path of 15 cm using a mixture of 1 volume of *concentrated ammonia R*, 25 volumes of *methanol R* and 75 volumes of *methylene chloride R*. Dry the plate at 100 °C to 105 °C and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Loss on drying (*2.2.32*)
Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Not more than 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.200 g in 50 mL of *acetone R*. Titrate with 0.1 M tetrabutylammonium hydroxide using a 4 g/L solution of *thymol blue R* in *methanol R* as indicator.

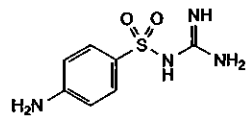
1 mL of 0.1 M tetrabutylammonium hydroxide is equivalent to 26.73 mg of $C_{11}H_{13}N_3O_3S$.

STORAGE
Store protected from light.

Ph Eur

Sulfaguanidine

(Ph. Eur. monograph 1476)



$C_7H_{10}N_4O_2S$ 214.3 *57-67-0*

Action and use
Sulfonamide antibacterial.

Ph Eur

DEFINITION
Sulfaguanidine contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (4-aminophenylsulfonyl)guanidine, calculated with reference to the dried substance.

CHARACTERS
A white or almost white, fine crystalline powder, very slightly soluble in water, slightly soluble in acetone, very slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride. It dissolves in dilute solutions of mineral acids.

IDENTIFICATION
First identification: A, B.

Second identification: A, C, D, E.

A. Melting point (*2.2.14*): 189 °C to 193 °C, determined on the dried substance.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *sulfaguanidine CRS*.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 5 mg in 10 mL of *1 M hydrochloric acid*. Dilute 1 mL of the solution to 10 mL with *water R*. The solution, without further acidification, gives the reaction of primary aromatic amines (*2.3.1*).

E. Suspend 0.1 g in 2 mL of *water R*, add 1 mL of *α-naphthol solution R* and 2 mL of a mixture of equal volumes of *water R* and *strong sodium hypochlorite solution R*. A red colour develops.

TESTS
Solution S
To 2.5 g, add 40 mL of *carbon dioxide-free water R*. Heat at about 70 °C for 5 min. Cool while stirring in iced water for about 15 min, filter and dilute to 50 mL with *carbon dioxide-free water R*.

Acidity
To 20 mL of solution S, add 0.1 mL of *bromothymol blue solution R1*. Not more than 0.2 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Related substances
Examine by thin layer chromatography (*2.2.27*), using a *TLC silica gel GF_{254} plate R*.

Test solution (a) Dissolve 50 mg of the substance to be examined in *acetone R* and dilute to 5 mL with the same solvent.

Test solution (b) Dilute 2 mL of test solution (a) to 10 mL with *acetone R*.

Reference solution (a) Dissolve 10 mg of *sulfaguanidine CRS* in *acetone R* and dilute to 5 mL with the same solvent.

Reference solution (b) Dilute 5 mL of test solution (b) to 200 mL with *acetone R*.

Reference solution (c) Dilute 5 mL of reference solution (b) to 10 mL with *acetone R*.

Reference solution (d) Dissolve 10 mg of *sulfanilamide R* in test solution (b) and dilute to 5 mL with the same solution.

Apply to the plate 10 µL of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *anhydrous formic acid R*, 20 volumes of *methanol R* and 70 volumes of *methylene chloride R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent) and at most one such spot is more intense than the spot in the chromatogram obtained with reference solution (c) (0.25 per cent). The test is not valid unless the chromatogram obtained with reference solution (d) shows two clearly separated principal spots.

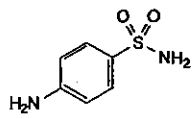
Loss on drying (*2.2.32*)
Not more than 8.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Not more than 0.1 per cent, determined on 1.0 g.

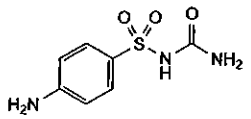
ASSAY
Dissolve 0.175 g in 50 mL of *dilute hydrochloric acid R*. Cool the solution in iced water. Carry out the determination of primary aromatic amino-nitrogen (*2.5.8*), determining the end-point electrometrically.

1 mL of *0.1 M sodium nitrite* is equivalent to 21.42 mg of $C_7H_{10}N_4O_2S$.

STORAGE
Store protected from light.

IMPURITIES


A. 4-aminobenzenesulfonamide (sulfanilamide),

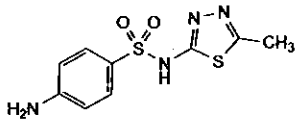


B. *N*-[(4-aminophenyl)sulfonyl]urea (sulfacarbamide).

Ph Eur

Sulfamethizole

(Ph. Eur. monograph 0637)



$C_9H_{10}N_4O_2S_2$ 270.3 *144-82-1*

Action and use
Sulfonamide antibacterial.

Ph Eur

DEFINITION
4-Amino-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)benzene-1-sulfonamide.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or yellowish-white, crystalline powder or crystals.

Solubility
Very slightly soluble in water, soluble in acetone, sparingly soluble in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides and in dilute mineral acids.

mp
About 210 °C.

IDENTIFICATION
First identification: A.
Second identification: B.

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *sulfamethizole CRS.*

B. Thin-layer chromatography (*2.2.27*).
Test solution Dissolve 30 mg of the substance to be examined in *acetone R* and dilute to 3 mL with the same solvent.
Reference solution Dissolve 30 mg of *sulfamethizole CRS* in *acetone R* and dilute to 3 mL with the same solvent.
Plate *TLC silica gel F_{254} plate R.*
Mobile phase *methanol R, methylene chloride R* (15:85 *V/V*).
Application 2 µL; the volume may be adapted based on the type of plate used.
Development Over 3/4 of the plate.
Drying At 100-105 °C.
Detection A Examine in ultraviolet light at 254 nm.
Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

Detection B Spray with a 40 g/L solution of *copper acetate R* and examine in daylight.
Results B The principal spot in the chromatogram obtained with the test solution is similar in colour to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Appearance of solution
The solution is not more intensely coloured than reference solution Y_5, BY_5 or GY_5 (*2.2.2, Method II*).
Dissolve 1.0 g in a mixture of 5 mL of *dilute sodium hydroxide solution R* and 5 mL of *water R.*

Acidity
To 1.25 g add 25 mL of *carbon dioxide-free water R* and heat at 70 °C for 5 min. Cool for about 15 min in iced water and filter. To 20 mL of the filtrate add 0.1 mL of *bromothymol blue solution R1.* Not more than 0.5 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Related substances
Liquid chromatography (*2.2.29*).
Solvent mixture *methanol R, water R* (15:85 *V/V*).
Test solution Dissolve 20.0 mg of the substance to be examined in 1 mL of *methanol R,* sonicate if necessary and dilute to 20.0 mL with the solvent mixture.
Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.
Reference solution (b) Dissolve 2 mg of *sulfamethizole for system suitability CRS* (containing impurities B, C and D) in 0.1 mL of *methanol R,* sonicate if necessary and dilute to 2 mL with the solvent mixture.
Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography compatible with 100 per cent aqueous mobile phases R* (5 µm);
— *temperature:* 30 °C.
Mobile phase:
— *mobile phase A: acetic acid R, methanol R, water for chromatography R* (1:14:85 *V/V/V*);
— *mobile phase B: acetic acid R, methanol R* (1:99 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	100	0
20 - 40	100 → 30	0 → 70
40 - 45	30	70

Flow rate 1.0 mL/min.
Detection Spectrophotometer at 254 nm.
Injection 20 µL.
Identification of impurities Use the chromatogram supplied with *sulfamethizole for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B, C and D.
Relative retention With reference to sulfamethizole (retention time = about 17 min): impurity B = about 0.23; impurity C = about 0.24; impurity D = about 1.9.
System suitability Reference solution (b):
— *resolution:* minimum 1.5 between the peaks due to impurities B and C.

Calculation of percentage contents:
— *correction factor*: multiply the peak area of impurity D by 1.4;
— for each impurity, use the concentration of sulfamethizole in reference solution (a).

Limits:
— *impurity D*: maximum 0.2 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.5 per cent;
— *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

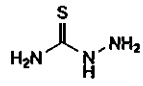
ASSAY
Carry out the determination of primary aromatic amino-nitrogen (*2.5.8*), using 0.2500 g and determining the end-point electrometrically.

1 mL of *0.1 M sodium nitrite* is equivalent to 27.03 mg of $C_9H_{10}N_4O_2S_2$.

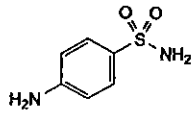
STORAGE
Protected from light.

IMPURITIES
Specified impurities D.

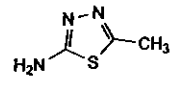
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) A, B, C.



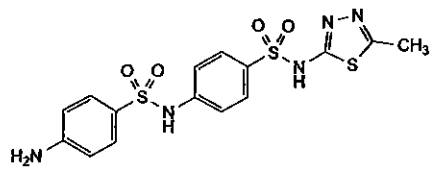
A. hydrazinecarbothioamide (thiosemicarbazide),



B. 4-aminobenzene-1-sulfonamide (sulfanilamide),



C. 5-methyl-1,3,4-thiadiazol-2-amine,

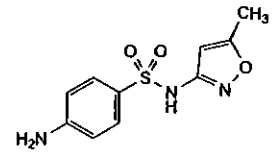


D. 4-(4-aminobenzene-1-sulfonamido)-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)benzene-1-sulfonamide.

Ph Eur

Sulfamethoxazole

(Ph. Eur. monograph 0108)



$C_{10}H_{11}N_3O_3S$ 253.3 *723-46-6*

Action and use
Sulfonamide antibacterial.

Preparations
Co-trimoxazole Infusion
Co-trimoxazole Oral Suspension
Paediatric Co-trimoxazole Oral Suspension
Co-trimoxazole Tablets
Co-trimoxazole Dispersible Tablets
Paediatric Co-trimoxazole Tablets

Ph Eur

DEFINITION
4-Amino-*N*-(5-methylisoxazol-3-yl)benzenesulfonamide.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, freely soluble in acetone, sparingly soluble in ethanol (96 per cent). It dissolves in dilute solutions of sodium hydroxide and in dilute acids.

IDENTIFICATION
First identification: A, B.
Second identification: A, C, D.

A. Melting point (*2.2.14*): 169 °C to 172 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *sulfamethoxazole CRS.*

C. Thin-layer chromatography (*2.2.27*).
Test solution Dissolve 20 mg of the substance to be examined in 3 mL of a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R* and dilute to 5 mL with the same mixture of solvents.
Reference solution Dissolve 20 mg of *sulfamethoxazole CRS* in 3 mL of a mixture of 2 volumes of *concentrated ammonia R* and 48 volumes of *methanol R* and dilute to 5 mL with the same mixture of solvents.
Plate *TLC silica gel F_{254} plate R.*
Mobile phase *dilute ammonia R1, water R, nitromethane R, dioxan R* (3:5:41:51 *V/V/V/V*).
Application 5 µL.
Development Over 3/4 of the plate.
Drying At 100-105 °C.
Detection Examine in ultraviolet light at 254 nm.
Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve about 5 mg in 10 mL of *1 M hydrochloric acid*. Dilute 1 mL of the solution to 10 mL with *water R*. The solution, without further acidification, gives the reaction of primary aromatic amines (*2.3.1*).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5, BY_5 or GY_5 (*2.2.2, Method II*).

Dissolve 1.0 g in a mixture of 5 mL of *dilute sodium hydroxide solution R* and 5 mL of *water R*.

Acidity

To 1.25 g, finely powdered, add 25 mL of *water R*. Heat at 70 °C for 5 min. Cool in iced water for about 15 min and filter. To 20 mL of the filtrate add 0.1 mL of *bromothymol blue solution R1*. Not more than 0.3 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in 45 mL of the mobile phase, sonicate at about 45 °C for 10 min, cool and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 10.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve 1 mg of the substance to be examined and 1 mg of *sulfamethoxazole impurity A CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (c) Dissolve 1.0 mg of *sulfamethoxazole impurity F CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 100.0 mL with the mobile phase.

Column:
- *size*: *l* = 0.25 m, Ø = 4.0 mm,
- *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm),
- *temperature*: 30 °C.

Mobile phase Mix 35 volumes of *methanol R2* and 65 volumes of a 13.6 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 5.3 with a 20 g/L solution of *potassium hydroxide R*.

Flow rate 0.9 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µL.

Run time 3 times the retention time of sulfamethoxazole.

Relative retention With reference to sulfamethoxazole (retention time = about 10 min): impurity D = about 0.3; impurity E = about 0.35; impurity F = about 0.45; impurity C = about 0.5; impurity A = about 1.2; impurity B = about 2.0.

System suitability Reference solution (b):
- *resolution*: minimum 3.5 between the peaks due to sulfamethoxazole and impurity A.

Limits:
- *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
- *impurity F*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent),
- *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
- *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent),
- *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.025 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Carry out the assay of primary aromatic amino-nitrogen (*2.5.8*), using 0.200 g and determining the end-point electrometrically.

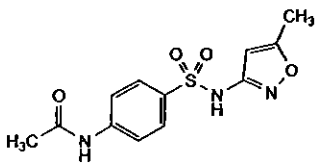
1 mL of *0.1 M sodium nitrite* is equivalent to 25.33 mg of $C_{10}H_{11}N_3O_3S$.

STORAGE

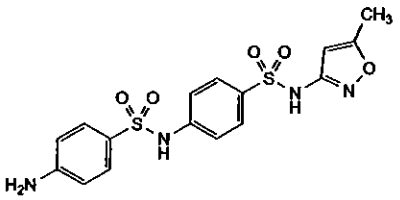
Protected from light.

IMPURITIES

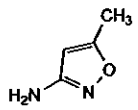
Specified impurities *A, B, C, D, E, F.*



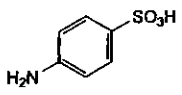
A. *N*-[4-[(5-methylisoxazol-3-yl)sulfamoyl]phenyl]acetamide,



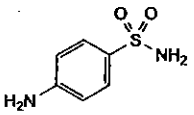
B. 4-[[(4-aminophenyl)sulfonyl]amino]-*N*-(5-methylisoxazol-3-yl)benzenesulfonamide,



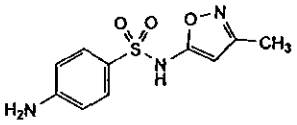
C. 5-methylisoxazol-3-amine,



D. 4-aminobenzenesulfonic acid (sulfanilic acid),



E. 4-aminobenzenesulfonamide (sulfanilamide),

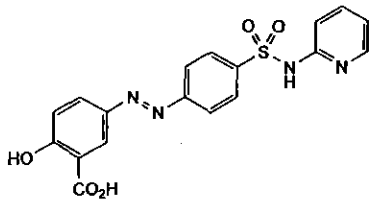


F. 4-amino-*N*-(3-methylisoxazol-5-yl)benzenesulfonamide.

Ph Eur

Sulfasalazine

(Ph. Eur. monograph 0863)



$C_{18}H_{14}N_4O_5S$ 398.4 *599-79-1*

Action and use
Sulfonamide aminosalicylate; treatment of ulcerative colitis.

Preparations
Sulfasalazine Gastro-resistant Tablets
Sulfasalazine Tablets

Ph Eur

DEFINITION
2-Hydroxy-5-[2-[4-(pyridin-2-ylsulfamoyl)phenyl] diazenyl]benzoic acid.

Content
97.0 per cent to 101.5 per cent (dried substance).

CHARACTERS
Appearance
Bright yellow or brownish-yellow, fine powder.

Solubility
Practically insoluble in water, very slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *sulfasalazine CRS*.

TESTS
Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in *dilute ammonia R3* and dilute to 25.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *dilute ammonia R3*.

Reference solution (b) Dissolve 1.0 mg of *sulfasalazine derivative for resolution CRS* in 10.0 mL of reference solution (a). Dilute 1.0 mL of this solution to 10.0 mL with reference solution (a).

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: in a 1000 mL volumetric flask dissolve 1.13 g of *sodium dihydrogen phosphate R* and 2.5 g of *sodium acetate R* in 900 mL of *water R*; adjust to pH 4.8 with *glacial acetic acid R* and dilute to 1000 mL with *water R*;
— *mobile phase B*: mobile phase A, *methanol R* (10:40 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	60 → 45	40 → 55
15 - 25	45	55
25 - 60	45 → 0	55 → 100
60 - 65	0	100

Flow rate 1 mL/min.

Detection Spectrophotometer at 320 nm.

Injection 20 µL.

Relative retention With reference to sulfasalazine: impurity H = about 0.16; impurity I = about 0.28; impurity C = about 0.80; impurity F = about 0.85; impurity G = about 1.39; impurity E = about 1.63; impurity B = about 1.85; impurity D = about 1.90; impurity A = about 2.00.

System suitability Reference solution (b):
— *resolution*: minimum 3.0 between the peaks due to sulfasalazine and sulfasalazine derivative for resolution.

Limits:
— *impurities A, B, C, D, E, F, G, I*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent);
— *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (4 per cent);
— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent); disregard any peak with a retention time less than 6 min (due to impurities H and J).

Impurities H and J
Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in *dilute ammonia R3* and dilute to 25.0 mL with the same solvent.

Reference solution (a) Dissolve 5.0 mg of *salicylic acid R* (impurity H) and 5.0 mg of *sulfapyridine CRS* (impurity J) in *dilute ammonia R3* and dilute to 10.0 mL with the same solvent.

Reference solution (b) Dilute 2.0 mL of reference solution (a) to 100.0 mL with *dilute ammonia R3*.

Column:
— *size*: *l* = 0.25, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mobile phase B (described in the test for related substances), mobile phase A (described in the test for related substances) (30:70 *V/V*).

Flow rate 1 mL/min.

Detection Spectrophotometer at 300 nm.

Injection 20 µL of the test solution and reference solution (b).

Run time 10 min.

Retention time Impurity H = about 6 min; impurity J = about 7 min.

System suitability Reference solution (b):
— *resolution*: minimum 2 between the peaks due to impurities H and J.

Limits:
— *impurities H, J*: for each impurity, not more than 0.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Chlorides (*2.4.4*)
Maximum 140 ppm.

To 1.25 g add 50 mL of *distilled water R*. Heat at about 70 °C for 5 min. Cool and filter. To 20 mL of the filtrate add 1 mL of *nitric acid R*, allow to stand for 5 min and filter to obtain a clear solution.

Sulfates (*2.4.13*)
Maximum 400 ppm.

To 20 mL of the filtrate prepared for the test for chlorides add 1 mL of *dilute hydrochloric acid R*, allow to stand for 5 min and filter.

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulfated ash (*2.4.14*)
Maximum 0.5 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in *0.1 M sodium hydroxide* and dilute to 100.0 mL with the same solvent. Transfer 5.0 mL of this solution to a 1000 mL volumetric flask containing about 750 mL of *water R*. Add 20.0 mL of a 6 g/L solution of *glacial acetic acid R* and dilute to 1000.0 mL with *water R*. Prepare a standard solution at the same time and in the same manner using 0.150 g of *sulfasalazine CRS*. Measure the absorbance (*2.2.25*) of the 2 solutions at the absorption maximum at 359 nm.

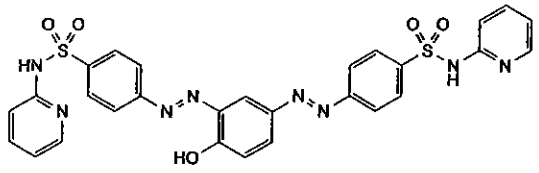
Calculate the content of $C_{18}H_{14}N_4O_5S$ from the absorbances measured and the concentration of the solutions.

STORAGE

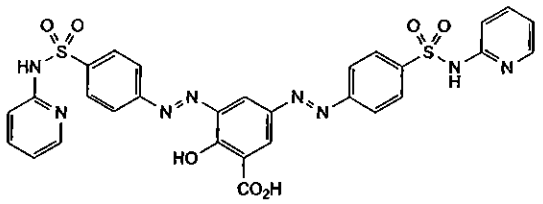
Protected from light.

IMPURITIES

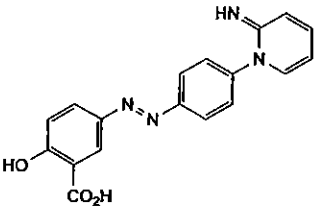
Specified impurities *A, B, C, D, E, F, G, H, I, J.*



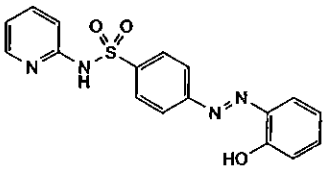
A. 4,4′-[(4-hydroxy-1,3-phenylene)bis(diazenediyl)]bis[*N*-(pyridin-2-yl)benzenesulfonamide],



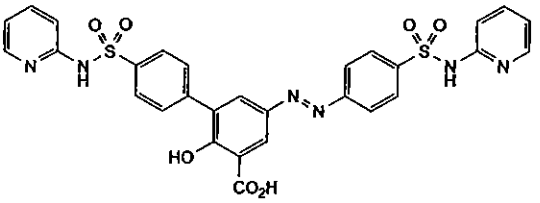
B. 2-hydroxy-3,5-bis[2-[4-(pyridin-2-ylsulfamoyl)phenyl]diazenyl]benzoic acid,



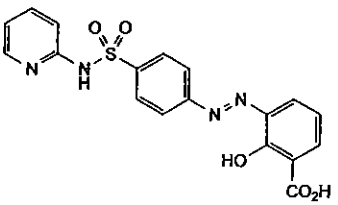
C. 2-hydroxy-5-[2-[4-(2-iminopyridin-1(2*H*)-yl)phenyl]diazenyl]benzoic acid,



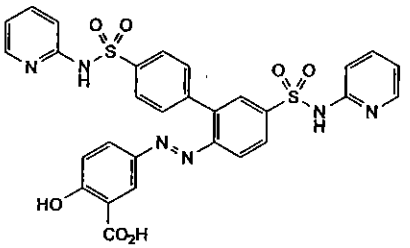
D. 4-[2-(2-hydroxyphenyl)diazenyl]-*N*-(pyridin-2-yl)benzenesulfonamide,



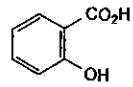
E. 2-hydroxy-4′-(pyridin-2-ylsulfamoyl)-5-[2-[4-(pyridin-2-ylsulfamoyl)phenyl]diazenyl]biphenyl-3-carboxylic acid,



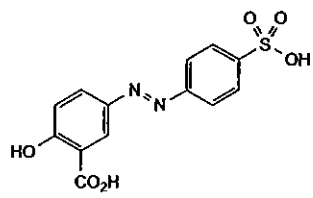
F. 2-hydroxy-3-[2-[4-(pyridin-2-ylsulfamoyl)phenyl]diazenyl] benzoic acid,



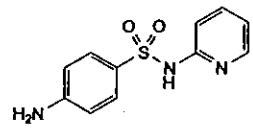
G. 5-[2-[4′,5-bis(pyridin-2-ylsulfamoyl)biphenyl-2-yl]diazenyl]-2-hydroxybenzoic acid,



H. 2-hydroxybenzenecarboxylic acid (salicylic acid),



I. 2-hydroxy-5-[2-(4-sulfophenyl)diazenyl]benzoic acid,

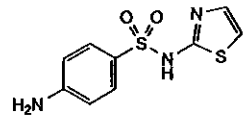


J. 4-amino-*N*-(pyridin-2-yl)benzenesulfonamide (sulfapyridine).

Ph Eur

Sulfathiazole

(Ph. Eur. monograph 0742)



$C_9H_9N_3O_2S_2$ 255.3 *72-14-0*

Action and use
Sulfonamide antibacterial.

Ph Eur

DEFINITION
Sulfathiazole contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 4-amino-*N*-(thiazol-2-yl)benzenesulfonamide, calculated with reference to the dried substance.

CHARACTERS
A white or slightly yellowish, crystalline powder, practically insoluble in water, slightly soluble in alcohol, practically insoluble in methylene chloride. It dissolves in dilute solutions of alkali hydroxides and in dilute mineral acids.

IDENTIFICATION
First identification: A, B.

Second identification: A, C, D, E.

A. Melting point (*2.2.14*): 200 °C to 203 °C. Melting may occur at about 175 °C, followed by solidification and a second melting between 200 °C and 203 °C.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *sulfathiazole CRS*. Examine the substances prepared as discs. If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *alcohol R*, evaporate to dryness *in vacuo* and record the spectra again using the residues.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 10 mg in a mixture of 10 mL of *water R* and 2 mL of *0.1 M sodium hydroxide* and add 0.5 mL of *copper sulfate solution R*. A greyish-blue or purple precipitate is formed.

E. Dissolve about 5 mg in 10 mL of *1 M hydrochloric acid*. Dilute 1 mL of the solution to 10 mL with *water R*. The solution, without further addition of acid, gives the reaction of primary aromatic amines (*2.3.1*).

TESTS
Appearance of solution
Dissolve 1.0 g in 10 mL of *1 M sodium hydroxide*.
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution GY_4 (*2.2.2, Method II*).

Acidity
To 1.0 g add 50 mL of *carbon dioxide-free water R*. Heat to 70 °C for 5 min. Cool rapidly to 20 °C and filter. To 25 mL of the filtrate add 0.1 mL of *bromothymol blue solution R1*. Not more than 0.1 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Related substances
Examine by thin-layer chromatography (*2.2.27*), using *silica gel H R* as the coating substance.

Test solution (a) Dissolve 0.10 g of the substance to be examined in a mixture of 1 volume of *concentrated ammonia R* and 9 volumes of *alcohol R* and dilute to 10 mL with the same mixture of solvents.

Test solution (b) Dilute 1 mL of test solution (a) to 5 mL with a mixture of 1 volume of *concentrated ammonia R* and 9 volumes of *alcohol R*.

Reference solution (a) Dissolve 20 mg of *sulfathiazole CRS* in a mixture of 1 volume of *concentrated ammonia R* and 9 volumes of *alcohol R* and dilute to 10 mL with the same mixture of solvents.

Reference solution (b) Dissolve 50 mg of *sulfanilamide R* in a mixture of 1 volume of *concentrated ammonia R* and 9 volumes of *alcohol R* and dilute to 100 mL with the same mixture of solvents. Dilute 1 mL of this solution to 10 mL with the same mixture of solvents.

Apply to the plate 10 µL of each solution. Develop over a path of 15 cm using a mixture of 18 volumes of *ammonia R* and 90 volumes of *butanol R*. Dry the plate at 100 °C to 105 °C for 10 min and spray with a 1 g/L solution of *dimethylaminobenzaldehyde R* in *alcohol R* containing 1 per cent *V/V* of *hydrochloric acid R*. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Loss on drying (*2.2.32*)
Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Not more than 0.1 per cent, determined on 1.0 g.

ASSAY
Carry out the determination of primary aromatic amino-nitrogen (*2.5.8*), using 0.200 g, determining the end-point electrometrically.

1 mL of *0.1 M sodium nitrite* is equivalent to 25.53 mg of $C_9H_9N_3O_2S_2$.

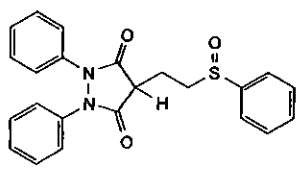
STORAGE

Store protected from light.

Ph Eur

Sulfinpyrazone

(Ph. Eur. monograph 0790)



$C_{23}H_{20}N_2O_3S$ 404.5 *57-96-5*

Action and use

Uricosuric.

Ph Eur

DEFINITION

1,2-Diphenyl-4-[2-(phenylsulfinyl)ethyl]pyrazolidine-3,5-dione.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Very slightly soluble in water, sparingly soluble in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification: A, C.

Second identification: A, B, D.

A. Melting point (*2.2.16*): 131 °C to 135 °C.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 30.0 mg in *0.01 M sodium hydroxide* and dilute to 100.0 mL with the same alkaline solution. Dilute 1.0 mL of this solution to 20.0 mL with *0.01 M sodium hydroxide.*

Spectral range 230-350 nm.

Absorption maximum At 260 nm.

Specific absorbance at the absorption maximum 530 to 580.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *sulfinpyrazone CRS.*

D. Dissolve about 10 mg in 3 mL of *acetone R* and add a mixture of 0.2 mL of *ferric chloride solution R2* and 3 mL of *water R.* A red to violet colour develops.

TESTS

Appearance of solution in acetone

The solution is clear (*2.2.1*) and its absorbance (*2.2.25*) at 420 nm using a path length of 4 cm is not greater than 0.10.

Dissolve 1.25 g in *acetone R* and dilute to 25 mL with the same solvent.

Appearance of solution in 1 M sodium hydroxide

The solution is clear (*2.2.1*) and its absorbance (*2.2.25*) at 420 nm using a path length of 4 cm is not greater than 0.15.

Dissolve 1.25 g, heating gently if necessary, in 25 mL of *1 M sodium hydroxide.*

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Solvent mixture *water R, acetonitrile R* (10:40 *V/V*).

Test solution Dissolve 10.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture.

Reference solution (b) Dissolve 5.0 mg of *sulfinpyrazone impurity A CRS* and 5.0 mg of *sulfinpyrazone impurity B CRS* in the solvent mixture and dilute to 5.0 mL with the solvent mixture.

Reference solution (c) Dilute 1.0 mL of reference solution (b) to 100.0 mL with the solvent mixture.

Reference solution (d) Dissolve the contents of a vial of *sulfinpyrazone for system suitability CRS* (containing impurity C) in 1.0 mL of the solvent mixture.

Reference solution (e) Dilute 1.0 mL of reference solution (a) to 10.0 mL with the solvent mixture.

Column:

— *size: l* = 0.125 m, Ø = 4.6 mm;

— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *tetrahydrofuran for chromatography R, acetonitrile R,* 0.3 per cent *V/V* solution of *phosphoric acid R* (7:35:58 *V/V/V*).

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 235 nm.

Injection 20 µl.

Run time 7 times the retention time of sulfinpyrazone.

Identification of impurities Use the chromatogram supplied with *sulfinpyrazone for system suitability CRS* and the chromatogram obtained with reference solution (d) to identify the peak due to impurity C; use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and B.

Relative retention With reference to sulfinpyrazone (retention time = about 3.5 min): impurity C = about 0.8; impurity A = about 1.6; impurity B = about 4.8.

System suitability Reference solution (d):

— *resolution*: minimum 2.0 between the peaks due to impurity C and sulfinpyrazone.

Limits:

— *impurities A, B*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (1.0 per cent);

— *impurity C*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (e) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (e) (0.10 per cent);

— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (e) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

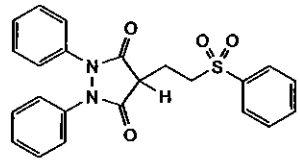
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.300 g in 25 mL of *acetone R*. Add 0.5 mL of *bromothymol blue solution R1*. Titrate with *0.1 M sodium hydroxide* until the colour changes from yellow to blue.

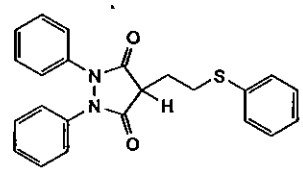
1 mL of *0.1 M sodium hydroxide* is equivalent to 40.45 mg of $C_{23}H_{20}N_2O_3S$.

STORAGE
Protected from light.

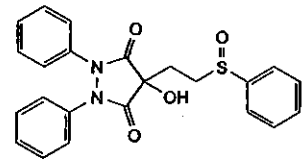
IMPURITIES
Specified impurities *A, B, C.*



A. 1,2-diphenyl-4-[2-(phenylsulfonyl)ethyl]pyrazolidine-3,5-dione,



B. 1,2-diphenyl-4-[2-(phenylsulfanyl)ethyl]pyrazolidine-3,5-dione,

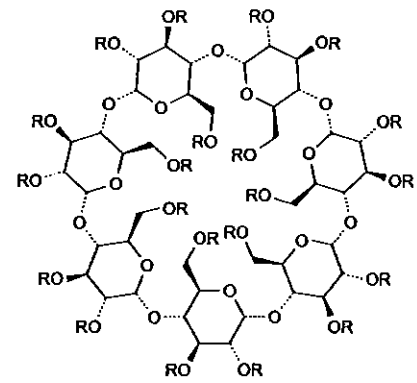


C. 4-hydroxy-1,2-diphenyl-4-[2-(phenylsulfinyl) ethyl]pyrazolidine-3,5-dione.

Ph Eur

Sulfobutylbetadex Sodium

(Ph. Eur. monograph 2804)



R = H or $[CH_2]_4$-SO_3Na

$C_{42}H_{70-n}O_{35}(C_4H_8SO_3Na)_n$ 2163 when n = 6.5 *182410-00-0*

Ph Eur

DEFINITION
Sulfobutylbetadex sodium (β-cyclodextrin, sodium 4-sulfonatobutyl ether) is the sodium salt of a partially substituted poly(sulfobutyl) ether of betadex.

Content
— *sulfobutylbetadex sodium*: 95.0 per cent to 105.0 per cent (anhydrous substance);
— *average number of sulfobutyl groups per cyclodextrin ring, expressed as average degree of substitution (DS)*: 5.9 to 6.6.

CHARACTERS
Appearance
White or almost white, hygroscopic powder.

Solubility
Freely soluble in water, practically insoluble in anhydrous ethanol and in methylene chloride.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *sulfobutylbetadex sodium CRS.*

Results The spectrum obtained with the substance to be examined shows the same absorption bands as the spectrum obtained with *sulfobutylbetadex sodium CRS*. Due to differences in the degree of substitution of the substance, the relative intensity of some absorption bands may vary.

B. It gives reaction (a) of sodium (*2.3.1*).

TESTS
Solution S
Dissolve 7.50 g in *carbon dioxide-free water R* and dilute to 50.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)
5.0 to 7.5 for solution S.

Use intensive nitrogen purging to avoid carbon dioxide absorption during measurement.

Reducing sugars
Maximum 0.05 per cent.

Reagent solution Dissolve 1.0 g of *triphenyltetrazolium chloride R* in 100 mL of *aldehyde-free methanol R*.

Test solution Dissolve 0.45 g of the substance to be examined in 2.0 mL of *dimethyl sulfoxide R* in a test tube and add 0.5 mL of a 40 g/L solution of *sodium hydroxide R* and 7.5 mL of the reagent solution. Mix and store at room temperature.

Reference solution Prepare at the same time and in the same manner as for the test solution, using 0.10 mL of a 2.25 g/L solution of *glucose R* instead of the substance to be examined.

After 1 h, measure the absorbance (*2.2.25*) of the solutions at the absorption maximum at 482 nm. The absorbance of the test solution is not greater than that of the reference solution.

Impurities A, C and D

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.300 g of the substance to be examined in *water R* and dilute to 5.0 mL with the same solvent.

Reference solution (a) Dissolve 15.0 mg of *betadex CRS* (impurity A) in *water R* and dilute to 5.0 mL with the same solvent. Dilute 1.0 mL of the solution to 50.0 mL with *water R*.

Reference solution (b) Dissolve 15.0 mg of *sulfobutylbetadex impurity C CRS* in *water R* and dilute to 5.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with *water R*.

Reference solution (c) Dissolve 15.0 mg of *sulfobutylbetadex impurity D CRS* in *water R* and dilute to 5.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with *water R*.

Reference solution (d) Dissolve 0.300 g of *sulfobutylbetadex sodium CRS* in 1.0 mL of reference solution (b) and 0.5 mL of reference solution (c), and dilute to 5.0 mL with *water R*.

Column:
- *size*: *l* = 0.15 m, Ø = 4.0 mm;
- *stationary phase*: *4-dimethylaminobenzylcarbamidesilyl silica gel for chromatography R* (3 µm);
- *temperature*: 25 °C.

Mobile phase:
- *mobile phase A*: to 4.0 mL of *triethylamine R* add 900 mL of *water for chromatography R* and adjust to pH 4.5 with *anhydrous formic acid R*; dilute to 1000 mL with *water for chromatography R*;
- *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	90	10
2 - 8	90 → 60	10 → 40
8 - 14	60 → 40	40 → 60
14 - 20	40	60

Flow rate 1.1 mL/min.

Detection Evaporative light-scattering detector; the following settings have been found to be suitable; if the detector has different setting parameters, adjust the detector settings so as to comply with the system suitability criteria.
- *carrier gas*: *nitrogen R*;
- *flow rate*: 1.5 L/min;
- *evaporator temperature*: 50 °C.

Equilibration At initial isocratic conditions for 10 min.

Injection 10 µL of the test solution and reference solutions (a) and (d).

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity A; use the chromatogram obtained with reference solution (d) to identify the peaks due to impurities C and D.

Relative retention With reference to impurity A (retention time = about 2.9 min): impurity C = about 0.7; impurity D = about 2.0; sulfobutylbetadex elutes as several peaks after impurity D unless heart-cutting is applied.

System suitability:
- *resolution*: minimum 2.0 between the peaks due to impurities C and A in the chromatogram obtained with reference solution (d);
- *repeatability*: maximum relative standard deviation of 3.0 per cent for the area of the peak due to impurity A determined on 5 injections of reference solution (a), using the initial isocratic conditions for 4 min.

Limits:
- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.1 per cent);
- *impurity D*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.05 per cent);
- disregard the peak due to the sodium counter-ion (corresponding to the dead volume of the column) and any peak eluting after impurity D (due to sulfobutylbetadex).

Impurity B

Gas chromatography (*2.2.28*). *Prepare the solutions immediately before use.*

Internal standard solution Dissolve 25.0 mg of *diethyl sulfone R* in 10.0 mL of *dimethylformamide R* and dilute to 1000.0 mL with *water R*. Dilute 1.0 mL of the solution to 100.0 mL with *water R*.

Test solutions (a), (b), (c) and (d) In 4 test tubes with screw caps, prepare 4 solutions as indicated in Table 2804.-1. Mix each test tube on a vortex mixer for 30 s and allow to stand for at least 5 min or until complete separation of the phases. Decant the organic phase into a vial and seal.

Reference solution (a) Dissolve 50.0 mg of *sulfobutylbetadex impurity B CRS* in 5.0 mL of *dimethylformamide R* and dilute with *water R* to obtain a concentration of 2.0 µg/mL.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 2.0 mL with *water R*.

Reference solution (c) Dilute 1.0 mL of reference solution (a) to 4.0 mL with *water R*.

Blank solution Prepare as described for test solution (d), but omitting the substance to be examined.

Column:
- *material*: fused silica;
- *size*: *l* = 30 m, Ø = 0.32 mm;
- *stationary phase*: *cyanopropyl(7)phenyl(7)methyl(86) polysiloxane R* (film thickness 0.5 µm).

Carrier gas *helium for chromatography R*.

Flow rate 2.4 mL/min.

Table 2804.-1.

	Substance to be examined (g)	Internal standard solution (mL)	Solvent/solution (mL)	*Methylene chloride R* (mL)	Final content of *sulfobutylbetadex impurity B CRS* (µg)
Blank solution	0	4.0	*Water R*, 1.0	1.0	0
Test solution (a)	1.000	4.0	Reference solution (a), 1.0	1.0	2.0
Test solution (b)	1.000	4.0	Reference solution (b), 1.0	1.0	1.0
Test solution (c)	1.000	4.0	Reference solution (c), 1.0	1.0	0.5
Test solution (d)	1.000	4.0	*Water R*, 1.0	1.0	0

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 10	100 → 200
	10 - 11.5	200 → 250
	11.5 - 16.5	250
Injection port		250
Detector		270

Detection Flame ionisation.

Injection 1 µL.

Relative retention With reference to the internal standard (retention time = about 6.5 min): impurity B = about 1.4.

System suitability:

— *repeatability*: maximum relative standard deviation of 10.0 per cent for the corrected ratios (as described under Calculation of content) determined on 5 injections of test solution (b).

Calculation of content Calculate the ratio of the area of the peak due to impurity B to the area of the peak due to the internal standard in the chromatograms obtained with test solutions (a), (b), (c) and (d) respectively. Subtract from these values the ratio of the area of the peak due to impurity B to the area of the peak due to the internal standard in the chromatogram obtained with the blank solution (diethyl sulfone may contain trace amounts of impurity B). Plot the added quantity of impurity B (in micrograms) as the abscissa and the corrected ratios as the ordinates. Extrapolate the line joining the points on the graph until it meets the *x*-axis. The distance between this point and the origin corresponds to the content of impurity B in micrograms per gram (ppm) of the substance to be examined.

Limit:

— *impurity B*: maximum 0.5 ppm.

Chlorides (*2.4.4*)

Maximum 0.12 per cent (corresponding to 0.20 per cent expressed as sodium chloride).

Dissolve a quantity of the substance to be examined corresponding to 42 mg of anhydrous substance in 15.0 mL of *water R*.

Water (*2.5.12*)

Maximum 10.0 per cent, determined on 0.200 g.

Microbial contamination

If intended for use in the manufacture of parenteral preparations:

— TAMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

If not intended for use in the manufacture of parenteral preparations:

— TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*);

— TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*);

— absence of *Escherichia coli* (*2.6.13*);

— absence of *Salmonella* (*2.6.13*).

ASSAY

Sulfobutylbetadex sodium

Size-exclusion chromatography (*2.2.30*).

Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution Dissolve 0.100 g of *sulfobutylbetadex sodium CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase.

Column:

— *size*: *l* = 0.30 m, Ø = 7.8 mm;

— *stationary phase*: *polymethacrylate gel R*.

Mobile phase Dissolve 10.11 g of *potassium nitrate R* in a mixture of 1 volume of *acetonitrile for chromatography R* and 4 volumes of *water for chromatography R* and dilute to 1000 mL with the same mixture of solvents.

Flow rate 1.0 mL/min.

Detection Differential refractometer maintained at 35 ± 2 °C.

Injection 20 µL.

Between-run rinsing Rinse the column with a mixture of 1 volume of *acetonitrile for chromatography R* and 9 volumes of *water for chromatography R*.

System suitability Reference solution:

— *repeatability*: maximum relative standard deviation of 2.0 per cent for the area of the peak due to sulfobutylbetadex, determined on 5 injections;

— *symmetry factor*: maximum 1.8.

Calculate the percentage content of sulfobutylbetadex sodium taking into account the assigned content of *sulfobutylbetadex sodium CRS*.

Average degree of substitution

Nuclear magnetic resonance spectrometry (*2.2.33*).

The average degree of substitution (*DS*) is calculated from the ratio between the signals of the inner-chain protons of the sulfobutyl ether groups (-O-CH_2-CH_2-CH_2-CH_2-SO_3H) and the signals of the glycosidic protons attached to the C1 carbon of the anhydroglucose units.

Test solution Introduce not less than 10.0 mg of the substance to be examined, previously dried for at least 1 day in a desiccator, into a 5 mm NMR tube. Add about 0.70 mL of *deuterium oxide R1*. Cap the tube and mix thoroughly.

Apparatus FT-NMR spectrometer operating at minimum 250 MHz and suitable for recording at 25 °C.

Acquisition of ^1H NMR spectra The following parameters may be used:

— *sweep width*: 10 ppm (0 to + 10.0 ppm);
— *irradiation frequency offset*: none;
— *time domain*: 32 K;
— *pulse width*: 90°;
— *pulse delay*: 15 s;
— *dummy scans*: 2;
— *number of scans*: 16.

Call the integration sub-routine after phase corrections and baseline correction between − 1 ppm and + 9 ppm.

The signal of exchangeable protons (solvent) at + 4.8 ppm is used as reference.

Make a zero filling at least twice in size relative to the acquisition data file and transform the FID to the spectrum without any correction of the Gaussian broadening factor (GB = 0) and with a line broadening factor not greater than 0.3 Hz (LB ≤ 0.3). Adjust integral level and tilting.

Measure the peak areas of the signals between 1.43 ppm and 2.12 ppm (A_1, -O-CH_2-**CH_2**-CH_2-CH_2-SO_3H), and the signals of the glycosidic protons between 4.95 ppm and 5.41 ppm (A_2, -O-**CH**-O-).

Calculate the average degree of substitution (*DS*) using the following expression:

$$\frac{7 \times A_1}{4 \times A_2}$$

A_1 = area of the peaks due to the inner methylene protons (indicated in bold) of the sulfobutyl ether functional group;
A_2 = area of the peaks due to the glycosidic protons (indicated in bold).

STORAGE

In an airtight container.

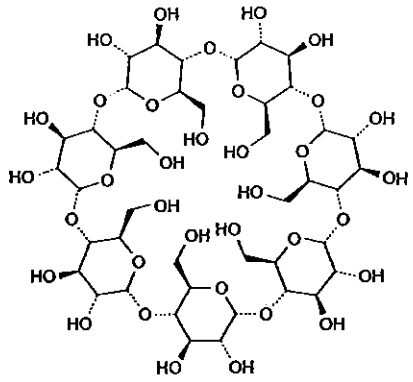
LABELLING

The label states:

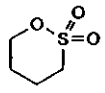
— the average degree of substitution (*DS*);
— where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

IMPURITIES

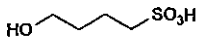
Specified impurities *A, B, C, D.*



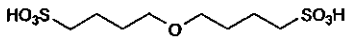
A. cycloheptakis-(1→4)-(α-D-glucopyranosyl) (betadex or cyclomaltoheptaose or β-cyclodextrin),



B. 1,2λ^6-oxathiane-2,2-dione,



C. 4-hydroxybutane-1-sulfonic acid,

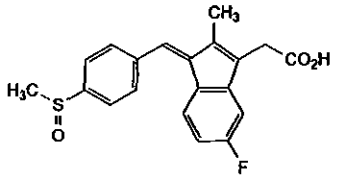


D. 4,4′-oxydi(butane-1-sulfonic acid).

Ph Eur

Sulindac

(Ph. Eur. monograph 0864)



and enantiomer

$C_{20}H_{17}FO_3S$ 356.4 *38194-50-2*

Action and use
Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Preparation
Sulindac Tablets

Ph Eur

DEFINITION

[(1*Z*)-6-Fluoro-3-[[4-[(*RS*)-methanesulfinyl]phenyl] methylidene]-2-methyl-3*H*-inden-1-yl]acetic acid.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
Yellow, crystalline powder.

Solubility
Very slightly soluble in water, soluble in methylene chloride, sparingly soluble in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *sulindac CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of hot *methanol R*, evaporate to dryness and record new spectra using the residues.

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.10 g of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 4 mg of *sulindac for system suitability CRS* (containing impurities B, C and D) in the mobile phase and dilute to 2 mL with the mobile phase.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *silica gel for chromatography R* (10 µm).

Mobile phase *glacial acetic acid R, ethanol (96 per cent) R, ethyl acetate R, methylene chloride R* (1:4:100:400 *V/V/V/V*).

Flow rate 2 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µL.

Run time 2.5 times the retention time of sulindac.

Identification of impurities Use the chromatogram supplied with *sulindac for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B, C and D.

Relative retention With reference to sulindac (retention time = about 10 min): impurity C = about 0.20; impurity B = about 0.25; impurity D = about 0.7.

System suitability Reference solution (b):
- *resolution*: minimum 2.0 between the peaks due to impurities C and B.

Calculation of percentage contents:
- *correction factor*: multiply the peak area of impurity C by 2;
- for each impurity, use the concentration of sulindac in reference solution (a).

Limits:
- *impurity C*: maximum 0.5 per cent;
- *impurity B*: maximum 0.4 per cent;
- *impurity D*: maximum 0.2 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 1.0 per cent;
- *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C at a pressure not exceeding 0.7 kPa.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 50 mL of *methanol R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M sodium hydroxide* is equivalent to 35.64 mg of $C_{20}H_{17}FO_3S$.

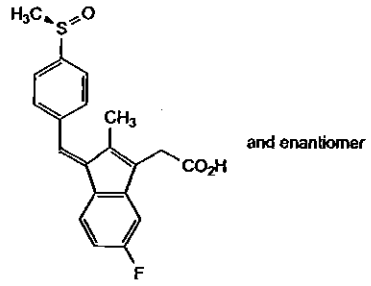
STORAGE

Protected from light.

IMPURITIES

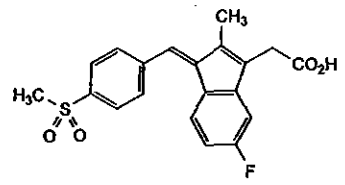
Specified impurities *B, C, D.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A.*

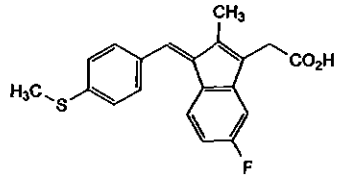


and enantiomer

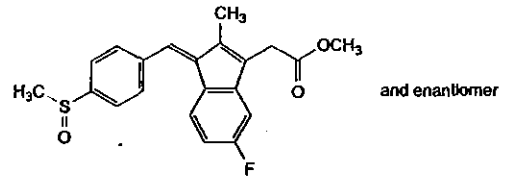
A. [(1*E*)-6-fluoro-3-[[4-[(*RS*)-methanesulfinyl]phenyl] methylidene]-2-methyl-3*H*-inden-1-yl]acetic acid,



B. [(1*Z*)-6-fluoro-3-[[4-(methanesulfonyl)phenyl] methylidene]-2-methyl-3*H*-inden-1-yl]acetic acid,



C. [(1*Z*)-6-fluoro-2-methyl-3-[[4-(methylsulfanyl)phenyl] methylidene]-3*H*-inden-1-yl]acetic acid,



and enantiomer

D. methyl [(1*Z*)-6-fluoro-3-[[4-[(*RS*)-methanesulfinyl]phenyl] methylidene]-2-methyl-3*H*-inden-1-yl]acetate.

Ph Eur

Sulfur

Sulfur for External Use

Sulphur for External Use

(Ph. Eur. monograph 0953)

S	32.06	*7704-34-9*

Action and use
Keratolytic.

Ph Eur

DEFINITION

Content
99.0 per cent to 101.0 per cent.

CHARACTERS

Appearance
Yellow powder.

Solubility
Practically insoluble in water, soluble in carbon disulfide, slightly soluble in vegetable oils.

mp
About 120 °C.

The size of most of the particles is not greater than 20 µm and that of almost all the particles is not greater than 40 µm.

IDENTIFICATION

A. Heated in the presence of air, it burns with a blue flame, emitting sulfur dioxide which changes the colour of moistened *blue litmus paper R* to red.

B. Heat 0.1 g with 0.5 mL of *bromine water R* until decolourised. Add 5 mL of *water R* and filter. The solution gives reaction (a) of sulfates (*2.3.1*).

TESTS

Solution S
To 5 g add 50 mL of *carbon dioxide-free water R* prepared from *distilled water R*. Allow to stand for 30 min with frequent shaking and filter.

Appearance of solution
Solution S is colourless (*2.2.2, Method II*).

Odour (*2.3.4*)
It has no perceptible odour of hydrogen sulfide.

Acidity or alkalinity
To 5 mL of solution S add 0.1 mL of *phenolphthalein solution R1*. The solution is colourless. Add 0.2 mL of *0.01 M sodium hydroxide*. The solution is red. Add 0.3 mL of *0.01 M hydrochloric acid*. The solution is colourless. Add 0.15 mL of *methyl red solution R*. The solution is orange-red.

Chlorides (*2.4.4*)
Maximum 100 ppm.

Dilute 5 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 100 ppm, determined on solution S.

Sulfides
To 10 mL of solution S add 2 mL of *buffer solution pH 3.5 R* and 1 mL of a freshly prepared 1.6 g/L solution of *lead nitrate R* in *carbon dioxide-free water R*. Shake. After 1 min any colour in the solution is not more intense than that in a reference solution prepared at the same time using 1 mL of *lead standard solution (10 ppm Pb) R*, 9 mL of *carbon dioxide-free water R*, 2 mL of *buffer solution pH 3.5 R* and 1.2 mL of *thioacetamide reagent R*.

Sulfated ash (*2.4.14*)
Maximum 0.2 per cent, determined on 1.0 g.

ASSAY
Carry out the oxygen-flask method (*2.5.10*), using 60.0 mg in a 1000 mL combustion flask. Absorb the combustion products in a mixture of 5 mL of *dilute hydrogen peroxide solution R* and 10 mL of *water R*. Heat to boiling, boil gently for 2 min and cool. Using 0.2 mL of *phenolphthalein solution R* as indicator, titrate with *0.1 M sodium hydroxide* until the colour changes from colourless to red. Carry out a blank titration under the same conditions.

1 mL of *0.1 M sodium hydroxide* is equivalent to 1.603 mg of S.

STORAGE
Protected from light.

Ph Eur

Sulfuric Acid

Sulphuric Acid

(Ph. Eur. monograph 1572)

H_2SO_4	98.1	*7664-93-9*

Preparation
Dilute Sulfuric Acid

Ph Eur

DEFINITION

Content
95.0 per cent *m/m* to 100.5 per cent *m/m*.

CHARACTERS

Appearance
Colourless, oily liquid, very hygroscopic.

Solubility
Miscible with water and with ethanol (96 per cent) producing intense heat.

Relative density
About 1.84.

IDENTIFICATION

A. Carefully add 1 mL to 100 mL of *water R*. The solution is strongly acid (*2.2.4*).

B. The solution obtained in identification test A gives reaction (a) of sulfates (*2.3.1*).

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Carefully pour, while cooling, 5 mL into 30 mL of *water R* and dilute to 50 mL with the same solvent.

Chlorides (*2.4.4*)
Maximum 50 ppm.

Mix carefully, while cooling, 3.3 g with 30 mL of *water R*. Neutralise with *ammonia R* and dilute to 50 mL with *water R*.

Nitrates
Add 5 mL to 5 mL of *water R*. Cool to room temperature and add 0.5 mL of *indigo carmine solution R*. The blue colour persists for at least 1 min.

Iron (*2.4.9*)
Maximum 25 ppm.

Cautiously evaporate 10.0 g and ignite to dull redness. Dissolve the ignition residue in 1 mL of *dilute hydrochloric acid R* with gentle heating and dilute to 25 mL with *water R*. Dilute 1 mL of this solution to 10 mL with *water R*.

ASSAY

Weigh accurately a ground-glass-stoppered flask containing 30 mL of *water R*. Introduce 0.2 mL, cool and weigh again. Titrate with *1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *1 M sodium hydroxide* is equivalent to 49.04 mg of H_2SO_4.

STORAGE

In an airtight container.

Ph Eur

Dilute Sulfuric Acid

Dilute Sulphuric Acid

DEFINITION

Dilute Sulfuric Acid is prepared by adding 104 g of Sulfuric Acid to 896 g of Purified Water with constant stirring and cooling. It contains not less than 9.5% and not more than 10.5% w/w of H_2SO_4.

Weight per mL

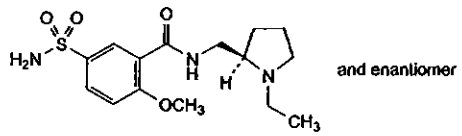
1.062 g to 1.072 g, Appendix V G.

ASSAY

To 10 g add 40 mL of *water* and titrate with *1M sodium hydroxide VS* using *methyl orange solution* as indicator. Each mL of *1M sodium hydroxide VS* is equivalent to 49.04 mg of H_2SO_4.

Sulpiride

(Ph. Eur. monograph 1045)



and enantiomer

$C_{15}H_{23}N_3O_4S$ 341.4 *15676-16-1*

Action and use

Dopamine receptor antagonist; neuroleptic.

Preparation

Sulpiride Tablets

Ph Eur

DEFINITION

N-{[(2*RS*)-1-Ethylpyrrolidin-2-yl)]methyl}-2-methoxy-5-sulfamoylbenzamide.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, sparingly soluble in methanol, slightly soluble in ethanol (96 per cent) and in methylene chloride. It dissolves in dilute solutions of mineral acids and alkali hydroxides.

IDENTIFICATION

First identification: B.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 177 °C to 181 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *sulpiride CRS.*

C. Examine the chromatograms obtained in the test for impurity A.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To about 1 mg in a porcelain dish, add 0.5 mL of *sulfuric acid R* and 0.05 mL of *formaldehyde solution R*. Examined in ultraviolet light at 365 nm, the solution shows blue fluorescence.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method I*).

Dissolve 1.0 g in *dilute acetic acid R* and dilute to 10 mL with the same acid.

Impurity A. Thin-layer chromatography (*2.2.27*)

Test solution (a) Dissolve 0.20 g of the substance to be examined in *methanol R*, sonicate until dissolution is complete and dilute to 10.0 mL with the same solvent.

Test solution (b) Dilute 1.0 mL of test solution (a) to 10.0 mL with *methanol R*.

Reference solution (a) Dissolve 20 mg of *sulpiride CRS* in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (b) Dissolve 5.0 mg of *sulpiride impurity A CRS* in *methanol R* and dilute to 25.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with *methanol R*.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *concentrated ammonia R, dioxan R, methanol R, methylene chloride R* (2:10:14:90 *V/V/V/V*).

Application 10 µL.

Development Over 1/2 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm for identification test C and then spray with *ninhydrin solution R*; heat at 100-105 °C for 15 min and examine in daylight.

Limit Test solution (a):

— *impurity A*: any spot due to impurity A is not more intense than the corresponding spot in the chromatogram obtained with reference solution (b) (0.1 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 5 mg of the substance to be examined and 5 mg of *sulpiride impurity B CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 10 volumes of *acetonitrile R*, 10 volumes of *methanol R* and 80 volumes of a solution containing 6.8 g/L of *potassium dihydrogen phosphate R* and 1 g/L of *sodium octanesulfonate R*, previously adjusted to pH 3.3 with *phosphoric acid R*.

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 240 nm.

Injection 10 µL.

Run time Twice the retention time of sulpiride.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity B.

Relative retention With reference to sulpiride (retention time = about 15 min): impurity B = about 0.7.

System suitability Reference solution (b):
— *resolution*: minimum 2.5 between the peaks due to impurity B and sulpiride.

Calculation of percentage contents:
— for each impurity, use the concentration of sulpiride in reference solution (a).

Limits:
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.3 per cent;
— *reporting threshold*: 0.05 per cent.

Chlorides (*2.4.4*)
Maximum 100 ppm.

Shake 1.0 g with 20 mL of *water R*. Filter through a sintered-glass filter (40) (*2.1.2*). To 10 mL of the filtrate add 5 mL of *water R*.

Iron (*2.4.9*)
Maximum 10 ppm.

Ignite 1.0 g in a silica crucible. To the residue add 1 mL of *1 M hydrochloric acid*, 3 mL of *water R* and 0.1 mL of *nitric acid R*. Heat on a water-bath for about 5 min. Place the solution in a test-tube. Rinse the crucible with 4 mL of *water R*. Collect the rinsings in the test-tube and dilute to 10 mL with *water R*.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

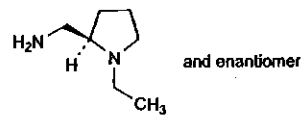
Dissolve 0.250 g in 80 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 34.14 mg of $C_{15}H_{23}N_3O_4S$.

IMPURITIES

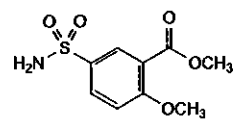
Specified impurities *A*.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D, E, F, G.*

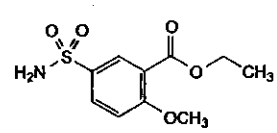


and enantiomer

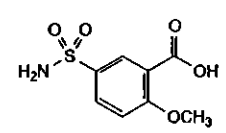
A. [(2*RS*)-1-ethylpyrrolidin-2-yl]methanamine,



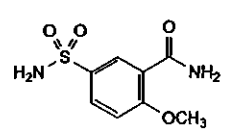
B. methyl 2-methoxy-5-sulfamoylbenzoate,



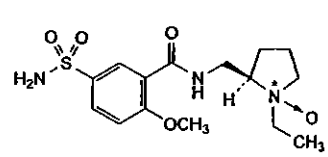
C. ethyl 2-methoxy-5-sulfamoylbenzoate,



D. 2-methoxy-5-sulfamoylbenzoic acid,

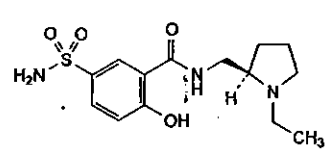


E. 2-methoxy-5-sulfamoylbenzamide,



and enantiomer

F. *N*-[[(2*RS*)-1-ethyl-1-oxidopyrrolidin-2-yl]methyl]-2-methoxy-5-sulfamoylbenzamide,



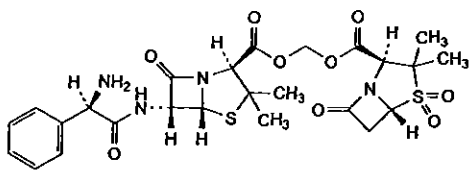
and enantiomer

G. *N*-[[(2*RS*)-1-éthylpyrrolidin-2-yl]méthyl]-2-hydroxy-5-sulfamoylbenzamide.

Ph Eur

Sultamicillin

(Ph. Eur. monograph 2211)



$C_{25}H_{30}N_4O_9S_2$ 594.7 *76497-13-7*

Action and use
Beta-lactamase inhibitor.

Ph Eur

DEFINITION

Methylene (2*S*,5*R*,6*R*)-6-[[(2*R*)-aminophenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (2*S*,5*R*)-3,3-dimethyl-4,4,7-trioxo-4λ⁶-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate.

Semi-synthetic product derived from a fermentation product.

Content
96.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, slightly hygroscopic, crystalline powder.

Solubility
Practically insoluble in water, very slightly soluble in methanol, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *sultamicillin CRS.*

TESTS

Specific optical rotation (*2.2.7*)
+ 190 to + 210 (anhydrous substance).

Dissolve 0.500 g in *dimethylformamide R* and dilute to 50.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use or keep at 2-8 °C for not more than 6 h.*

Solution A *methanol R1, acetonitrile R1* (20:80 *V/V*).

Solution B Dissolve 1.56 g of *sodium dihydrogen phosphate R* in 900 mL of *water R*. Add 7.0 mL of *phosphoric acid R* and dilute to 1000 mL with *water R*.

Blank solution Solution B, solution A (30:70 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in 35 mL of solution A and sonicate for about 1 min. Add 13 mL of solution B, mix and sonicate for about 1 min. Dilute to 50.0 mL with solution B and mix.

Reference solution (a) Dissolve 70.0 mg of *sultamicillin tosilate CRS* in 35 mL of solution A and sonicate for about 1 min. Add 13 mL of solution B, mix and sonicate for about 1 min. Dilute to 50.0 mL with solution B and mix.

Reference solution (b) Suspend 15 mg of *sultamicillin tosilate CRS* in 20 mL of a 0.4 g/L solution of *sodium hydroxide R* and sonicate in an ultrasonic bath for about 5 min. Add 20 mL of a 0.36 g/L solution of *hydrochloric acid R* and dilute to 100 mL with *water R*.

Reference solution (c) Dilute 1.0 mL of reference solution (a) to 100.0 mL with the blank solution.

Reference solution (d) Dissolve 17.3 mg of *ampicillin trihydrate CRS* (impurity C) and 15.0 mg of *sulbactam CRS* (impurity A) in *water R* and dilute to 50.0 mL with the same solvent. Dilute 1.0 mL of this solution to 100.0 mL with *water R*.

Reference solution (e) Dissolve 5 mg of *sultamicillin for peak identification CRS* (containing impurity G) in 7.0 mL of solution A and sonicate for about 1 min. Dilute to 10.0 mL with solution B, mix and sonicate for about 1 min.

Column:
— *size: l* = 0.10 m, Ø = 4.6 mm;
— *stationary phase: octadecylsilyl silica gel for chromatography R* (3.5 µm);
— *temperature*: 25 °C.

Mobile phase:
— *mobile phase A*: 4.68 g/L solution of *sodium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*;
— *mobile phase B*: *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	95 → 30	5 → 70
15 - 16	30	70
16 - 16.5	30 → 95	70 → 5
16.5 - 20	95	5

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 5 µL of the blank solution, the test solution and reference solutions (b), (c), (d) and (e).

Identification of impurities Use the chromatogram supplied with *sultamicillin for peak identification CRS* and the chromatogram obtained with reference solution (e) to identify the peak due to impurity G.

Relative retention With reference to sultamicillin (retention time = about 9.3 min): impurity A = about 0.41; ampicillin penicilloic acid = about 0.47; impurity B = about 0.50; impurity C = about 0.55; impurity D = about 0.94; impurity E = about 1.09; impurity F = about 1.26; impurity G = about 1.42.

System suitability Reference solution (b):
— *resolution*: minimum 2.5 between the peaks due to ampicillin penicilloic acid and impurity B and minimum 2.5 between the peaks due to impurities B and C.

Limits:
— *impurity G*: not more than the area of the peak due to sultamicillin in the chromatogram obtained with reference solution (c) (1.0 per cent);
— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.3 per cent);
— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.3 per cent);
— *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.3 per cent);
— *impurities D, E, F*: for each impurity, not more than 0.3 times the area of the peak due to sultamicillin in the chromatogram obtained with reference solution (c) (0.3 per cent);

— *any other impurity*: for each impurity, not more than 0.3 times the area of the peak due to sultamicillin in the chromatogram obtained with reference solution (c) (0.3 per cent);
— *total*: not more than 3 times the area of the peak due to sultamicillin in the chromatogram obtained with reference solution (c) (3.0 per cent);
— *disregard limit*: 0.1 times the area of the peak due to sultamicillin in the chromatogram obtained with reference solution (c) (0.1 per cent).

Ethyl acetate

Head-space gas chromatography (*2.2.28*).

Test solution Dissolve 0.200 g in 7.0 mL of a mixture of 1 volume of *water R* and 99 volumes of *dimethylformamide R*.

Reference solution Dissolve 0.200 g of *ethyl acetate R* in 240 mL of a mixture of 1 volume of *water R* and 99 volumes of *dimethylformamide R* and dilute to 250.0 mL with the same mixture of solvents. Dilute 5.0 mL of this solution to 7.0 mL with a mixture of 1 volume of *water R* and 99 volumes of *dimethylformamide R*.

Close the vials immediately with a tight rubber membrane stopper coated with polytetrafluoroethylene and secure with an aluminium crimped cap. Shake to obtain a homogeneous solution.

Column:
— *material*: fused silica;
— *size*: *l* = 50 m, Ø = 0.32 mm;
— *stationary phase*: *methylpolysiloxane R* (film thickness: 1.8 µm or 3 µm).

Carrier gas *helium for chromatography R*.

Linear velocity 35 cm/s.

Split ratio 1:5.

Static head-space conditions that may be used:
— *equilibration temperature*: 105 °C;
— *equilibration time*: 45 min;
— *transfer-line temperature*: 110 °C;
— *pressurisation time*: 30 s.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 6	70
	6 - 16	70 → 220
	16 - 18	220
Injection port		140
Detector		250

Detection Flame ionisation.

Injection 1 mL.

Relative retention With reference to dimethylformamide (retention time = about 14 min): ethyl acetate = about 0.7.

Limit:
— *ethyl acetate*: maximum 2.5 per cent.

Water (*2.5.12*)

Maximum 1.0 per cent, determined on 0.50 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (a).

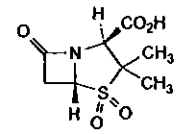
Calculate the percentage content of sultamicillin ($C_{25}H_{30}N_4O_9S_2$) from the declared content of $C_{25}H_{30}N_4O_9S_2$ in *sultamicillin tosilate CRS* and by multiplying the sultamicillin tosilate content by 0.7752.

STORAGE

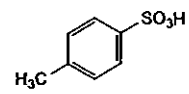
In an airtight container.

IMPURITIES

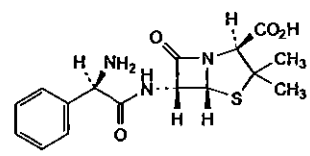
Specified impurities *A, B, C, D, E, F, G.*



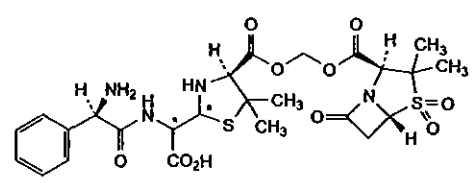
A. (2*S*,5*R*)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylic acid 4,4-dioxide (sulbactam),



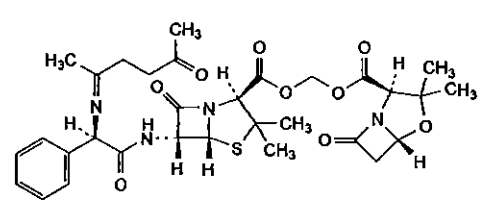
B. 4-methylbenzenesulfonic acid (*p*-toluenesulfonic acid),



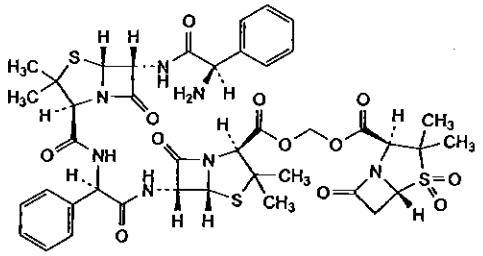
C. (2*S*,5*R*,6*R*)-6-[[(2*R*)-2-amino-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (ampicillin),



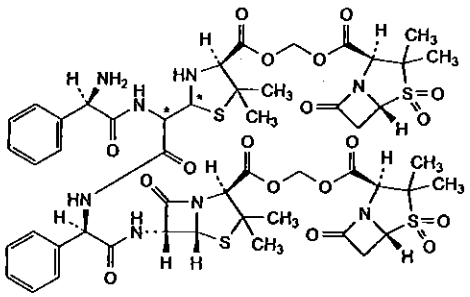
D. [[(2*R*)-aminophenylacetyl]amino][(4*S*)-4-[[[[[(2*S*,5*R*)-3,3-dimethyl-4,4,7-trioxo-4λ⁶-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]oxy]methoxy]carbonyl]-5,5-dimethylthiazolidin-2-yl]acetic acid (penicilloic acids of sultamicillin),



E. methylene (2*S*,5*R*,6*R*)-3,3-dimethyl-6-[[(2*R*)-[(1-methyl-4-oxopentylidene)amino]phenylacetyl]amino]-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (2*S*,5*R*)-3,3-dimethyl-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylate,



F. methylene (2*S*,5*R*,6*R*)-6-[[(2*R*)-[[[(2*S*,5*R*,6*R*)-6-[[(2*R*)-aminophenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]amino]phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (2*S*,5*R*)-3,3-dimethyl-4,4,7-trioxo-4λ⁶-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (ampicillin sultamicillin amide),

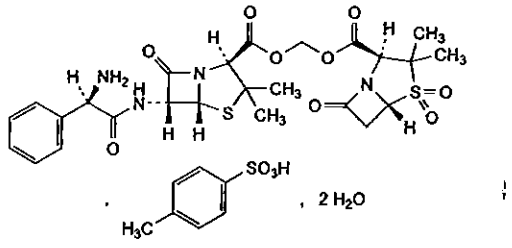


G. methylene (2*S*,5*R*,6*R*)-6-[[(2*R*)-[[[[(2*R*)-aminophenylacetyl]amino][(4*S*)-4-[[[[[(2*S*,5*R*)-3,3-dimethyl-4,4,7-trioxo-4λ⁶-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]oxy]methoxy]carbonyl]-5,5-dimethylthiazolidin-2-yl]acetyl]amino]phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (2*S*,5*R*)-3,3-dimethyl-4,4,7-trioxo-4λ⁶-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (sultamicillin dimer).

Ph Eur

Sultamicillin Tosilate Dihydrate

(Ph. Eur. monograph 2212)



$C_{32}H_{38}N_4O_{12}S_3,2H_2O$ 803

Action and use
Beta-lactamase inhibitor.

Ph Eur

DEFINITION
4-Methylbenzenesulfonate of methylene (2*S*,5*R*,6*R*)-6-[[(2*R*)-aminophenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (2*S*,5*R*)-3,3-dimethyl-4,4,7-trioxo-4λ⁶-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate dihydrate.

Semi-synthetic product derived from a fermentation product.

Content
95.0 per cent to 102.0 per cent (anhydrous substance).

PRODUCTION
It is considered that alkyl toluenesulfonate esters are genotoxic and are potential impurities in sultamicillin tosilate dihydrate. The manufacturing process should be developed taking into consideration the principles of quality risk management, together with considerations of the quality of starting materials, process capability and validation.
The general method *2.5.40. Methyl, ethyl and isopropyl toluenesulfonate in active substances* is available to assist manufacturers.

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison sultamicillin tosilate CRS.

TESTS
Specific optical rotation (*2.2.7*)
+ 178 to + 195 (anhydrous substance).
Dissolve 1.000 g in *dimethylformamide R* and dilute to 50.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use or keep at 2-8 °C for not more than 6 h.*
Solution A *methanol R1, acetonitrile R1* (20:80 *V/V*).
Solution B Dissolve 1.56 g of *sodium dihydrogen phosphate R* in 900 mL of *water R*. Add 7.0 mL of *phosphoric acid R* and dilute to 1000 mL with *water R*.
Blank solution Solution B, solution A (30:70 *V/V*).
Test solution Dissolve 70.0 mg of the substance to be examined in 35 mL of solution A and sonicate for about 1 min. Add 13 mL of solution B, mix and sonicate for about 1 min. Dilute to 50.0 mL with solution B and mix.
Reference solution (a) Dissolve 70.0 mg of *sultamicillin tosilate CRS* in 35 mL of solution A and sonicate for about 1 min. Add 13 mL of solution B, mix and sonicate for about 1 min. Dilute to 50.0 mL with solution B and mix.
Reference solution (b) Suspend 15 mg of the substance to be examined in 20 mL of a 0.4 g/L solution of *sodium hydroxide R* and sonicate in an ultrasonic bath for about 5 min. Add 20 mL of a 0.36 g/L solution of *hydrochloric acid R* and dilute to 100.0 mL with *water R*.
Reference solution (c) Dissolve 0.200 g of the substance to be examined in 70.0 mL of solution A and sonicate for about 1 min. Add 25.0 mL of solution B, mix and sonicate for about 1 min. Dilute to 100.0 mL with solution B and mix. Dilute 1.0 mL of this solution to 100.0 mL with the blank solution.
Reference solution (d) Dissolve 32.3 mg of *ampicillin trihydrate CRS* (impurity B) and 7.0 mg of *sulbactam CRS* (impurity A) in *water R* and dilute to 1000 mL with the same solvent.

Column:
— *size*: *l* = 0.10 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3.5 µm);
— *temperature*: 25 °C.

Mobile phase:
— *mobile phase A*: 4.68 g/L solution of *sodium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*;
— *mobile phase B*: *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	95 → 30	5 → 70
15 - 16	30	70
16 - 16.5	30 → 95	70 → 5
16.5 - 20	95	5

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 5 µL of the blank solution, the test solution and reference solutions (b), (c) and (d).

Relative retention With reference to sultamicillin (retention time = about 9.3 min): impurity A = about 0.41; ampicillin penicilloic acid = about 0.47; tosilate = about 0.50; impurity B = about 0.55; impurity C = about 0.94; impurity D = about 1.09; impurity F = about 1.23; impurity E = about 1.26; impurity G = about 1.42.

System suitability Reference solution (b):
— *resolution*: minimum 2.5 between the peaks due to ampicillin penicilloic acid and tosilate and minimum 2.5 between the peaks due to tosilate and impurity B.

Limits:
— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (2.0 per cent);
— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.5 per cent);
— *impurities C, D, E, F, G*: for each impurity, not more than 0.5 times the area of the peak due to sultamicillin in the chromatogram obtained with reference solution (c) (0.5 per cent);
— *any other impurity*: for each impurity, not more than 0.5 times the area of the peak due to sultamicillin in the chromatogram obtained with reference solution (c) (0.5 per cent);
— *total*: not more than 4 times the area of the peak due to sultamicillin in the chromatogram obtained with reference solution (c) (4.0 per cent);
— *disregard limit*: 0.1 times the area of the peak due to sultamicillin in the chromatogram obtained with reference solution (c) (0.1 per cent).

Ethyl acetate

Head-space gas chromatography (*2.2.28*).

Test solution Dissolve 0.200 g in 7.0 mL of a mixture of 1 volume of *water R* and 99 volumes of *dimethylformamide R*.

Reference solution Dissolve 0.200 g of *ethyl acetate R* in 240 mL of a mixture of 1 volume of *water R* and 99 volumes of *dimethylformamide R* and dilute to 250.0 mL with the same mixture of solvents. Dilute 5.0 mL of this solution to 7.0 mL with a mixture of 1 volume of *water R* and 99 volumes of *dimethylformamide R*.

Immediately close the vials with a tight rubber membrane stopper coated with polytetrafluoroethylene and secure with an aluminium crimped cap. Shake to obtain a homogeneous solution.

Column:
— *material*: fused silica;
— *size*: *l* = 50 m, Ø = 0.32 mm;
— *stationary phase*: *methylpolysiloxane R* (film thickness: 1.8 µm or 3 µm).

Carrier gas *helium for chromatography R*.

Linear velocity 35 cm/s.

Split ratio 1:5.

Static head-space conditions that may be used:
— *equilibration temperature*: 105 °C;
— *equilibration time*: 45 min;
— *transfer-line temperature*: 110 °C;
— *pressurisation time*: 30 s.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 6	70
	6 - 16	70 → 220
	16 - 18	220
Injection port		140
Detector		250

Detection Flame ionisation.

Injection 1 mL.

Relative retention With reference to dimethylformamide (retention time = about 14 min): ethyl acetate = about 0.7.

Limit:
— *ethyl acetate*: maximum 2.0 per cent.

Water (*2.5.12*)

4.0 per cent to 6.0 per cent, determined on 0.200 g.

Sulfated ash (*2.4.14*)

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

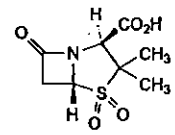
Calculate the percentage content of sultamicillin tosilate ($C_{32}H_{38}N_4O_{12}S_3$) from the declared content of *sultamicillin tosilate CRS*.

STORAGE

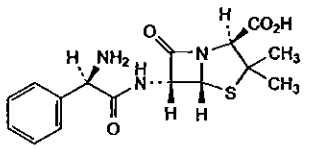
In an airtight container.

IMPURITIES

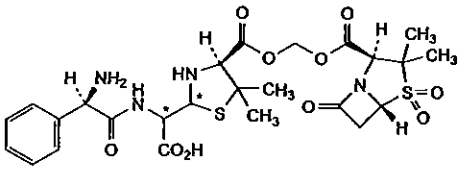
Specified impurities *A, B, C, D, E, F, G.*



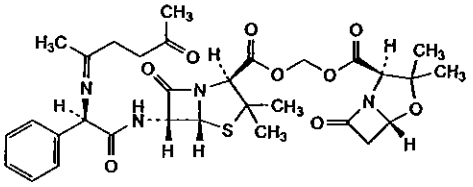
A. (2*S*,5*R*)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylic acid 4,4-dioxide (sulbactam),



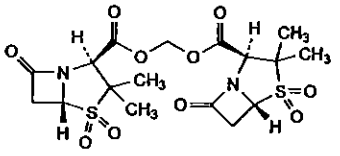
B. (2*S*,5*R*,6*R*)-6-[[(2*R*)-2-amino-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (ampicillin),



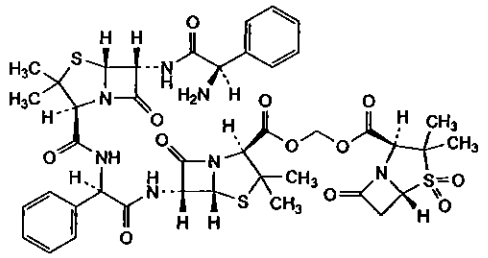
C. [[(2*R*)-aminophenylacetyl]amino][(4*S*)-4-[[[[[(2*S*,5*R*)-3,3-dimethyl-4,4,7-trioxo-4λ⁶-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]oxy]methoxy]carbonyl]-5,5-dimethylthiazolidin-2-yl]acetic acid (penicilloic acids of sultamicillin),



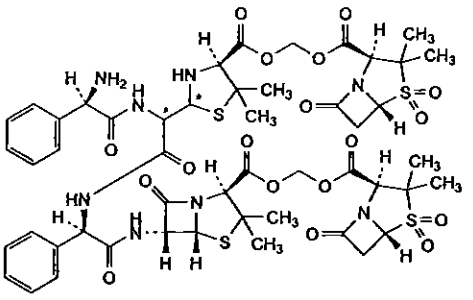
D. methylene (2*S*,5*R*,6*R*)-3,3-dimethyl-6-[[(2*R*)-[(1-methyl-4-oxopentylidene)amino]phenylacetyl]amino]-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (2*S*,5*R*)-3,3-dimethyl-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylate,



E. methylene bis[(2*S*,5*R*)-3,3-dimethyl-4,4,7-trioxo-4λ⁶-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate] (sulbactam methylene ester),



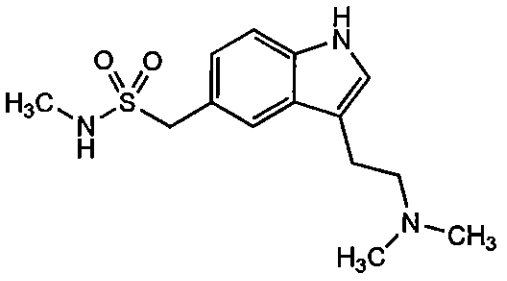
F. methylene (2*S*,5*R*,6*R*)-6-[[(2*R*)-[[[(2*S*,5*R*,6*R*)-6-[[(2*R*)-aminophenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]amino]-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (2*S*,5*R*)-3,3-dimethyl-4,4,7-trioxo-4λ⁶-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (ampicillin sultamicillin amide),



G. methylene (2*S*,5*R*,6*R*)-6-[[(2*R*)-[[[[(2*R*)-amino-phenylacetyl]amino][(4*S*)-4-[[[[[(2*S*,5*R*)-3,3-dimethyl-4,4,7-trioxo-4λ⁶-thia-1-azabicyclo[3.2.0]hept-2-yl]-carbonyl]oxy]methoxy]carbonyl]-5,5-dimethylthiazolidin-2-yl]acetyl]amino]phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (2*S*,5*R*)-3,3-dimethyl-4,4,7-trioxo-4λ⁶-thia-1-azabicyclo [3.2.0]heptane-2-carboxylate (sultamicillin dimer).

Ph Eur

Sumatriptan



$C_{14}H_{21}N_3O_2S$ 295.4 *103628-46-2*

Action and use
Serotonin $5HT_1$ receptor agonist; treatment of migraine.

Preparation
Sumatriptan Nasal Spray

DEFINITION
Sumatriptan is 3-(2-dimethylaminoethyl)indol-5-yl-*N*-methylmethanesulfonamide. It contains not less than 97.5% and not more than 102.0% of $C_{14}H_{21}N_3O_2S$, calculated with reference to the anhydrous substance.

CHARACTERISTICS
A white to pale yellow powder. Very slightly soluble in *water*.

IDENTIFICATION
The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of sumatriptan *(RS 414)*.

TESTS
The total impurity content in the test for Impurities A and H and the test for Related substances is not greater than 1.5%.

Impurities A and H
Carry out the method for *liquid chromatography*, Appendix III D. Prepare the solutions in a mixture containing 3 volumes of 0.025M *sodium dihydrogen orthophosphate*, the pH of which has been adjusted to 6.5, and 1 volume of *acetonitrile* (solution A).

(1) 0.2% w/v of the substance being examined.

(2) Dilute 1 volume of solution (1) to 100 volumes.

(3) Dilute the contents of a vial of *sumatriptan for system suitability EPCRS* to 1 mL with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Spherisorb silica S5W is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2.0 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 282 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1) allow the chromatography to proceed for 5 times the retention time of the principal peak.

MOBILE PHASE

10 volumes of 10M *ammonium acetate* and 90 volumes of *methanol.*

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) resembles that supplied with *sumatriptan for system suitability EPCRS* and the *resolution* between impurity A and sumatriptan is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.6%; taking into account the correction factor of 0.6 for impurity A);

the area of any peak due to impurity H is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%).

Related substances

Carry out the method for *liquid chromatography,* Appendix III D. Prepare the solutions in a mixture containing 3 volumes of 0.025M *sodium dihydrogen orthophosphate,* the pH of which has been adjusted to 6.5, and 1 volume of *acetonitrile* (solution A).

(1) 0.2% w/v of the substance being examined.

(2) Dilute 1 volume of solution (1) to 100 volumes and further dilute 1 volume of the resulting solution to 10 volumes.

(3) Dilute the contents of a vial of *sumatriptan impurity mixture EPCRS* to 1 mL with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 282 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mix 25 volumes of *acetonitrile* with 75 volumes of a solution containing 0.97 g of *dibutylamine,* 0.735 g of *orthophosphoric acid* and 2.93 g of *sodium dihydrogen orthophosphate* in 750 mL *water,* adjust to pH 6.5 with 10M *sodium hydroxide* and dilute to 1000 mL with *water.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity C and sumatriptan is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the areas of any peaks corresponding to impurities B, C and D are not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5% of each);

the area of any peak corresponding to impurity E is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Water

Not more than 1.0% w/w, Appendix IX C. Use 1.0 g.

ASSAY

Carry out the method for *liquid chromatography,* Appendix III D. Prepare solutions (1) and (2) in a mixture containing 3 volumes of 0.025M *sodium dihydrogen orthophosphate,* the pH of which has been adjusted to 6.5, and 1 volume of *acetonitrile* (solution A).

(1) 0.01% w/v of the substance being examined.

(2) 0.014% w/v of *sumatriptan succinate BPCRS.*

(3) Dilute the contents of a vial of *sumatriptan impurity mixture EPCRS* to 1 mL with the mobile phase .

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity C and sumatriptan is at least 1.5.

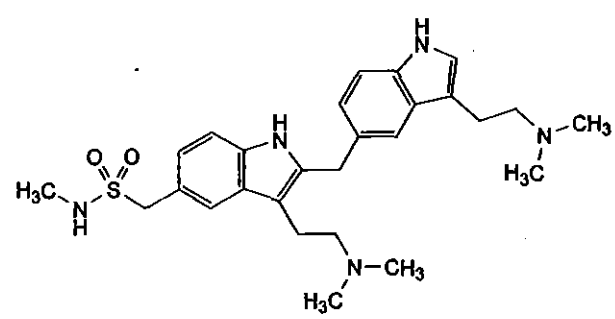
DETERMINATION OF CONTENT

Calculate the content of $C_{14}H_{21}N_3O_2S$ using the declared content of $C_{14}H_{21}N_3O_2S,C_4H_6O_4$ in *sumatriptan succinate BPCRS.* Each 1 mg of $C_{14}H_{21}N_3O_2S$ is equivalent to 1.4 mg of $C_{14}H_{21}N_3O_2S,C_4H_6O_4$.

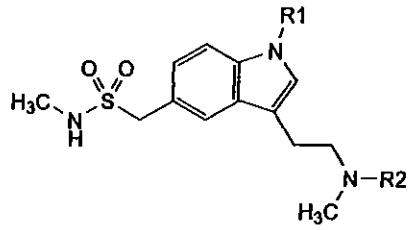
STORAGE

Sumatriptan should be protected from light.

IMPURITIES

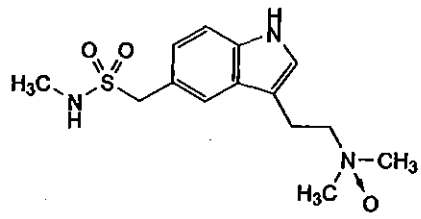


A. [3-[2-(dimethylamino)ethyl]-2-[[3-[2-(dimethylamino) ethyl]-1*H*-indol-5-yl]methyl]-1*H*-indol-5-yl]-*N*-methylmethanesulfonamide,

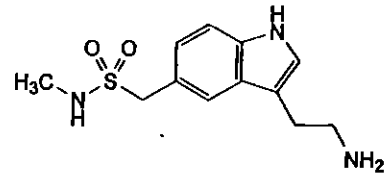


B. R1 = R2 = H: *N*-methyl[3-[2-(methylamino)ethyl]-1*H*-indol-5-yl]methanesulfonamide,

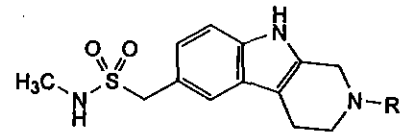
C. R1 = CH_2-OH, R2 = CH_3: [3-[2-(dimethylamino)ethyl]-1-(hydroxymethyl)-1*H*-indol-5-yl]-*N*-methylmethanesulfonamide,



D. *N*,*N*-dimethyl-2-[5-[(methylsulfamoyl)methyl]-1*H*-indol-3-yl]ethanamine *N*-oxide,

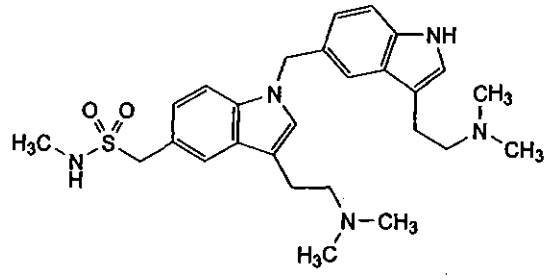


E. [3-(2-aminoethyl)-1*H*-indol-5-yl]-*N*-methylmethanesulfonamide,



F. R = H: *N*-methyl(2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-6-yl)methanesulfonamide,

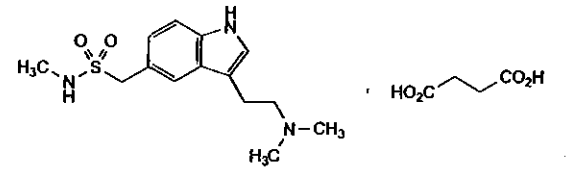
G. R = CH_3: *N*-methyl(2-methyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-6-yl)methanesulfonamide,



H. [3-[2-(dimethylamino)ethyl]-1-[[3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl]methyl]-1*H*-indol-5-yl]-*N*-methylmethanesulfonamide.

Sumatriptan Succinate

(Ph. Eur. monograph 1573)



$C_{18}H_{27}N_3O_6S$ 413.5 *103628-48-4*

Action and use
Serotonin $5HT_1$ receptor agonist; treatment of migraine.

Preparations
Sumatriptan Injection
Sumatriptan Tablets

Ph Eur

DEFINITION

1-[3-[2-(Dimethylamino)ethyl]-1*H*-indol-5-yl]-*N*-methylmethanesulfonamide hydrogen butanedioate.

Content
97.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Freely soluble in water, sparingly soluble in methanol, practically insoluble in methylene chloride.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *sumatriptan succinate CRS.*

TESTS

Solution S
Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 25.0 mL with the same solvent.

pH (*2.2.3*)
4.5 to 5.3.

Dilute 2.5 mL of solution S to 10 mL with *carbon dioxide-free water R.*

Absorbance (*2.2.25*)
Maximum 0.10, determined at 440 nm on solution S.

Impurities A and H
Liquid chromatography (*2.2.29*).

Test solution Dissolve 30.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 3 mg of *sumatriptan for system suitability CRS* (containing impurities A and H) in the mobile phase and dilute to 1.0 mL with the mobile phase.

Column:
— *size:* l = 0.25 m, Ø = 4.6 mm;
— *stationary phase: silica gel for chromatography R* (5 µm).

Mobile phase Mix 10 volumes of a 771 g/L solution of *ammonium acetate R* and 90 volumes of *methanol R.*

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 282 nm.

Injection 20 µL.

Run time 5 times the retention time of sumatriptan.

Identification of impurities Use the chromatogram supplied with *sumatriptan for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and H.

Relative retention With reference to sumatriptan (retention time = about 2 min): impurity A = about 1.9; impurity H = about 2.6.

System suitability Reference solution (b):
— *resolution*: minimum 3.0 between the peaks due to impurities A and H.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity A by 0.6;
— *impurity A*: not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.6 per cent);
— *impurity H*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent).

Related substances
Liquid chromatography (*2.2.29*).

Solution A Dissolve 2.925 g of *sodium dihydrogen phosphate R* in 600 mL of *water R*, adjust to pH 6.5 with *strong sodium hydroxide solution R*, dilute to 750 mL with *water R*, add 250 mL of *acetonitrile R* and mix.

Test solution (a) Dissolve 30.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Test solution (b) Dissolve 15.0 mg of the substance to be examined in solution A and dilute to 100.0 mL with solution A.

Reference solution (a) Dilute 1.0 mL of test solution (a) to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve the contents of a vial of *sumatriptan impurity mixture CRS* (containing impurities B, C, D and E) in the mobile phase and dilute to 1 mL with the mobile phase.

Reference solution (c) Dissolve 15.0 mg of *sumatriptan succinate CRS* in solution A and dilute to 100.0 mL with solution A.

Column:
— *size*: *l* = 0.25 m, Ø = 4 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 25 volumes of *acetonitrile R* with 75 volumes of a solution prepared as follows: dissolve 0.970 g of *dibutylamine R*, 0.735 g of *phosphoric acid R* and 2.93 g of *sodium dihydrogen phosphate R* in 750 mL of *water R*, adjust to pH 6.5 with *strong sodium hydroxide solution R* and dilute to 1000 mL with *water R*.

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 282 nm.

Injection 10 µL of test solution (a) and reference solutions (a) and (b).

Run time 4 times the retention time of sumatriptan.

Identification of impurities Use the chromatogram supplied with *sumatriptan impurity mixture CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B, C, D and E.

Relative retention With reference to sumatriptan (retention time = about 7 min): impurity E = about 0.5; impurity B = about 0.6; impurity D = about 0.7; impurity C = about 0.8.

System suitability Reference solution (b):
— *resolution*: minimum 1.5 between the peaks due to impurity C and sumatriptan;
— the chromatogram shows 5 clearly separated peaks.

Limits:
— *impurities B, C, D*: for each impurity, not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *impurity E*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.6 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)
Maximum 1.0 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

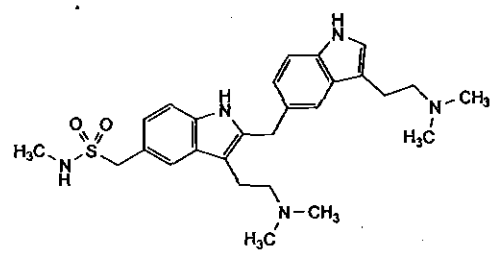
Injection Test solution (b) and reference solution (c).

Calculate the percentage content of $C_{18}H_{27}N_3O_6S$ taking into account the assigned content of *sumatriptan succinate CRS*.

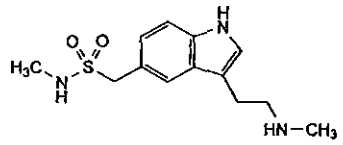
STORAGE
Protected from light.

IMPURITIES
Specified impurities *A, B, C, D, E, H.*

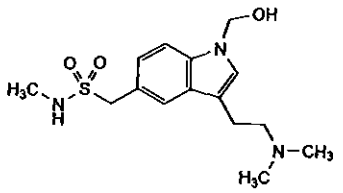
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *F, G.*



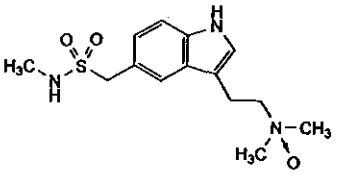
A. 1-[3-[2-(dimethylamino)ethyl]-2-[[3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl]methyl]-1*H*-indol-5-yl]-*N*-methylmethanesulfonamide,



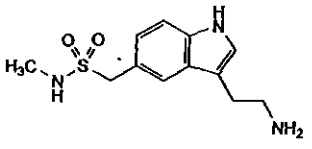
B. *N*-methyl-1-[3-[2-(methylamino)ethyl]-1*H*-indol-5-yl]methanesulfonamide,



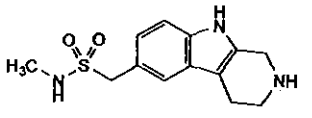
C. 1-[3-[2-(dimethylamino)ethyl]-1-(hydroxymethyl)-1*H*-indol-5-yl]-*N*-methylmethanesulfonamide,



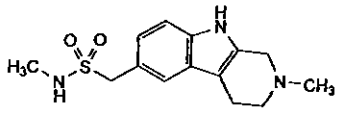
D. *N*,*N*-dimethyl-2-[5-[(methylsulfamoyl)methyl]-1*H*-indol-3-yl]ethan-1-amine *N*-oxide,



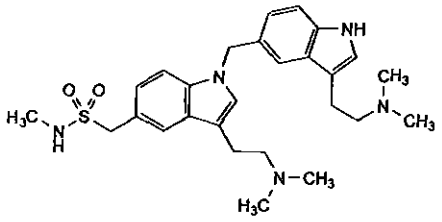
E. 1-[3-(2-aminoethyl)-1*H*-indol-5-yl]-*N*-methylmethanesulfonamide,



F. *N*-methyl-1-(2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-6-yl)methanesulfonamide,



G. *N*-methyl-1-(2-methyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-6-yl)methanesulfonamide,



H. 1-[3-[2-(dimethylamino)ethyl]-1-[[3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl]methyl]-1*H*-indol-5-yl]-*N*-methylmethanesulfon amide.

Ph Eur

Refined Sunflower Oil

(Ph. Eur. monograph 1371)

Ph Eur

DEFINITION

Fatty oil obtained from the seeds of *Helianthus annuus* L. by mechanical expression or by extraction. It is then refined. A suitable antioxidant may be added.

CHARACTERS

Appearance
Clear, light yellow liquid.

Solubility
Practically insoluble in water and in ethanol (96 per cent), miscible with light petroleum (bp: 40-60 °C).

Relative density
About 0.921.

Refractive index
About 1.474.

IDENTIFICATION

Identification of fatty oils by thin-layer chromatography (*2.3.2*).

Results The chromatogram obtained is similar to the corresponding chromatogram shown in Figure 2.3.2.-1.

TESTS

Acid value (*2.5.1*)
Maximum 0.5, determined on 10.0 g.

Peroxide value (*2.5.5, Method A*)
Maximum 10.0.

Unsaponifiable matter (*2.5.7*)
Maximum 1.5 per cent, determined on 5.0 g.

Alkaline impurities (*2.4.19*)
It complies with the test.

Composition of fatty acids (*2.4.22, Method A*)
Use the mixture of calibrating substances in Table 2.4.22.-3.

Composition of the fatty-acid fraction of the oil:
— *palmitic acid*: 4.0 per cent to 9.0 per cent,
— *stearic acid*: 1.0 per cent to 7.0 per cent,
— *oleic acid*: 14.0 per cent to 40.0 per cent,
— *linoleic acid*: 48.0 per cent to 74.0 per cent.

Water (*2.5.32*)
Maximum 0.1 per cent, determined on 1.00 g.

STORAGE

In an airtight, well-filled container, protected from light.

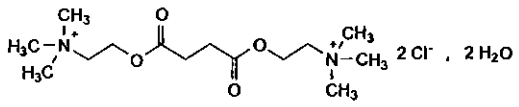
LABELLING

The label states whether the oil is obtained by mechanical expression or by extraction.

Ph Eur

Suxamethonium Chloride

(Ph. Eur. monograph 0248)



$C_{14}H_{30}Cl_2N_2O_4,2H_2O$ 397.3 *6101-15-1*

Action and use
Depolarizing neuromuscular blocker.

Preparation
Suxamethonium Chloride Injection

Ph Eur

DEFINITION
Suxamethonium chloride contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of 2,2′-[butanedioylbis(oxy)]bis(*N,N,N*-trimethylethanaminium) dichloride, calculated with reference to the anhydrous substance.

CHARACTERS
A white or almost white, crystalline powder, hygroscopic, freely soluble in water, slightly soluble in alcohol.

It melts at about 160 °C, determined without previous drying.

IDENTIFICATION
First identification: A, D.

Second identification: B, C, D.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *suxamethonium chloride CRS*. Examine the substances prepared as discs.

B. To 1 mL of solution S (see Tests) add 9 mL of *water R*, 10 mL of *dilute sulfuric acid R* and 30 mL of *ammonium reineckate solution R*. A pink precipitate is formed. Allow to stand for 30 min, filter, wash with *water R*, with *alcohol R* and then with *ether R* and dry at 80 °C. The melting point (*2.2.14*) of the precipitate is 180 °C to 185 °C.

C. Dissolve about 25 mg in 1 mL of *water R* and add 0.1 mL of a 10 g/L solution of *cobalt chloride R* and 0.1 mL of *potassium ferrocyanide solution R*. A green colour is produced.

D. About 20 mg gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S
Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*). Dilute 4 mL of solution S to 10 mL with *water R*. The solution is colourless (*2.2.2, Method II*).

pH (*2.2.3*)
Dilute 1 mL of solution S to 10 mL with *carbon dioxide-free water R*. The pH of the solution is 4.0 to 5.0.

Choline chloride
Examine by thin-layer chromatography (*2.2.27*), using *cellulose for chromatography R1* as the coating substance.

Test solution Dissolve 0.4 g of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 0.4 g of *suxamethonium chloride CRS* and 2 mg of *choline chloride R* in *methanol R* and dilute to 10 mL with the same solvent.

Apply to the plate 5 µL of each solution. Prepare the mobile phase as follows: shake together for 10 min, 10 volumes of *anhydrous formic acid R*, 40 volumes of *water R* and 50 volumes of *butanol R*; allow to stand and use the upper layer. Develop over a path of 15 cm. Dry the plate in a current of air and spray with *potassium iodobismuthate solution R*. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot corresponding to choline chloride in the chromatogram obtained with the reference solution (0.5 per cent). The test is not valid unless the chromatogram obtained with the reference solution shows two clearly separated spots.

Water (*2.5.12*)
8.0 per cent to 10.0 per cent, determined on 0.30 g by the semi-micro determination of water.

Sulfated ash (*2.4.14*)
Not more than 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.150 g in 50 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

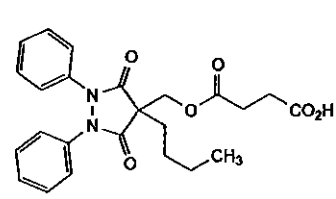
1 mL of *0.1 M perchloric acid* is equivalent to 18.07 mg of $C_{14}H_{30}Cl_2N_2O_4$.

STORAGE
Store in an airtight container, protected from light.

Ph Eur

Suxibuzone

(Ph. Eur. monograph 1574)



$C_{24}H_{26}N_2O_6$ 438.5 *27470-51-5*

Action and use
Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Ph Eur

DEFINITION
4-[(4-Butyl-3,5-dioxo-1,2-diphenylpyrazolidin-4-yl)methoxy]-4-oxobutanoic acid.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, freely soluble in acetone, soluble in ethanol (96 per cent), practically insoluble in cyclohexane.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *suxibuzone CRS.*

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 1 g in *anhydrous ethanol R* and dilute to 20 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.10 g of the substance to be examined in *acetonitrile R* and dilute to 25.0 mL with the same solvent.

Reference solution (a) Dissolve 2.8 mg of *phenylbutazone CRS* (impurity A), 2.8 mg of *suxibuzone impurity B CRS* and 2.8 mg of *suxibuzone impurity C CRS* in *acetonitrile R* and dilute to 10.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with *acetonitrile R.*

Reference solution (b) Dissolve 4 mg of *phenylbutazone CRS* (impurity A) in *acetonitrile R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with *acetonitrile R.*

Reference solution (c) Dissolve 10 mg of *phenylbutazone CRS* (impurity A) in *acetonitrile R* and dilute to 25.0 mL with the same solvent. Mix 10.0 mL of this solution with 1.0 mL of the test solution and dilute the mixture to 25.0 mL with *acetonitrile R.*

Column:

— *size*: *l* = 0.125 m, Ø = 4.0 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 44 volumes of *acetonitrile R* and 56 volumes of a solution prepared as follows: dissolve 6.7 g of *citric acid monohydrate R* and 2.4 g of *tris(hydroxymethyl) aminomethane R* in 950 mL of *water R*, adjust to pH 3.0 with *citric acid monohydrate R* and dilute to 1000 mL with *water R.*

Flow rate 1 mL/min.

Detection Spectrophotometer at 250 nm.

Injection 10 µL.

Relative retention With reference to suxibuzone (retention time = about 7 min): impurity C = 0.7; impurity A = 1.4; impurity B = 3.3.

System suitability Reference solution (c):

— *resolution*: minimum of 2.0 between the peaks due to suxibuzone and impurity A.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.7 per cent);

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.7 per cent);

— *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.7 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.1 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (a) (0.07 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven *in vacuo* at 60 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.400 g in previously neutralised *anhydrous ethanol R* and dilute to 10 mL with the same solvent. Carry out a potentiometric titration (*2.2.20*) using *0.1 M sodium hydroxide.*

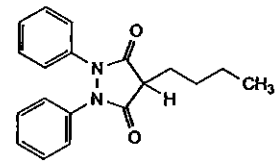
1 mL of *0.1 M sodium hydroxide* is equivalent to 43.85 mg of $C_{24}H_{26}N_2O_6$.

STORAGE

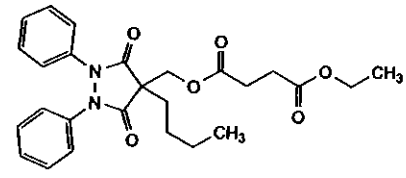
Protected from light.

IMPURITIES

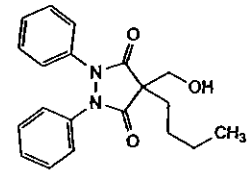
Specified impurities *A, B, C.*



A. 4-butyl-1,2-diphenylpyrazolidine-3,5-dione (phenylbutazone),



B. (4-butyl-3,5-dioxo-1,2-diphenylpyrazolidin-4-yl)methyl ethyl butanedioate,

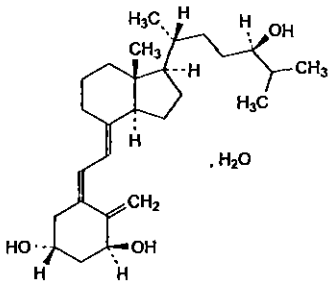


C. 4-butyl-4-(hydroxymethyl)-1,2-diphenyl-1,2-dihydro-4*H*-pyrazole-3,5-dione.

Ph Eur

Tacalcitol Monohydrate

(Ph. Eur. monograph 2272)



$C_{27}H_{44}O_3,H_2O$ 434.7 *93129-94-3*

Action and use
Vitamin D_3 analogue.

Ph Eur

DEFINITION

(5*Z*,7*E*)-(24*R*)-9,10-Secocholesta-5,7,10(19)-triene-1α,3β,24-triol.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

A reversible isomerisation to pre-tacalcitol takes place in solution, depending on temperature and time. The activity is due to both compounds.

It is sensitive to air, heat and light.

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, freely soluble in anhydrous ethanol and in ethyl acetate.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of tacalcitol monohydrate.*

B. It complies with test B for related substances (see Tests).

C. Water (see Tests).

TESTS

Related substances

A. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use avoiding exposure to light and air.*

Test solution Dissolve 5.0 mg of the substance to be examined in *acetonitrile R* and dilute to 25.0 mL with the same solvent.

Reference solution (a) Dissolve 5.0 mg of *tacalcitol monohydrate CRS* in *acetonitrile R* and dilute to 25.0 mL with the same solvent.

Reference solution (b) Heat 3 mL of the test solution at 80 °C for 30 min. Cool the solution to room temperature.

Reference solution (c) Dilute 1.0 mL of the test solution to 100.0 mL with *acetonitrile R*. Dilute 1.0 mL of this solution to 10.0 mL with *acetonitrile R*.

Reference solution (d) Dissolve the contents of a vial of *tacalcitol impurity A CRS* in 1 mL of the test solution.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *water for chromatography R, acetonitrile R* (40:60 *V/V*).

Flow rate 2 mL/min.

Detection Spectrophotometer at 265 nm.

Injection 50 µL of the test solution and reference solutions (b), (c) and (d).

Run time 2.5 times the retention time of tacalcitol.

Identification of impurities Use the chromatogram obtained with reference solution (d) to identify the peak due to impurity A; use the chromatogram obtained with reference solution (b) to identify the peak due to pre-tacalcitol.

Relative retention With reference to tacalcitol (retention time = about 11 min): pre-tacalcitol = about 0.8; impurity A = about 0.9.

System suitability Reference solution (d):
— *resolution*: minimum 1.5 between the peaks due to impurity A and tacalcitol.

Calculation of percentage contents:
— for each impurity, use the concentration of tacalcitol monohydrate in reference solution (c).

Limits:
— *impurity A*: maximum 0.3 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.5 per cent;
— *reporting threshold*: 0.05 per cent; disregard the peak due to pre-tacalcitol.

B. Liquid chromatography (*2.2.29*).

Test solution Dissolve 2.0 mg of the substance to be examined in the mobile phase and dilute to 20.0 mL with the mobile phase.

Reference solution (a) Dissolve the contents of a vial of *tacalcitol for system suitability CRS* (containing impurity B) in 0.5 mL of the mobile phase.

Reference solution (b) Dilute 5.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *amylose derivative of silica gel for chiral separation R* (10 µm).

Mobile phase *anhydrous ethanol R, heptane R* (14:86 *V/V*).

Flow rate 1 mL/min.

Detection Spectrophotometer at 265 nm.

Injection 50 µL.

Run time 2.5 times the retention time of tacalcitol.

Identification of impurities Use the chromatogram supplied with *tacalcitol for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peak due to impurity B.

Relative retention With reference to tacalcitol (retention time = about 12 min): impurity A = about 0.7; impurity B = about 0.85.

System suitability Reference solution (a):
— *resolution*: minimum 1.5 between the peaks due to impurity B and tacalcitol.

Calculation of percentage contents:
— for each impurity, use the concentration of tacalcitol monohydrate in reference solution (b).

Limits:
- *impurity B*: maximum 1.0 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- disregard any peak with a relative retention with reference to tacalcitol of about 0.7 (impurity A).

Water (*2.5.32*)
3.8 per cent to 4.8 per cent, determined on 10.0 mg by direct sample introduction.

ASSAY
Liquid chromatography (*2.2.29*) as described in test A for related substances with the following modification.

Injection Test solution and reference solutions (a) and (b).

Calculate the percentage content of $C_{27}H_{44}O_3$ taking into account the assigned content of *tacalcitol monohydrate CRS* and, if present, the peak due to pre-tacalcitol.

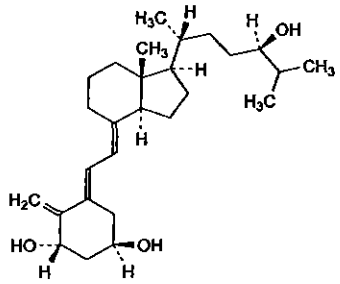
STORAGE
In an airtight container, under an inert gas, protected from light at a temperature not exceeding −15 °C.

IMPURITIES
Test A for related substances: A.

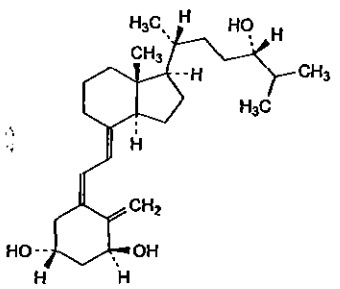
Test B for related substances: B, C.

Specified impurities *A, B.*

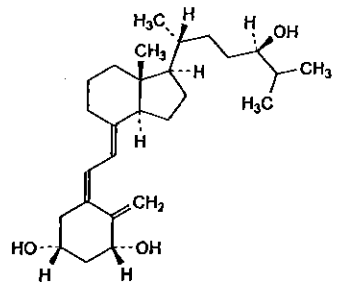
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C.*



A. (5*E*,7*E*)-(24*R*)-9,10-secocholesta-5,7,10(19)-triene-1α,3β,24-triol (*trans*-tacalcitol),



B. (5*Z*,7*E*)-(24*S*)-9,10-secocholesta-5,7,10(19)-triene-1α,3β,24-triol ((24*S*)-tacalcitol),

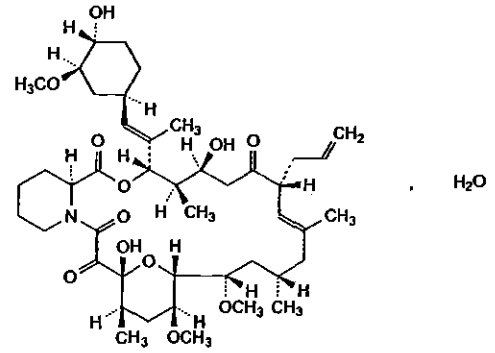


C. (5*Z*,7*E*)-(24*R*)-9,10-secocholesta-5,7,10(19)-triene-1β,3β,24-triol (1β-tacalcitol).

Ph Eur

Tacrolimus Monohydrate

(Ph. Eur. monograph 2244)



$C_{44}H_{69}NO_{12},H_2O$ 822 *109581-93-3*

Action and use
Calcineurin inhibitor; immunosuppressant.

Ph Eur

DEFINITION
(3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-5,19-dihydroxy-3-[(1*E*)-1-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]prop-1-en-2-yl]-14,16-dimethoxy-4,10,12,18-tetramethyl-8-(prop-2-en-1-yl)-3,4,5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-octadecahydro-7H-15,19-epoxypyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(23*H*)-tetrone monohydrate.

Substance produced by the growth of certain strains of *Streptomyces tsukubaensis*.

Content
97.0 per cent to 102.0 per cent for the sum of tacrolimus, tacrolimus compound I and tacrolimus compound II (anhydrous substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, soluble in ethanol (96 per cent), practically insoluble in heptane.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *tacrolimus monohydrate CRS.*

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution GY_7 (*2.2.2, Method II*).

Dissolve 0.20 g in *ethanol (96 per cent) R* and dilute to 20.0 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*). *Carry out the test protected from light. Allow the test and reference solutions to stand for 3 h at room temperature before use.*

Solvent mixture *water R, acetonitrile R* (30:70 *V/V*).

Test solution Dissolve 30.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (a) Dissolve 30.0 mg of *tacrolimus monohydrate CRS* in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 3.0 mg of *tacrolimus for system suitability CRS* (containing impurity A) in the solvent mixture and dilute to 1.0 mL with the solvent mixture.

Reference solution (c) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Column:
- *size: l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (3 µm);
- *temperature:* 60 °C.

Mobile phase:
- *mobile phase A*: mix 20 volumes of a mixture of 19 volumes of *1,1-dimethylethyl methyl ether R* and 81 volumes of *acetonitrile for chromatography R* with 80 volumes of a 0.04 per cent *V/V* solution of *phosphoric acid R*;
- *mobile phase B*: mix 20 volumes of a 0.04 per cent *V/V* solution of *phosphoric acid R* with 80 volumes of a mixture of 19 volumes of *1,1-dimethylethyl methyl ether R* and 81 volumes of *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	72	28
30 - 53	72 → 15	28 → 85

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 220 nm.

Autosampler Set at 4 °C.

Injection 20 µL of the test solution and reference solutions (b) and (c).

Identification of impurities Use the chromatogram supplied with *tacrolimus for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity A.

Relative retention With reference to tacrolimus (retention time = about 24 min): tacrolimus compound I = broad peak at about 0.5; impurity B = sharp peak at about 0.5; tacrolimus compound II = about 0.6; impurity A = about 0.9.

System suitability Reference solution (b):
- *resolution*: minimum 3.0 between the peaks due to impurity A and tacrolimus.

Integration The peak due to impurity B, which co-elutes with tacrolimus compound I, is a rider peak that is to be integrated using tangential skimming. It is necessary that the integration area of the peak due to tacrolimus compound I preserves the Gaussian peak shape.

Calculation of percentage contents:
- for each impurity, use the concentration of tacrolimus monohydrate in reference solution (c).

Limits:
- *impurity A*: maximum 0.5 per cent;
- *unspecified impurities*: for each impurity, maximum 0.15 per cent;
- *total*: maximum 1.0 per cent;
- *reporting threshold*: 0.05 per cent; disregard the peaks due to tacrolimus compound I and tacrolimus compound II.

Water (*2.5.12*)

1.5 per cent to 4.0 per cent, determined on 0.250 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

Calculate the percentage content of the sum of tacrolimus, tacrolimus compound I and tacrolimus compound II taking into account the assigned content of *tacrolimus monohydrate CRS*.

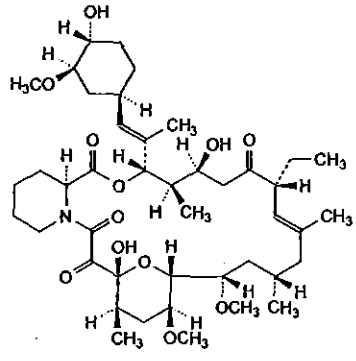
STORAGE

In an airtight container. If the substance is sterile, the container is also sterile and tamper-evident.

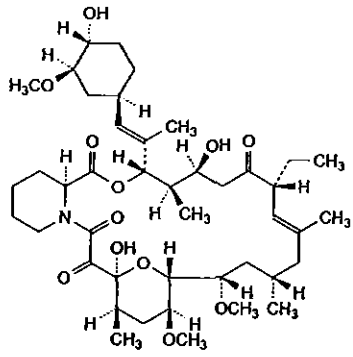
IMPURITIES

Specified impurities *A.*

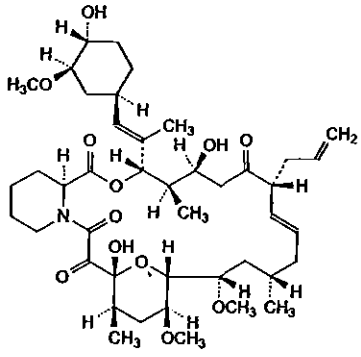
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D, E, H, I.*



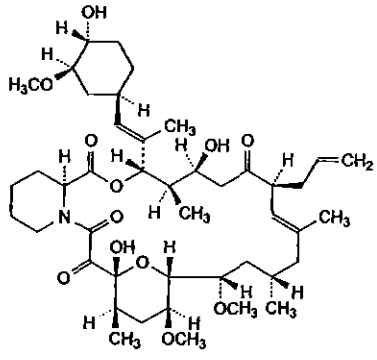
A. (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-8-ethyl-5,19-dihydroxy-3-[(1*E*)-1-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]prop-1-en-2-yl]-14,16-dimethoxy-4,10,12,18-tetramethyl-3,4,5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-octadecahydro-7*H*-15,19-epoxypyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(23*H*)-tetrone,



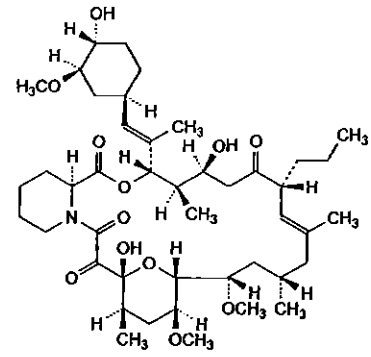
B. (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*S*,26a*S*)-8-ethyl-5,19-dihydroxy-3-[(1*E*)-1-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]prop-1-en-2-yl]-14,16-dimethoxy-4,10,12,18-tetramethyl-3,4,5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-octadecahydro-7*H*-15,19-epoxypyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(23*H*)-tetrone,



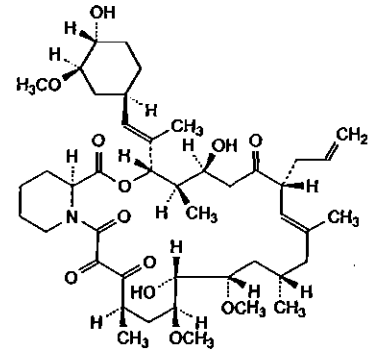
C. (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-5,19-dihydroxy-3-[(1*E*)-1-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]prop-1-en-2-yl]-14,16-dimethoxy-4,12,18-trimethyl-8-(prop-2-en-1-yl)-3,4,5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-octadecahydro-7*H*-15,19-epoxypyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(23*H*)-tetrone,



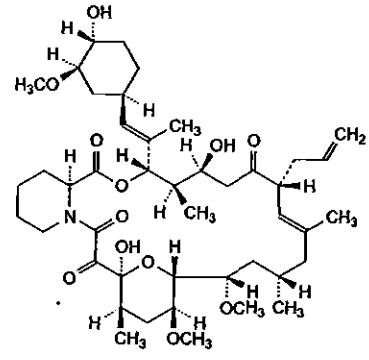
D. (3*S*,4*R*,5*S*,8*S*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-5,19-dihydroxy-3-[(1*E*)-1-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]prop-1-en-2-yl]-14,16-dimethoxy-4,10,12,18-tetramethyl-8-(prop-2-en-1-yl)-3,4,5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-octadecahydro-7*H*-15,19-epoxypyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(23*H*)-tetrone,



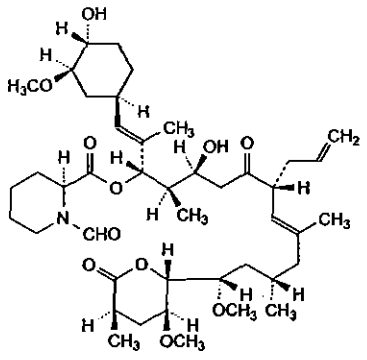
E. (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-5,19-dihydroxy-3-[(1*E*)-1-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]prop-1-en-2-yl]-14,16-dimethoxy-4,10,12,18-tetramethyl-8-propyl-3,4,5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-octadecahydro-7*H*-15,19-epoxypyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(23*H*)-tetrone,



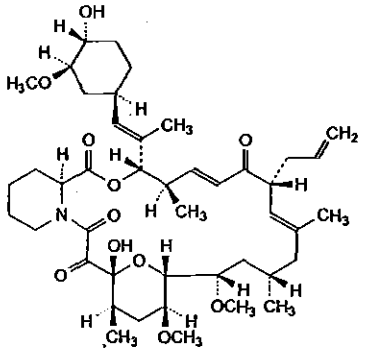
F. (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-5,15-dihydroxy-3-[(1*E*)-1-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]prop-1-en-2-yl]-14,16-dimethoxy-4,10,12,18-tetramethyl-8-(prop-2-en-1-yl)-3,4,5,6,11,12,13,14,15,16,17,18,24,25,26,26a-hexadecahydro-7*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,19,20,21(8*H*,23*H*)-pentone (tacrolimus compound I),



G. (3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*S*,26a*S*)-5,19-dihydroxy-3-[(1*E*)-1-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]prop-1-en-2-yl]-14,16-dimethoxy-4,10,12,18-tetramethyl-8-(prop-2-en-1-yl)-3,4,5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-octadecahydro-7*H*-15,19-epoxypyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(23*H*)-tetrone (tacrolimus compound II),



H. (1*E*,3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*)-5-hydroxy-1-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-14-methoxy-14-[(2*R*,3*S*,5*R*)-3-methoxy-5-methyl-6-oxooxan-2-yl]-2,4,10,12-tetramethyl-7-oxo-8-(prop-2-en-1-yl)tetradeca-1,9-dien-3-yl (2*S*)-1-formylpiperidine-2-carboxylate,

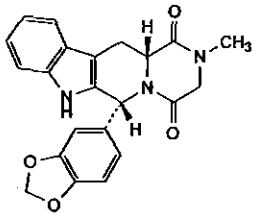


I. (3*S*,4*R*,5*E*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-19-hydroxy-3-[(1*E*)-1-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]prop-1-en-2-yl]-14,16-dimethoxy-4,10,12,18-tetramethyl-8-(prop-2-en-1-yl)-3,4,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-7*H*-15,19-epoxypyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(23*H*)-tetrone.

Ph Eur

Tadalafil

(Ph. Eur. monograph 2606)



$C_{22}H_{19}N_3O_4$ 389.4 *171596-29-5*

Action and use
Selective inhibitor of cyclic GMP-specific phosphodiesterase (Type V) with vasodilator action; treatment of erectile dysfunction.

Ph Eur

DEFINITION
(6*R*,12a*R*)-6-(1,3-Benzodioxol-5-yl)-2-methyl-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]-pyrido[3,4-*b*]indole-1,4-dione.

Content
97.5 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Practically insoluble in water, freely soluble in dimethyl sulfoxide, slightly soluble in methylene chloride.

IDENTIFICATION
Carry out either tests A, B or tests A, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *tadalafil CRS.*

B. Liquid chromatography (*2.2.29*) as described in the test for impurities A, B and C with the following modification.

Injection Test solution and reference solution (a).

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

C. Specific optical rotation (*2.2.7*): + 78.0 to + 84.0 (dried substance).

Dissolve 0.250 g in *dimethyl sulfoxide R* and dilute to 25.0 mL with the same solvent.

TESTS

Impurities A, B and C
Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R, hexane R, 2-propanol R* (20:40:40 *V/V/V*).

Solution A Dissolve 27 g of *tetrabutylammonium hydroxide R* in *methanol R* and dilute to 100.0 mL with the same solvent.

Test solution Dissolve 25.0 mg of the substance to be examined in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Reference solution (a) Dissolve 25.0 mg of *tadalafil CRS* in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) In order to prepare impurity A *in situ*, dissolve 25 mg of the substance to be examined in 40 mL of the solvent mixture. Add 1 mL of solution A, mix well and allow to stand for 20 min. Add 1 mL of *trifluoroacetic acid R* and dilute to 100 mL with the solvent mixture.

Reference solution (d) To 1 mL of the test solution add 1 mL of reference solution (c) and dilute to 50 mL with the solvent mixture.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *amylose derivative of silica gel for chiral separation R* (10 µm);
— *temperature*: 30 °C.

Mobile phase *hexane R, 2-propanol R1* (50:50 *V/V*).

Flow rate 0.75 mL/min.

Detection Spectrophotometer at 222 nm.

Injection 20 µL of the test solution and reference solutions (b) and (d).

Run time 2.2 times the retention time of tadalafil.

Identification of impurities Use the chromatogram obtained with reference solution (d) to identify the peak due to impurity A.

Relative retention With reference to tadalafil (retention time = about 11 min): impurity A = about 0.8.

System suitability Reference solution (d)
— *resolution*: minimum 2.0 between the peaks due to impurity A and tadalafil.

Limits:
— *impurity A*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent).

Related substances

Liquid chromatography (*2.2.29*). *Do not use sonication during the preparation of the solutions.*

Solvent mixture *acetonitrile R, 2-propanol R* (50:50 *V/V*).

Solution A Dissolve 27 g of *tetrabutylammonium hydroxide R* in *methanol R* and dilute to 100.0 mL with the same solvent.

Test solution (a) Dissolve 40 mg of the substance to be examined in 50 mL of *acetonitrile R* and dilute to 100.0 mL with mobile phase A.

Test solution (b) Dissolve 50.0 mg of the substance to be examined in 50 mL of *acetonitrile R* and dilute to 100.0 mL with mobile phase A. To 10.0 mL of this solution add 25.0 mL of *acetonitrile R* and dilute to 50.0 mL with mobile phase A.

Reference solution (a) To 1.0 mL of test solution (a) add 50 mL of *acetonitrile R* and dilute to 100.0 mL with mobile phase A. To 1.0 mL of this solution add 5 mL of *acetonitrile R* and dilute to 10.0 mL with mobile phase A.

Reference solution (b) In order to prepare impurity A *in situ*, dissolve 4 mg of the substance to be examined in 50 mL of the solvent mixture. Add 1 mL of solution A, mix, and allow to stand for 40 min. Add 1 mL of *trifluoroacetic acid R* and dilute to 100 mL with the solvent mixture.

Reference solution (c) Dilute 1 mL of reference solution (b) to 50 mL with test solution (a).

Reference solution (d) Dissolve 50.0 mg of *tadalafil CRS* in 50 mL of *acetonitrile R* and dilute to 100.0 mL with mobile phase A. To 10.0 mL of this solution add 25.0 mL of *acetonitrile R* and dilute to 50.0 mL with mobile phase A.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: mix 1.0 mL of *trifluoroacetic acid R* with *water for chromatography R* and dilute to 1000 mL with the same solvent;
— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	85	15
3 - 30	85 → 5	15 → 95
30 - 33	5	95

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 285 nm.

Injection 20 µL of test solution (a) and reference solutions (a) and (c).

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peak due to impurities A + C.

Relative retention With reference to tadalafil (retention time = about 16 min): impurities A and C = about 1.03.

System suitability Reference solution (c):
— *peak-to-valley ratio*: minimum 3.3, where H_p = height above the baseline of the peak due to impurities A + C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to tadalafil.

Limits:
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent); disregard any peak due to impurity A and/or C.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 105 °C for 3 h.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Mobile phase *acetonitrile R*, mobile phase A (45:55 *V/V*).

Flow rate 1.5 mL/min.

Injection Test solution (b) and reference solution (d).

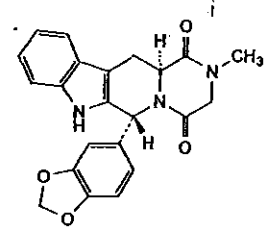
Run time Twice the retention time of tadalafil (retention time = about 4.5 min).

Calculate the percentage content of $C_{22}H_{19}N_3O_4$ from the declared content of *tadalafil CRS*.

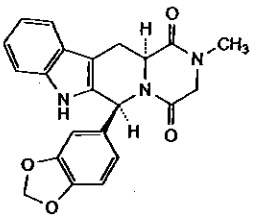
IMPURITIES

Specified impurities *A*.

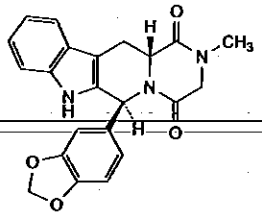
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D, E, F, G, H, I.*



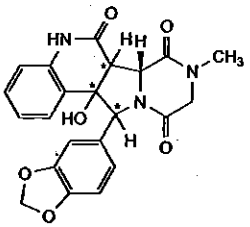
A. (6*R*,12a*S*)-6-(1,3-benzodioxol-5-yl)-2-methyl-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione,



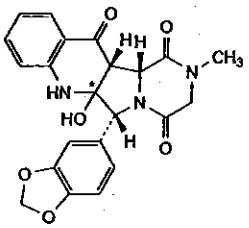
B. (6*S*,12a*S*)-6-(1,3-benzodioxol-5-yl)-2-methyl-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione,



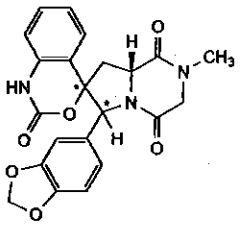
C. (6*S*,12a*R*)-6-(1,3-benzodioxol-5-yl)-2-methyl-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione,



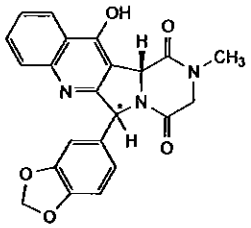
D. (6b*R*)-12-(1,3-benzodioxol-5-yl)-12a-hydroxy-8-methyl-6a,6b,8,9,12,12a-hexahydropyrazino[1′,2′:1,2]pyrrolo[3,4-*c*]quinoline-6,7,10(5*H*)-trione,



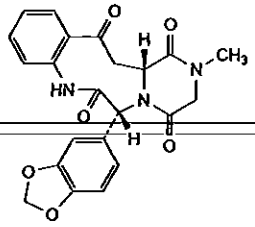
E. (6*R*,12a*R*,12b*R*)-6-(1,3-benzodioxol-5-yl)-6a-hydroxy-2-methyl-2,3,6a,7,12a,12b-hexahydropyrazino[1′,2′:1,5]pyrrolo[3,4-*b*]quinoline-1,4,12(6*H*)-trione,



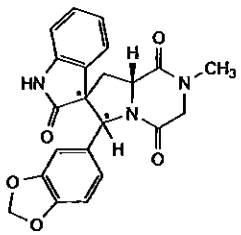
F. (8a′*R*)-6′-(1,3-benzodioxol-5-yl)-2′-methyl-2′,3′,8′,8a′-tetrahydro-6′*H*-spiro[3,1-benzoxazine-4,7′-pyrrolo[1,2-*a*]pyrazine]-1′,2,4′(1*H*)-trione,



G. (12b*R*)-6-(1,3-benzodioxol-5-yl)-12-hydroxy-2-methyl-2,3,6,12b-tetrahydropyrazino[1′,2′:1,5]pyrrolo[3,4-*b*]quinoline-1,4-dione,



H. (6*R*,14a*R*)-6-(1,3-benzodioxol-5-yl)-2-methyl-2,3,14,14a-tetrahydropyrazino[1,2-*d*][1,4]benzodiazonine-1,4,7,13(6*H*,8*H*)-tetrone,



I. (8a′*R*)-6′-(1,3-benzodioxol-5-yl)-2′-methyl-2′,3′,8′,8a′-tetrahydro-6′*H*-spiro[indole-3,7′-pyrrolo[1,2-*a*]pyrazine]-1′,2,4′(1*H*)-trione.

Ph Eur

Purified Talc

(Talc, Ph. Eur. monograph 0438)

14807-96-6

Preparation
Talc Dusting Powder

Ph Eur

DEFINITION
Powdered, selected, natural, hydrated magnesium silicate. Pure talc has the formula $Mg_3Si_4O_{10}(OH)_2$ (M_r 379.3). It may contain variable amounts of associated minerals among which chlorites (hydrated aluminium and magnesium silicates), magnesite (magnesium carbonate), calcite (calcium carbonate) and dolomite (calcium and magnesium carbonate) are predominant.

PRODUCTION
Talc derived from deposits that are known to contain associated asbestos is not suitable for pharmaceutical use. The manufacturer is responsible for demonstrating by the test for amphiboles and serpentines that the product is free from asbestos. The presence of amphiboles and of serpentines is revealed by X-ray diffraction or by infrared spectrophotometry (see A and B). If detected, the specific

morphological criteria of asbestos are investigated by a suitable method of optical microscopy to determine whether tremolite asbestos or chrysotile is present, as described below.

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs of *potassium bromide R*.

In the range 740 cm^{-1} to 760 cm^{-1} using scale expansion, any absorption band at 758 ± 1 cm^{-1} may indicate the presence of tremolite or of chlorite. If the absorption band remains after ignition of the substance to be examined at 850 ± 50 °C for at least 30 min, it indicates the presence of the tremolite. In the range 600 cm^{-1} to 650 cm^{-1} using scale expansion, any absorption band or shoulder may indicate the presence of serpentines.

B. X-ray diffraction.

Preparation Place the sample on the sample holder; pack and smooth its surface with a polished glass microscope slide.

Radiation Cu Kα monochromatic, 40 kV, 24-30 mA.

Incident slit 1°.

Detection slit 0.2°.

Goniometer speed 1/10° 2θ/min.

Scanning range 10-13° 2θ and 24-26° 2θ.

Sample Not oriented.

Results The presence of amphiboles is detected by a diffraction peak at 10.5 ± 0.1° 2θ, the presence of serpentines is detected by diffraction peaks at 24.3 ± 0.1° 2θ and at 12.1 ± 0.1° 2θ.

If, by one of the 2 methods, amphiboles and/or serpentine are detected, examine by a suitable method of optical microscopy to determine the asbestos character.

The presence of asbestos is shown if the following 2 criteria are met:

— a range of length to width ratios of 20:1 to 100:1, or higher for fibres longer than 5 µm;
— capability of splitting into very thin fibrils;

and if at least 2 of the following 4 criteria are met:

— parallel fibres occurring in bundles;
— fibre bundles displaying frayed ends;
— fibres in the form of thin needles;
— matted masses of individual fibres and/or fibres showing curvature.

CHARACTERS

Appearance

Light, homogeneous, white or almost white powder, greasy to the touch (non abrasive).

Solubility

Practically insoluble in water, in ethanol (96 per cent) and in dilute solutions of acids and alkali hydroxides.

IDENTIFICATION

First identification: A.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs of *potassium bromide R*.

Absorption bands At 3677 ± 2 cm^{-1}, 1018 ± 2 cm^{-1} and 669 ± 2 cm^{-1}.

B. In a platinum crucible, melt a mixture of 0.2 g of *anhydrous sodium carbonate R* and 2.0 g of *potassium carbonate R*. To the melted mass add 0.1 g of the substance to be examined and heat until the mixture is completely melted. Allow to cool and transfer the melted mass into an evaporating dish with 50 mL of hot *water R*. Add *hydrochloric acid R* until effervescence ceases. Add 10 mL of *hydrochloric acid R* and evaporate to dryness on a water-bath. Allow to cool. Add 20 mL of *water R*, heat to boiling and filter (the residue is used for identification test C). To 5 mL of the filtrate add 1 mL of *ammonia R* and 1 mL of *ammonium chloride solution R* and filter. To the filtrate add 1 mL of *disodium hydrogen phosphate solution R*. A white, crystalline precipitate is formed.

C. The residue obtained in identification test B gives the reaction of silicates (*2.3.1*).

TESTS

Solution S1

Weigh 10.0 g into a conical flask fitted with a reflux condenser, gradually add 50 mL of *0.5 M hydrochloric acid* while stirring and heat on a water-bath for 30 min. Allow to cool. Transfer the mixture to a beaker and allow the undissolved material to settle. Filter the supernatant through medium-speed filter paper into a 100 mL volumetric flask, retaining as much as possible of the insoluble material in the beaker. Wash the residue and the beaker with 3 quantities, each of 10 mL, of hot *water R*. Wash the filter with 15 mL of hot *water R*, allow the filtrate to cool and dilute to 100.0 mL with the same solvent.

Solution S2

Perchlorates mixed with heavy metals are known to be explosive. Take proper precautions while performing this procedure Weigh 0.5 g in a 100 mL polytetrafluoroethylene dish, add 5 mL of *hydrochloric acid R*, 5 mL of *lead-free nitric acid R* and 5 mL of *perchloric acid R*. Stir gently then add 35 mL of *hydrofluoric acid R* and evaporate slowly to dryness on a hot plate. To the residue, add 5 mL of *hydrochloric acid R*, cover with a watch-glass, heat to boiling and allow to cool. Rinse the watch-glass and the dish with *water R*. Transfer into a volumetric flask, rinse the dish with *water R* and dilute to 50.0 mL with the same solvent.

Acidity or alkalinity

Boil 2.5 g with 50 mL of *carbon dioxide-free water R* under reflux. Filter *in vacuo*. To 10 mL of the filtrate add 0.1 mL of *bromothymol blue solution R1*; not more than 0.4 mL of *0.01 M hydrochloric acid* is required to change the colour of the indicator to green. To 10 mL of the filtrate add 0.1 mL of *phenolphthalein solution R1*; not more than 0.3 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink.

Water-soluble substances

Maximum 0.2 per cent.

To 10.0 g add 50 mL of *carbon dioxide-free water R*, heat to boiling and maintain boiling under a reflux condenser for 30 min. Allow to cool, filter through a medium-speed filter paper and dilute to 50.0 mL with *carbon dioxide-free water R*. Take 25.0 mL of the filtrate, evaporate to dryness and heat at 105 °C for 1 h. The residue weighs a maximum of 10 mg.

Aluminium

Maximum 2.0 per cent.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution To 5.0 mL of solution S2 add 10 mL of a 25.34 g/L solution of *caesium chloride R*, 10.0 mL of *hydrochloric acid R* and dilute to 100.0 mL with *water R*.

Reference solutions Into 4 identical volumetric flasks, each containing 10.0 mL of *hydrochloric acid R* and 10 mL of a 25.34 g/L solution of *caesium chloride R*, introduce respectively 5.0 mL, 10.0 mL, 15.0 mL and 20.0 mL of *aluminium standard solution (100 ppm Al) R* and dilute to 100.0 mL with *water R*.

Source Aluminium hollow-cathode lamp.

Wavelength 309.3 nm.

Atomisation device Nitrous oxide-acetylene flame.

Calcium

Maximum 0.9 per cent.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution To 5.0 mL of solution S2 add 10.0 mL of *hydrochloric acid R*, 10 mL of *lanthanum chloride solution R* and dilute to 100.0 mL with *water R*.

Reference solutions Into 4 identical volumetric flasks, each containing 10.0 mL of *hydrochloric acid R* and 10 mL of *lanthanum chloride solution R*, introduce respectively 1.0 mL, 2.0 mL, 3.0 mL and 5.0 mL of *calcium standard solution (100 ppm Ca) R1* and dilute to 100.0 mL with *water R*.

Source Calcium hollow-cathode lamp.

Wavelength 422.7 nm.

Atomisation device Nitrous oxide-acetylene flame.

Iron

Maximum 0.25 per cent.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution To 2.5 mL of solution S1, add 50.0 mL of *0.5 M hydrochloric acid* and dilute to 100.0 mL with *water R*.

Reference solutions Into 4 identical volumetric flasks, each containing 50.0 mL of *0.5 M hydrochloric acid*, introduce respectively 2.0 mL, 2.5 mL, 3.0 mL and 4.0 mL of *iron standard solution (250 ppm Fe) R* and dilute to 100.0 mL with *water R*.

Source Iron hollow-cathode lamp.

Wavelength 248.3 nm.

Atomisation device Air-acetylene flame.

Correction Deuterium lamp.

Lead

Maximum 10 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Use solution S1.

Reference solutions Into 4 identical volumetric flasks, each containing 50.0 mL of *0.5 M hydrochloric acid*, introduce respectively 5.0 mL, 7.5 mL, 10.0 mL and 12.5 mL of *lead standard solution (10 ppm Pb) R1* and dilute to 100.0 mL with *water R*.

Source Lead hollow-cathode lamp.

Wavelength 217.0 nm.

Atomisation device Air-acetylene flame.

Magnesium

17.0 per cent to 19.5 per cent.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Dilute 0.5 mL of solution S2 to 100.0 mL with *water R*. To 4.0 mL of the solution, add 10.0 mL of *hydrochloric acid R*, 10 mL of *lanthanum chloride solution R* and dilute to 100.0 mL with *water R*.

Reference solutions Into 4 identical volumetric flasks, each containing 10.0 mL of *hydrochloric acid R* and 10 mL of *lanthanum chloride solution R*, introduce respectively 2.5 mL, 3.0 mL, 4.0 mL and 5.0 mL of *magnesium standard solution (10 ppm Mg) R1* and dilute to 100.0 mL with *water R*.

Source Magnesium hollow-cathode lamp.

Wavelength 285.2 nm.

Atomisation device Air-acetylene flame.

Loss on ignition

Maximum 7.0 per cent, determined on 1.00 g by ignition to constant weight at 1050-1100 °C.

Microbial contamination

If intended for cutaneous administration:
— TAMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

If intended for oral administration:
— TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*);
— TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

LABELLING

The label states, where applicable, that the substance is suitable for oral or cutaneous administration.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for talc used as antisticking agent or glidant in tablets and capsules or as antiadhesive in coated and film-coated tablets.

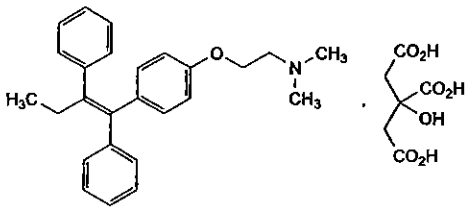
Particle-size distribution (*2.9.31*)

Specific surface area (*2.9.26*)

Ph Eur

Tamoxifen Citrate

(Ph. Eur. monograph 1046)



$C_{32}H_{37}NO_8$ 563.6 *54965-24-1*

Action and use

Selective estrogen receptor modulator.

Preparations

Tamoxifen Oral Solution

Tamoxifen Tablets

Ph Eur

DEFINITION

2-[4-[(*Z*)-1,2-Diphenylbut-1-en-1-yl]phenoxy]-*N*,*N*-dimethylethan-1-amine dihydrogen 2-hydroxypropane-1,2,3-tricarboxylate.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Slightly soluble in water, soluble in methanol, slightly soluble in acetone.

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification: B.

Second identification: A, C.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 20 mg in *methanol R* and dilute to 50.0 mL with the same solvent. Dilute 5.0 mL of the solution to 100.0 mL with *methanol R*.

Spectral range 220-350 nm.

Absorption maxima At 237 nm and 275 nm.

Absorbance ratio A_{237}/A_{275} = 1.45 to 1.65.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *tamoxifen citrate CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *acetone R*, evaporate to dryness and record new spectra using the residues.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dissolve 10 mg of *tamoxifen citrate CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 10 mg of *clomifene citrate CRS* and 10 mg of *tamoxifen citrate CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *triethylamine R, toluene R* (10:90 *V/V*).

Application 5 µL.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use and protect from light.*

Test solution Dissolve 15 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dissolve 3 mg of *tamoxifen citrate for performance test CRS* (containing impurities A and F) in the mobile phase and dilute to 2.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 40 volumes of *acetonitrile R* and 60 volumes of *water R* containing 0.9 g/L of *sodium dihydrogen phosphate R* and 4.8 g/L of *N,N-dimethyloctylamine R*; adjust to pH 3.0 with *phosphoric acid R*.

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 240 nm.

Injection 10 µL.

Run time Twice the retention time of tamoxifen.

Identification of impurities Use the chromatogram supplied with *tamoxifen citrate for performance test CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A and F.

Relative retention With reference to tamoxifen (retention time = about 20 min): impurity A = about 0.8; impurity F = about 0.9.

System suitability Reference solution (a):

— *resolution*: minimum 3.0 between the peaks due to impurities A and F; baseline separation between the peaks due to impurity F and tamoxifen.

Limits:

— *impurity A*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *impurity F*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak due to the citrate.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 65 °C for 4 h.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

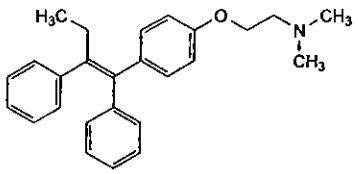
Dissolve 0.400 g in 75 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 56.36 mg of $C_{32}H_{37}NO_8$.

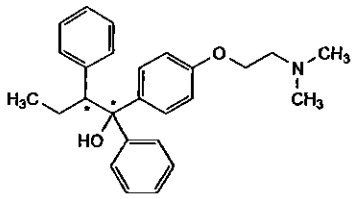
IMPURITIES

Specified impurities *A, F.*

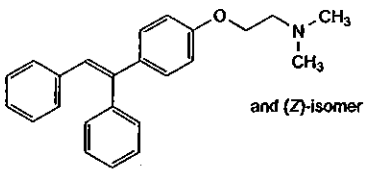
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D, E, G, H.*



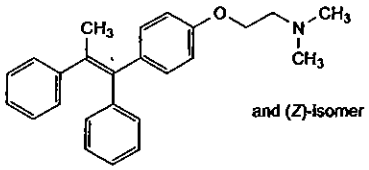
A. 2-[4-[(*E*)-1,2-diphenylbut-1-en-1-yl]phenoxy]-*N*,*N*-dimethylethan-1-amine ((*E*)-isomer),



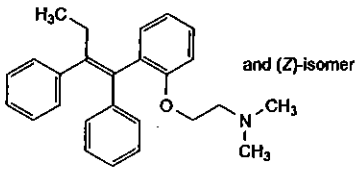
B. 1-[4-[2-(dimethylamino)ethoxy]phenyl]-1,2-diphenylbutan-1-ol,



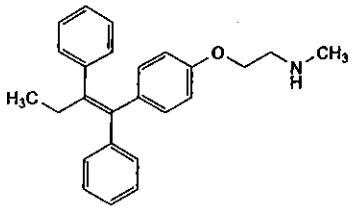
C. 2-[4-[(*EZ*)-1,2-diphenyleth-1-en-1-yl]phenoxy]-*N*,*N*-dimethylethan-1-amine,



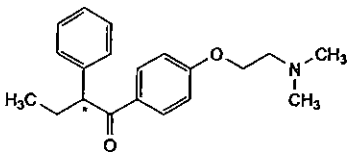
D. 2-[4-[(*EZ*)-1,2-diphenylprop-1-en-1-yl]phenoxy]-*N*,*N*-dimethylethan-1-amine,



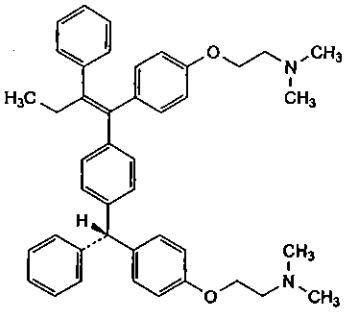
E. 2-[2-[(*EZ*)-1,2-diphenylbut-1-en-1-yl]phenoxy]-*N*,*N*-dimethylethan-1-amine,



F. 2-[4-[(*Z*)-1,2-diphenylbut-1-en-1-yl]phenoxy]-*N*-methylethan-1-amine,



G. 1-[4-[2-(dimethylamino)ethoxy]phenyl]-2-phenylbutan-1-one,

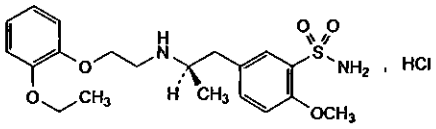


H. 2-[4-[(*RS*)-[4-[(*Z*)-1-[4-[2-(dimethylamino)ethoxy]phenyl]-2-phenylbut-1-en-1-yl]phenyl](phenyl)methyl]phenoxy]-*N*,*N*-dimethylethan-1-amine.

Ph Eur

Tamsulosin Hydrochloride

(Ph. Eur. monograph 2131)



$C_{20}H_{29}ClN_2O_5S$ 445.0 *106463-17-6*

Action and use
Alpha-1-adrenoceptor antagonist.

Preparations
Tamsulosin Prolonged-release Capsules
Tamsulosin Prolonged-release Tablets

Ph Eur

DEFINITION
5-[(2*R*)-2-[[2-(2-Ethoxyphenoxy)ethyl]amino]propyl]-2-methoxybenzenesulfonamide hydrochloride.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Slightly soluble in water, freely soluble in formic acid, slightly soluble in anhydrous ethanol.

mp
About 230 °C.

IDENTIFICATION
Carry out either tests A, C, D or tests A, B, D.

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison tamsulosin hydrochloride CRS.

B. Specific optical rotation (*2.2.7*): −20.5 to −17.5 (dried substance).
Dissolve with heating 0.15 g in *water R* and dilute to 20.0 mL with the same solvent.

C. Enantiomeric purity (see Tests).

D. Dissolve with heating 0.75 g in *water R* and dilute to 100.0 mL with the same solvent. Take 5 mL of the solution and cool in an ice-bath. Add 3 mL of *dilute nitric acid R* and

shake. Allow to stand at room temperature for 30 min and filter. The filtrate gives reaction (a) of chlorides (*2.3.1*).

TESTS

Related substances

A. Impurities eluting before tamsulosin. Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 4 mg of *tamsulosin impurity D CRS* and 4 mg of the substance to be examined in the mobile phase and dilute to 20 mL with the mobile phase. Dilute 2 mL of the solution to 20 mL with the mobile phase.

Reference solution (c) Dissolve 4 mg of *tamsulosin impurity H CRS* and 4 mg of the substance to be examined in the mobile phase and dilute to 20 mL with the mobile phase. Dilute 2 mL of the solution to 20 mL with the mobile phase.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase Dissolve 3.0 g of *sodium hydroxide R* in a mixture of 8.7 mL of *perchloric acid R* and 1900 mL of *water for chromatography R*; adjust to pH 2.0 with *0.5 M sodium hydroxide* and dilute to 2000 mL with *water for chromatography R*; to 1400 mL of this solution add 600 mL of *acetonitrile for chromatography R*.

Flow rate 1.3 mL/min.

Detection Spectrophotometer at 225 nm.

Injection 10 µL of the test solution and reference solutions (a) and (b).

Run time 1.5 times the retention time of tamsulosin (retention time = about 6 min).

System suitability Reference solution (b):
- *resolution*: minimum 6.0 between the peaks due to impurity D and tamsulosin.

Limits:
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

B. Impurities eluting after tamsulosin. Liquid chromatography (*2.2.29*) as described in test A with the following modifications.

Mobile phase Dissolve 3.0 g of *sodium hydroxide R* in a mixture of 8.7 mL of *perchloric acid R* and 1900 mL of *water for chromatography R*; adjust to pH 2.0 with *0.5 M sodium hydroxide* and dilute to 2000 mL with *water for chromatography R*; add 2000 mL of *acetonitrile for chromatography R*.

Flow rate 1.0 mL/min.

Injection 10 µL of the test solution and reference solutions (a) and (c).

Run time 5 times the retention time of tamsulosin (retention time = about 2.5 min).

System suitability Reference solution (c):
- *resolution*: minimum 2.0 between the peaks due to tamsulosin and impurity H.

Limits:
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *sum of impurities eluting before tamsulosin in test A and after tamsulosin in test B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Enantiomeric purity

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in *methanol R* and dilute to 25.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *methanol R*. Dilute 1.0 mL of this solution to 10.0 mL with *methanol R*.

Reference solution (b) Dissolve 5 mg of *tamsulosin racemate CRS* in *methanol R* and dilute to 25 mL with the same solvent. Dilute 2 mL of the solution to 10 mL with *methanol R*.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *amylose derivative of silica gel for chiral separation R*;
- *temperature*: 40 °C.

Mobile phase *diethylamine R, methanol R1, anhydrous ethanol R, hexane R* (1:150:200:650 *V/V/V/V*).

Flow rate 0.5 mL/min.

Detection Spectrophotometer at 225 nm.

Injection 10 µL.

Relative retention With reference to tamsulosin (retention time = about 14 min): impurity G = about 0.8.

System suitability Reference solution (b):
- *resolution*: minimum 2.0 between the peaks due to impurity G and tamsulosin.

Limit:
- *impurity G*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.350 g in 5.0 mL of *anhydrous formic acid R*, add 75 mL of a mixture of 2 volumes of *acetic anhydride R* and 3 volumes of *glacial acetic acid R*. Titrate immediately with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

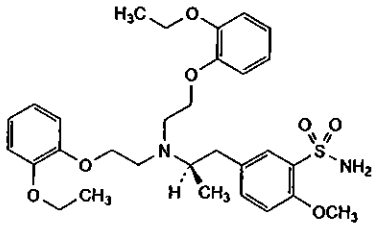
1 mL of *0.1 M perchloric acid* is equivalent to 44.50 mg of $C_{20}H_{29}ClN_2O_5S$.

IMPURITIES

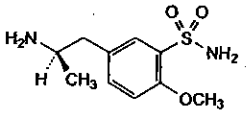
Specified impurities *G*.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests

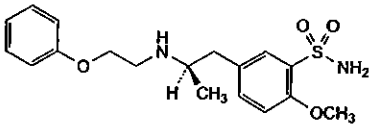
in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, E, F, H, I.*



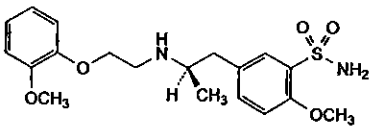
A. 5-[(2*R*)-2-[bis[2-(2-ethoxyphenoxy)ethyl]amino]propyl]-2-methoxybenzenesulfonamide,



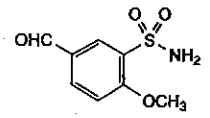
B. 5-[(2*R*)-2-aminopropyl]-2-methoxybenzenesulfonamide,



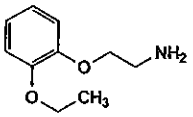
C. 2-methoxy-5-[(2*R*)-2-[(2-phenoxyethyl)amino] propyl]benzenesulfonamide,



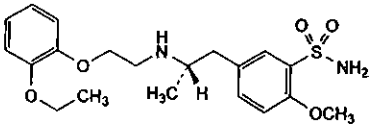
D. 2-methoxy-5-[(2*R*)-2-[[2-(2-methoxyphenoxy) ethyl]amino]propyl]benzenesulfonamide,



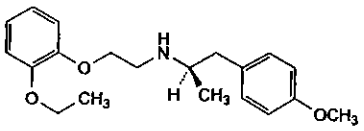
E. 5-formyl-2-methoxybenzenesulfonamide,



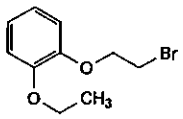
F. 2-(2-ethoxyphenoxy)ethanamine,



G. 5-[(2*S*)-2-[[2-(2-ethoxyphenoxy)ethyl]amino]propyl]-2-methoxybenzenesulfonamide,



H. (2*R*)-*N*-[2-(2-ethoxyphenoxy)ethyl]-1-(4-methoxyphenyl)propan-2-amine,



I. 1-(2-bromoethoxy)-2-ethoxybenzene.

Ph Eur

Tannic Acid

(Ph. Eur. monograph 1477)

Action and use
Astringent.

Ph Eur

DEFINITION
Mixture of esters of glucose with gallic acid and 3-galloylgallic acid.

CHARACTERS
Appearance
Yellowish-white or slightly brown amorphous light powder or shiny plates.

Solubility
Very soluble in water, freely soluble in acetone, in ethanol (96 per cent) and in glycerol (85 per cent), practically insoluble in methylene chloride.

IDENTIFICATION
A. Dilute 0.1 mL of solution S (see Tests) to 5 mL with *water R*. Add 0.1 mL of *ferric chloride solution R1*. A blackish-blue colour is produced which becomes green on the addition of 1 mL of *dilute sulfuric acid R*.

B. To 1 mL of solution S, add 3 mL of a 1 g/L solution of *gelatin R*. The mixture becomes turbid and a flocculent precipitate is formed.

C. Dilute 0.1 mL of solution S to 5 mL with *water R*. Add 0.3 mL of *barium hydroxide solution R*. A greenish-blue precipitate is formed.

TESTS
Solution S
Dissolve 4.0 g in *carbon dioxide-free water R* and dilute to 20 mL with the same solvent.

Appearance of solution
Solution S is not more opalescent than reference suspension II (*2.2.1*).

Dextrins, gum, salts, sugars
To 2 mL of solution S, add 2 mL of *ethanol (96 per cent) R*. The solution is clear. Add 1 mL of *ether R*. The solution remains clear for at least 10 min.

Resins
To 5 mL of solution S, add 5 mL of *water R*. The mixture remains clear (*2.2.1*) for at least 15 min.

Loss on drying (*2.2.32*)
Maximum 12.0 per cent, determined on 0.200 g by drying at 105 °C.

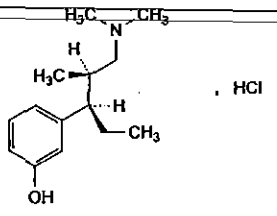
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

STORAGE
Protected from light.

Ph Eur

Tapentadol Hydrochloride

(Ph. Eur. monograph 3035)



$C_{14}H_{24}ClNO$ 257.8 *175591-09-0*

Action and use
μ-Opioid receptor (OP3, MOR) agonist and noradrenaline reuptake inhibitor; analgesic.

Ph Eur

DEFINITION
3-[(2*R*,3*R*)-1-(Dimethylamino)-2-methylpentan-3-yl]phenol hydrochloride.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

PRODUCTION
It is considered that alkyl methanesulfonate esters are genotoxic and are potential impurities in tapentadol hydrochloride. The manufacturing process should be developed taking into consideration the principles of quality risk management, together with considerations of the quality of starting materials, process capability and validation.
The general methods *2.5.37. Methyl, ethyl and isopropyl methanesulfonate in methanesulfonic acid, 2.5.38. Methyl, ethyl and isopropyl methanesulfonate in active substances* and *2.5.39. Methanesulfonyl chloride in methanesulfonic acid* are available to assist manufacturers.

CHARACTERS
Appearance
White or almost white powder.

Solubility
Freely soluble in water and in methanol, soluble in anhydrous ethanol, very slightly soluble in heptane.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *tapentadol hydrochloride CRS.*
B. It gives reaction (a) of chlorides (*2.3.1*).
C. Enantiomeric purity (see Tests).

TESTS
Enantiomeric purity
Liquid chromatography (*2.2.29*).
Solvent mixture *diethylamine R, 2-propanol R, heptane R* (0.2:10:89.8 *V/V/V*).
Test solution Dissolve 10.0 mg of the substance to be examined in a mixture of 40 μL of *diethylamine R* and 2 mL of *2-propanol R* using sonication and dilute to 20.0 mL with *heptane R*.
Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.
Reference solution (b) Dissolve 5 mg of *tapentadol impurity A CRS* and 5 mg of the substance to be examined in a mixture of 0.2 mL of *diethylamine R* and 10 mL of *2-propanol R* using sonication and dilute to 100 mL with *heptane R*.
Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: amylose derivative of silica gel for chiral separation R* (5 μm).
Mobile phase *diethylamine R, 2-propanol R, heptane R* (0.1:2:98 *V/V/V*).
Flow rate 1 mL/min.
Detection Spectrophotometer at 270 nm.
Injection 20 μL.
Run time 1.5 times the retention time of tapentadol.
Relative retention With reference to tapentadol (retention time = about 13 min): impurity A = about 1.2.
System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the peaks due to tapentadol and impurity A.
Limit:
— *impurity A*: maximum 1.0 per cent, calculated as the ratio of the area of the peak due to impurity A to the sum of the areas of the peaks due to tapentadol and impurity A;
— *reporting threshold*: 0.10 per cent (reference solution (a)).

Related substances
Liquid chromatography (*2.2.29*).
Solvent mixture *phosphoric acid R, methanol R, water R* (0.1:20:80 *V/V/V*).
Test solution (a) Dissolve 30.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.
Test solution (b) Dilute 5.0 mL of test solution (a) to 50.0 mL with the solvent mixture.
Reference solution (a) Dissolve 30.0 mg of *tapentadol hydrochloride CRS* in the solvent mixture and dilute to 50.0 mL with the solvent mixture. Dilute 5.0 mL of the solution to 50.0 mL with the solvent mixture.
Reference solution (b) Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.
Reference solution (c) Dissolve 3 mg of *tapentadol impurity C CRS* in the solvent mixture and dilute to 5 mL with the solvent mixture. Add 0.5 mL of the solution to 30 mg of the substance to be examined and dilute to 50 mL with the solvent mixture.
Column:
— *size: l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 μm);
— *temperature*: 35 °C.

Mobile phase:
— *mobile phase A: phosphoric acid R, methanol R2, water for chromatography R* (0.1:10:90 *V/V/V*);
— *mobile phase B: phosphoric acid R, water for chromatography R, methanol R2* (0.1:10:90 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	100	0
2 - 44	100 → 61	0 → 39
44 - 44.5	61 → 0	39 → 100

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µL of test solution (a) and reference solutions (b) and (c).

Relative retention With reference to tapentadol (retention time = about 15 min): impurity C = about 0.9.

System suitability Reference solution (c):
— *resolution*: minimum 1.5 between the peaks due to impurity C and tapentadol.

Calculation of percentage contents:
— for each impurity, use the concentration of tapentadol hydrochloride in reference solution (b).

Limits:
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.4 per cent;
— *reporting threshold*: 0.05 per cent.

Water (*2.5.12*)
Maximum 0.5 per cent, determined on 0.250 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

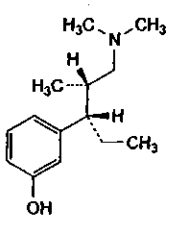
Injection Test solution (b) and reference solution (a).

Calculate the percentage content of $C_{14}H_{24}ClNO$ taking into account the assigned content of *tapentadol hydrochloride CRS*.

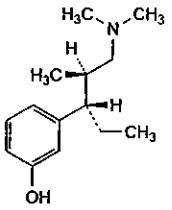
STORAGE
Protected from light.

IMPURITIES
Specified impurities *A.*

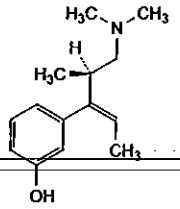
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D, E.*



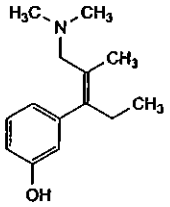
A. 3-[(2*S*,3*S*)-1-(dimethylamino)-2-methylpentan-3-yl] phenol,



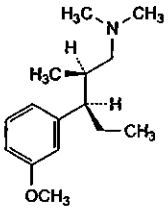
B. 3-[(2*R*,3*S*)-1-(dimethylamino)-2-methylpentan-3-yl] phenol,



C. 3-[(2*Z*,4*R*)-5-(dimethylamino)-4-methylpent-2-en-3-yl] phenol,



D. 3-[(2*Z*)-1-(dimethylamino)-2-methylpent-2-en-3-yl] phenol,



E. (2*R*,3*R*)-3-(3-methoxyphenyl)-*N*,*N*,2-trimethylpentan-1-amine.

Ph Eur

Tar

Action and use
Used in treatment of psoriasis.

DEFINITION
Tar is bituminous liquid obtained from the wood of various trees of the family Pinaceae by destructive distillation and is known in commerce as Stockholm Tar.

CHARACTERISTICS
Dark brown or nearly black, semi-liquid; denser than water.
Soluble in *ethanol (90%)*, in *ether* and in fixed and volatile oils.

IDENTIFICATION
A. The aqueous liquid obtained by shaking 1 g with 20 mL of *water* for 5 minutes is acidic to *litmus paper*.

B. Carefully add 0.5 g to 10 mL of *petroleum spirit (boiling range, 40° to 60°)* and allow to stand for 30 minutes. When examined in daylight no fluorescence is produced.

Coal Tar

Action and use
Used in treatment of psoriasis.

Preparations
Betamethasone Valerate and Coal Tar Paste
Coal Tar and Salicylic Acid Ointment
Coal Tar Solution
Strong Coal Tar Solution
Zinc and Coal Tar Paste

DEFINITION
Coal Tar is a product obtained from bituminous coal by destructive distillation at about 1000°.

CHARACTERISTICS
A nearly black, viscous liquid. On exposure to air, the viscosity gradually increases. It burns in air with a luminous, sooty flame. It has a weight per mL of about 1.15 g.

Slightly soluble in *water*; partly soluble in *ethanol*, in *ether* and in volatile oils.

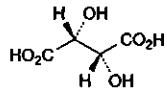
IDENTIFICATION
A. A saturated solution is alkaline to *litmus solution*.

B. Carefully add 0.5 g to 10 mL of *petroleum spirit (boiling range, 40° to 60°)* and allow to stand for 30 minutes. When examined in daylight, the supernatant liquid has a blue fluorescence which becomes more intense when viewed under *ultraviolet light (365 nm)*.

TESTS
Ash
Not more than 2.0%, Appendix XI J.

Tartaric Acid

(Ph. Eur. monograph 0460)



$C_4H_6O_6$ 150.1 *87-69-4*

Ph Eur

DEFINITION
(2*R*,3*R*)-2,3-Dihydroxybutanedioic acid.

The substance is of natural origin, obtained by extraction of lees during winemaking.

Content
99.5 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder or colourless crystals.

Solubility
Very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION
A. Solution S (see Tests) is strongly acid (*2.2.4*).

B. It gives the reactions of tartrates (*2.3.1*).

TESTS
Solution S
Dissolve 5.0 g in *distilled water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*)
+ 12.0 to + 12.8 (dried substance).

Dissolve 5.00 g in *water R* and dilute to 25.0 mL with the same solvent.

Oxalic acid
Maximum 360 ppm, calculated as anhydrous oxalic acid.

Dissolve 0.80 g in 4 mL of *water R*. Add 3 mL of *hydrochloric acid R* and 1 g of *zinc R* in granules and boil for 1 min. Allow to stand for 2 min. Collect the liquid in a test-tube containing 0.25 mL of a 10 g/L solution of *phenylhydrazine hydrochloride R* and heat to boiling. Cool rapidly, transfer to a graduated cylinder and add an equal volume of *hydrochloric acid R* and 0.25 mL of a 50 g/L solution of *potassium ferricyanide R*. Shake and allow to stand for 30 min. Any pink colour in the solution is not more intense than that in a standard prepared at the same time in the same manner using 4 mL of a 0.1 g/L solution of *oxalic acid R*.

Chlorides (*2.4.4*)
Maximum 100 ppm.

Dilute 5 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 150 ppm.

Dilute 10 mL of solution S to 15 mL with *distilled water R*.

Calcium (*2.4.3*)
Maximum 200 ppm.

To 5 mL of solution S add 10 mL of a 50 g/L solution of *sodium acetate R* in *distilled water R*.

Loss on drying (*2.2.32*)
Maximum 0.2 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

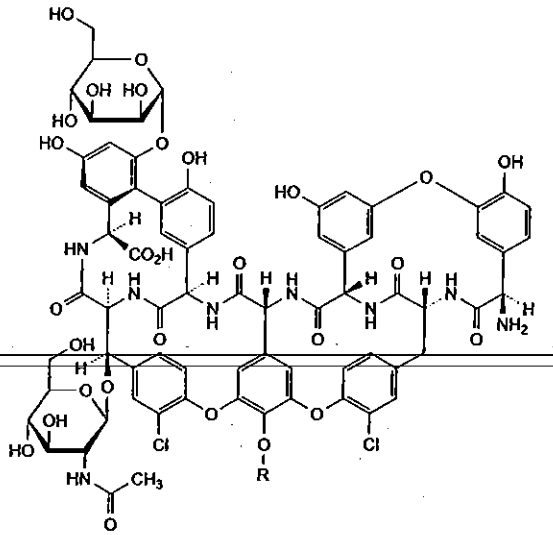
ASSAY
Dissolve 0.650 g in 25 mL of *water R*. Titrate with *1 M sodium hydroxide* using 0.5 mL of *phenolphthalein solution R* as indicator, until a pink colour is obtained.

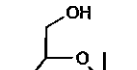
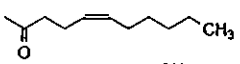
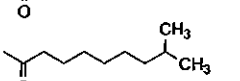
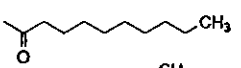
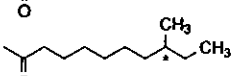
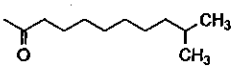
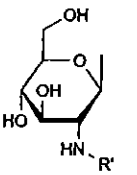
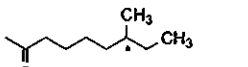
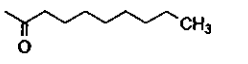
1 mL of *1 M sodium hydroxide* is equivalent to 75.05 mg of $C_4H_6O_6$.

Ph Eur

Teicoplanin

(Ph. Eur. monograph 2358)



teicoplanin	R	R'
$A_{2\text{-}1}$ $C_{88}H_{95}Cl_2N_9O_{33}$ M_r 1878		
$A_{2\text{-}2}$ $C_{88}H_{97}Cl_2N_9O_{33}$ M_r 1880		
$A_{2\text{-}3}$ $C_{88}H_{97}Cl_2N_9O_{33}$ M_r 1880		
$A_{2\text{-}4}$ $C_{89}H_{99}Cl_2N_9O_{33}$ M_r 1894		
$A_{2\text{-}5}$ $C_{89}H_{99}Cl_2N_9O_{33}$ M_r 1894		
$A_{3\text{-}1}$ $C_{72}H_{68}Cl_2N_8O_{28}$ M_r 1564	H	
$A_{2\text{-}1a}$ $C_{87}H_{95}Cl_2N_9O_{33}$ M_r 1866		
$A_{2\text{-}1b}$ $C_{87}H_{95}Cl_2N_9O_{33}$ M_r 1866		

61036-62-2

Action and use
Glycopeptide antibacterial.

Ph Eur

DEFINITION

Mixture of glycopeptides produced by certain strains of *Actinoplanes teichomyceticus sp.*; the 6 principal components of the mixture are teicoplanins $A_{2\text{-}1}$ to $A_{2\text{-}5}$ and teicoplanin $A_{3\text{-}1}$, and 2 minor components are teicoplanins $A_{2\text{-}1a}$ and $A_{2\text{-}1b}$.

Fermentation product.

Potency
Minimum 900 IU/mg (anhydrous and sodium chloride-free substance).

CHARACTERS

Appearance
White or yellowish, amorphous powder.

Solubility
Freely soluble in water, sparingly soluble in dimethylformamide, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *teicoplanin for identification CRS.*

B. Examine the chromatograms obtained in the test for composition.

Results The principal peaks (teicoplanins $A_{3\text{-}1}$, $A_{2\text{-}1}$, $A_{2\text{-}2}$, $A_{2\text{-}3}$, $A_{2\text{-}4}$ and $A_{2\text{-}5}$) and the 2 minor peaks (teicoplanins $A_{2\text{-}1a}$ and $A_{2\text{-}1b}$) in the chromatogram obtained with the test solution are similar in retention time and size to the principal peaks in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_3 or B_4 (*2.2.2, Method I*).

Dissolve 0.8 g in 10 mL of *water R*.

pH (*2.2.3*)
6.5 to 7.5.

Dissolve 0.50 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Composition
Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution Dissolve 0.100 g of the substance to be examined in *water R* and dilute to 50.0 mL with the same solvent.

Reference solution (a) Dissolve 20 mg of *teicoplanin for identification CRS* in *water R* and dilute to 10.0 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 10.0 mL with *water R*. Dilute 1.0 mL of this solution to 20.0 mL with *water R*.

Reference solution (c) Dissolve 50.0 mg of *mesityl oxide CRS* (impurity A) in *water R* and dilute to 25.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with *water R*. Dilute 1.0 mL of this solution to 100.0 mL with *water R*.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: mix 900 mL of a 3.0 g/L solution of *anhydrous sodium dihydrogen phosphate R*, adjusted to pH 6.0 with a 40 g/L solution of *sodium hydroxide R*, and 100 mL of *acetonitrile R*;
— *mobile phase B*: mix 300 mL of a 3.0 g/L solution of *anhydrous sodium dihydrogen phosphate R*, adjusted to pH 6.0 with a 40 g/L solution of *sodium hydroxide R*, and 700 mL of *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	100 → 50	0 → 50
30 - 31	50 → 10	50 → 90
31 - 35	10	90

Flow rate 2.3 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL of the test solution and reference solutions (a) and (b).

Identification Use the chromatogram supplied with *teicoplanin for identification CRS* and the chromatogram obtained with reference solution (a) to identify the groups and components.

Relative retention With reference to teicoplanin A_{2-2} (retention time = about 18 min):
- teicoplanin A_3 group ≤ 0.70:
  - teicoplanin A_{3-1} = about 0.43.
- teicoplanin A_2 group > 0.70 including:
  - teicoplanin A_{2-1} group > 0.70 and < 1.00:
  - teicoplanin A_{2-1a} = about 0.85;
  - teicoplanin A_{2-1b} = about 0.88;
  - teicoplanin A_{2-1} = about 0.93;
  - teicoplanin A_{2-2} = 1.00;
  - teicoplanin A_{2-3} group > 1.00 and < 1.12:
  - teicoplanin A_{2-3} = about 1.03;
  - teicoplanin A_{2-4} = about 1.12;
  - teicoplanin A_{2-5} group > 1.12 and < 1.25:
  - teicoplanin A_{2-5} = about 1.15;
  - teicoplanin A_{2-6} group ≥ 1.25:
  - teicoplanin-like related substance RS A_{2-6a} = about 1.25;
  - teicoplanin-like related substance RS A_{2-6b} = about 1.30;
  - teicoplanin-like related substance RS A_{2-6c} = about 1.38.

System suitability:
- the chromatogram obtained with reference solution (a) is similar to the chromatogram supplied with *teicoplanin for identification CRS*;
- *resolution*: minimum 1.0 between the peaks due to teicoplanin A_{2-4} and teicoplanin A_{2-5} in the chromatogram obtained with reference solution (a);
- *signal-to-noise ratio*: minimum 40 for the peak due to teicoplanin A_{2-2} in the chromatogram obtained with reference solution (b).

Calculate the percentage contents using the following equations:

$$\text{teicoplanin } A_3 \text{ group} = \frac{0.83 \times S_3}{S_2 + 0.83 \times S_3} \times 100$$

$$\text{teicoplanin } A_2 \text{ group} = \frac{S_2}{S_2 + 0.83 \times S_3} \times 100$$

$$\text{teicoplanin } A_{2\text{-}1} \text{ group} = \frac{S_{2-1}}{S_2 + 0.83 \times S_3} \times 100$$

$$\text{teicoplanin } A_{2\text{-}1a} = \frac{A_{2-1a}}{S_2 + 0.83 \times S_3} \times 100$$

$$\text{teicoplanin } A_{2\text{-}1b} = \frac{A_{2-1b}}{S_2 + 0.83 \times S_3} \times 100$$

$$\text{teicoplanin } A_{2\text{-}1} = \frac{A_{2-1}}{S_2 + 0.83 \times S_3} \times 100$$

$$\text{teicoplanin } A_{2\text{-}2} = \frac{A_{2-2}}{S_2 + 0.83 \times S_3} \times 100$$

$$\text{teicoplanin } A_{2\text{-}3} \text{ group} = \frac{S_{2-3}}{S_2 + 0.83 \times S_3} \times 100$$

$$\text{teicoplanin } A_{2\text{-}3} = \frac{A_{2-3}}{S_2 + 0.83 \times S_3} \times 100$$

$$\text{teicoplanin } A_{2\text{-}4} = \frac{A_{2-4}}{S_2 + 0.83 \times S_3} \times 100$$

$$\text{teicoplanin } A_{2\text{-}5} \text{ group} = \frac{S_{2-5}}{S_2 + 0.83 \times S_3} \times 100$$

$$\text{teicoplanin } A_{2\text{-}5} = \frac{A_{2-5}}{S_2 + 0.83 \times S_3} \times 100$$

$$\text{teicoplanin } A_{2\text{-}6} \text{ group} = \frac{S_{2-6}}{S_2 + 0.83 \times S_3} \times 100$$

S_2 = sum of the areas of the peaks due to teicoplanin A_2 group in the chromatogram obtained with the test solution;

S_3 = sum of the areas of the peaks due to teicoplanin A_3 group in the chromatogram obtained with the test solution; disregard any peak due to impurity A;

S_{2-6} = sum of the areas of the peaks with a relative retention greater than or equal to 1.25 in the chromatogram obtained with the test solution;

S_{2-1} = sum of the areas of the peaks due to teicoplanin A_{2-1} group in the chromatogram obtained with the test solution;

A_{2-1a} = area of the peak due to teicoplanin A_{2-1a} in the chromatogram obtained with the test solution;

A_{2-1b} = area of the peak due to teicoplanin A_{2-1b} in the chromatogram obtained with the test solution;

A_{2-1} = area of the peak due to teicoplanin A_{2-1} in the chromatogram obtained with the test solution;

A_{2-2} = area of the peak due to teicoplanin A_{2-2} in the chromatogram obtained with the test solution;

S_{2-3} = sum of the areas of the peaks due to teicoplanin A_{2-3} group in the chromatogram obtained with the test solution;

A_{2-3} = area of the peak due to teicoplanin A_{2-3} in the chromatogram obtained with the test solution;

A_{2-4} = area of the peak due to teicoplanin A_{2-4} in the chromatogram obtained with the test solution;

S_{2-5} = sum of the areas of the peaks due to teicoplanin A_{2-5} group in the chromatogram obtained with the test solution;

A_{2-5} = area of the peak due to teicoplanin A_{2-5} in the chromatogram obtained with the test solution;

Limits:
- *teicoplanin A_2 group*: 84.0 per cent to 98.0 per cent;
- *teicoplanin A_{2-2}*: 37.0 per cent to 50.0 per cent;
- *teicoplanin A_{2-1} group*: 10.0 per cent to 19.0 per cent;
- *teicoplanin A_{2-5} group*: 7.0 per cent to 17.0 per cent;
- *teicoplanin A_{2-4}*: 7.0 per cent to 15.0 per cent;
- *teicoplanin A_{2-5}*: 7.0 per cent to 15.0 per cent;
- *teicoplanin A_{2-3} group*: 5.0 per cent to 11.0 per cent;
- *teicoplanin A_3 group*: 4.0 per cent to 12.0 per cent;
- *teicoplanin A_{2-3}*: 4.0 per cent to 8.5 per cent;
- *teicoplanin A_{2-1}*: 2.0 per cent to 7.0 per cent;
- *teicoplanin A_{2-1a}*: 0.5 per cent to 5.5 per cent;
- *teicoplanin A_{2-1b}*: 0.5 per cent to 4.0 per cent;
- *teicoplanin A_{2-6} group*: maximum 5.0 per cent;
- *disregard limit*: 0.25 per cent.

Related substances

Liquid chromatography (*2.2.29*) as described in the test for composition. Use the normalisation procedure.

Use the chromatogram obtained with reference solution (a) to identify all peaks present above the disregard limit as teicoplanin-like related substances. Any peak present in any part of the chromatogram obtained with the test solution that cannot be correlated to a peak above the disregard limit in reference solution (a) should be considered as a non-teicoplanin-like impurity, unless it is characterized by other means.

A teicoplanin-like related substance is defined as a substance that shares the same glycopeptide core structure of the parent molecule, composed of a linear heptapeptide aglycone, an α-D-mannose and an acetyl-β-D-glucosamine.

The R′ side chains in the teicoplanin-like related substances RS $A_{2\text{-}6a}$, RS $A_{2\text{-}6b}$ and RS $A_{2\text{-}6c}$ are unknown.

Calculate the percentage contents using the following equations:

$$\text{teicoplanin-like related substance (x)} = \frac{A_{RSTLx}}{(S2 + 0.83 \times S_3)} \times 100$$

A_{RSTLx} = area of the peak due to the teicoplanin-like related substance (x) in the chromatogram obtained with the test solution;

$$\text{non-teicoplanin-like impurity (x)} = \frac{A_{Ix}}{(S2 + 0.83 \times S_3)} \times 100$$

A_{Ix} = area of the peak due to the non-teicoplanin-like impurity (x) in the chromatogram obtained with the test solution.

Limits:
- — *teicoplanin-like related substance RS $A_{2\text{-}6c}$*: maximum 2.5 per cent;
- — *teicoplanin-like related substance RS $A_{2\text{-}6a}$*: maximum 1.5 per cent;
- — *teicoplanin-like related substance RS $A_{2\text{-}6b}$*: maximum 1.5 per cent;
- — *any non-teicoplanin-like impurity other than impurity A*: maximum 0.5 per cent;
- — *total non-teicoplanin-like impurities other than impurity A*: maximum 1.5 per cent.

Impurity A

Liquid chromatography (*2.2.29*) as described in the test for composition with the following modifications.

Injection 20 µL of the test solution and reference solution (c).

Relative retention With reference to teicoplanin $A_{2\text{-}2}$ (retention time = about 18 min): impurity A = about 0.6.

Calculation of percentage content:
- — for impurity A, use the concentration of impurity A in reference solution (c).

Limit:
- — *impurity A*: maximum 0.2 per cent.

Chlorides

Maximum 5.0 per cent, expressed as sodium chloride (anhydrous substance).

Dissolve 1.000 g in 300 mL of *water R*, stir and acidify with 2 mL of *nitric acid R*. Titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M silver nitrate* is equivalent to 5.844 mg of NaCl.

Water (*2.5.12*)

Maximum 15.0 per cent, determined on 0.300 g.

ASSAY

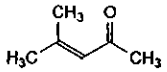
Carry out the microbiological assay of antibiotics (*2.7.2*), using the diffusion method. Use *teicoplanin CRS* as the reference substance.

STORAGE

Protected from light, at a temperature of 2 °C to 8 °C.

IMPURITIES

Specified impurities *A*.

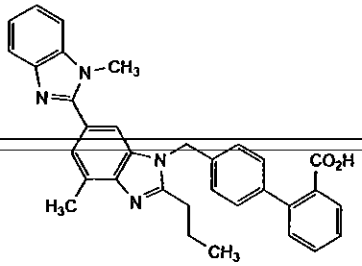


A. 4-methylpent-3-en-2-one (mesityl oxide).

Ph Eur

Telmisartan

(Ph. Eur. monograph 2154)



$C_{33}H_{30}N_4O_2$ 514.6 *144701-48-4*

Action and use

Angiotensin II (AT_1) receptor antagonist.

Preparation

Telmisartan Tablets

Ph Eur

DEFINITION

4′-[[4-Methyl-6-(1-methyl-1*H*-benzimidazol-2-yl)-2-propyl-1*H*-benzimidazol-1-yl]methyl][1,1′-biphenyl]-2-carboxylic acid.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or slightly yellowish, crystalline powder.

Solubility

Practically insoluble in water, slightly soluble in methanol, sparingly soluble in methylene chloride. It dissolves in a 40 g/L solution of *sodium hydroxide R*.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *telmisartan CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in hot *anhydrous ethanol R*, evaporate to dryness and record new spectra using the residues.

TESTS

Appearance of solution

The solution is not more intensely coloured than reference solution Y_4 (*2.2.2, Method II*).

Dissolve 0.5 g in a 40 g/L solution of *sodium hydroxide R* and dilute to 10 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution To 25 mg of the substance to be examined add about 5 mL of *methanol R* and 100 µL of a 40 g/L solution of *sodium hydroxide R*. Dissolve using sonication and dilute to 50.0 mL with *methanol R*.

Reference solution (a) Dilute 1.0 mL of the test solution to 10.0 mL with *methanol R*. Dilute 1.0 mL of this solution to 100.0 mL with *methanol R*.

Reference solution (b) Dissolve the contents of a vial of *telmisartan for system suitability CRS* (containing impurities A, B, C, E and F) in 2 mL of *methanol R*.

Reference solution (c) To 5 mg of *telmisartan for peak identification CRS* (containing impurity D) add about 5 mL of *methanol R* and 100 µL of a 40 g/L solution of *sodium hydroxide R*. Dissolve using sonication and dilute to 10 mL with *methanol R*.

Column:
- *size*: *l* = 0.125 m, Ø = 4.0 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm) with a pore size of 10 nm;
- *temperature*: 40 °C.

Mobile phase:
- *mobile phase A*: dissolve 2.0 g of *potassium dihydrogen phosphate R* and 3.8 g of *sodium pentanesulfonate monohydrate R1* in 900 mL of *water for chromatography R*, adjust to pH 3.0 with *dilute phosphoric acid R* and dilute to 1000 mL with *water for chromatography R*;
- *mobile phase B*: *methanol R1, acetonitrile for chromatography R* (20:80 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	70	30
3 - 28	70 → 20	30 → 80

Flow rate 1 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 10 µL.

Identification of impurities Use the chromatogram supplied with *telmisartan for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C, E and F; use the chromatogram supplied with *telmisartan for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peak due to impurity D.

Relative retention With reference to telmisartan (retention time = about 15 min): impurity A = about 0.2; impurity E = about 0.6; impurity F = about 0.7; impurity B = about 0.9; impurity C = about 1.5; impurity D = about 1.6.

System suitability Reference solution (b):
- the chromatogram obtained with reference solution (b) is similar to the chromatogram supplied with *telmisartan for system suitability CRS*;
- *resolution*: minimum 3.0 between the peaks due to impurity B and telmisartan.

Limits:
- *impurities C, D*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *impurities A, B*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

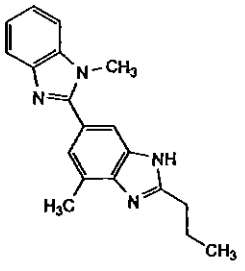
Dissolve 0.190 g in 5 mL of *anhydrous formic acid R*. Add 75 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 25.73 mg of $C_{33}H_{30}N_4O_2$.

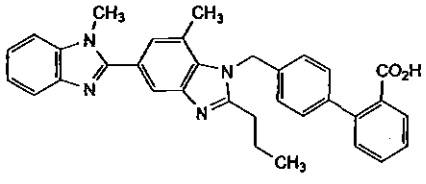
IMPURITIES

Specified impurities *A, B, C, D.*

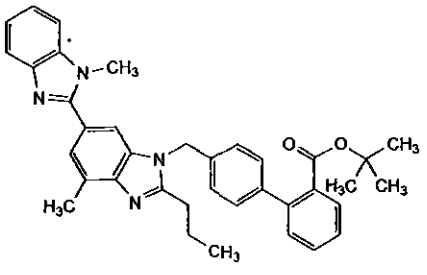
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *E, F, G, H, I, J.*



A. 4-methyl-6-(1-methyl-1*H*-benzimidazol-2-yl)-2-propyl-1*H*-benzimidazole,

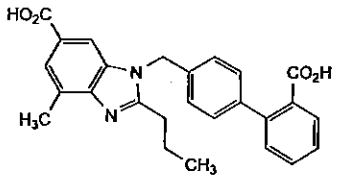


B. 4′-[[7-methyl-5-(1-methyl-1*H*-benzimidazol-2-yl)-2-propyl-1*H*-benzimidazol-1-yl]methyl][1,1′-biphenyl]-2-carboxylic acid,

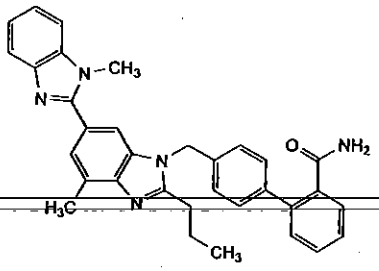


C. *tert*-butyl 4′-[[4-methyl-6-(1-methyl-1*H*-benzimidazol-2-yl)-2-propyl-1*H*-benzimidazol-1-yl]methyl][1,1′-biphenyl]-2-carboxylate,

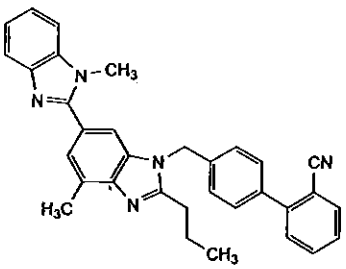
D. unknown structure,



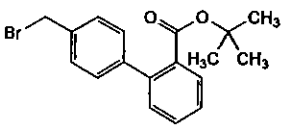
E. 1-[(2′-carboxy[1,1′-biphenyl]-4-yl)methyl]-4-methyl-2-propyl-1*H*-benzimidazole-6-carboxylic acid,



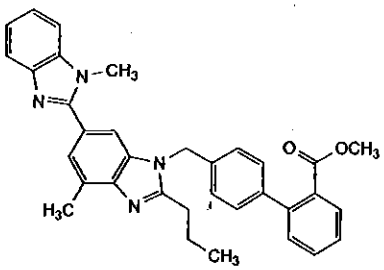
F. 4′-[[4-methyl-6-(1-methyl-1*H*-benzimidazol-2-yl)-2-propyl-1*H*-benzimidazol-1-yl]methyl][1,1′-biphenyl]-2-carboxamide,



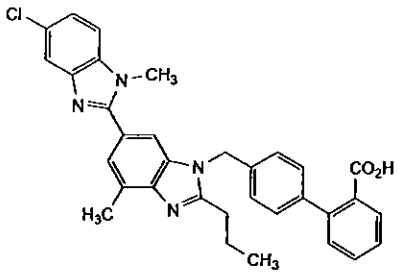
G. 4′-[[4-methyl-6-(1-methyl-1*H*-benzimidazol-2-yl)-2-propyl-1*H*-benzimidazol-1-yl]methyl][1,1′-biphenyl]-2-carbonitrile,



H. *tert*-butyl 4′-(bromomethyl)[1,1′-biphenyl]-2-carboxylate,



I. methyl 4′-[(1,7′-dimethyl-2′-propyl-1*H*,3′*H*-[2,5′-bibenzimidazol]-3′-yl)methyl][1,1′-biphenyl]-2-carboxylate,

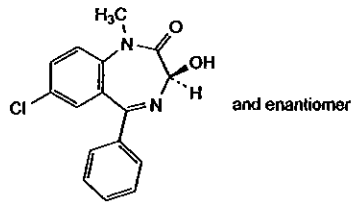


J. 4′-[(5-chloro-1,7′-dimethyl-2′-propyl-1*H*,3′*H*-[2,5′-bibenzimidazol]-3′-yl)methyl][1,1′-biphenyl]-2-carboxylic acid.

Ph Eur

Temazepam

(Ph. Eur. monograph 0954)



$C_{16}H_{13}ClN_2O_2$ 300.7 *846-50-4*

Action and use
Benzodiazepine.

Preparations
Temazepam Oral Solution
Temazepam Tablets

Ph Eur

DEFINITION

(3*RS*)-7-Chloro-3-hydroxy-1-methyl-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).
Comparison *temazepam CRS*.

TESTS

Impurity A
Maximum 0.05 per cent.

Dissolve 0.400 g in *methylene chloride R* and dilute to 20.0 mL with the same solvent. The absorbance (*2.2.25*) is not greater than 0.30 at 409 nm.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 10.0 mg of the substance to be examined in a mixture of 1 volume of *water R* and 9 volumes

of *methanol R* and dilute to 50.0 mL with the same mixture of solvents.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with a mixture of 1 volume of *water R* and 9 volumes of *methanol R*. Dilute 2.0 mL of this solution to 10.0 mL with a mixture of 1 volume of *water R* and 9 volumes of *methanol R*.

Reference solution (b) Dissolve 1 mg of *oxazepam R*, 1 mg of *temazepam impurity F CRS* and 1 mg of *temazepam impurity G CRS* in a mixture of 1 volume of *water R* and 9 volumes of *methanol R* and dilute to 25 mL with the same mixture of solvents.

Reference solution (c) Dissolve 1 mg of *temazepam impurity C CRS* and 1 mg of *temazepam impurity D CRS* with a mixture of 1 volume of *water R* and 9 volumes of *methanol R* and dilute to 25 mL with the same mixture of solvents.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3.5 µm).

Mobile phase:
- *mobile phase A*: solution containing 4.9 g/L of *sodium dihydrogen phosphate R* and 0.63 g/L of *disodium hydrogen phosphate dodecahydrate R* (pH 5.6);
- *mobile phase B*: *methanol R*;
- *mobile phase C*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Mobile phase C (per cent *V/V*)
0 - 18	54	39	7
18 - 25	54 → 22	39 → 63	7 → 15
25 - 31	22	63	15
31 - 37	22 → 54	63 → 39	15 → 7

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µL.

Relative retention With reference to temazepam (retention time = about 16 min): impurity E = about 0.55; impurity F = about 0.67; impurity G = about 0.73; impurity B = about 0.8; impurity D = about 1.2; impurity C = about 1.3; impurity A = about 1.5.

System suitability Reference solution (b):
- *resolution*: minimum 1.5 between the peaks due to impurity F and impurity G;
- *peak-to-valley ratio*: minimum 1.7, where H_p = height above the baseline of the peak due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity B.

Limits:
- *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity F = 3.2; impurity G = 3.1;
- *impurities B, C, D, E, F, G*: for each impurity, not more. than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

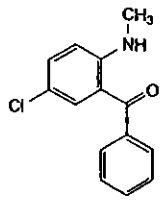
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.250 g in 50 mL of *nitroethane R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

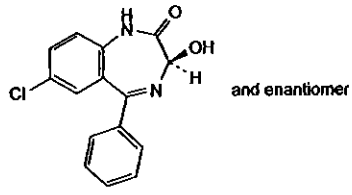
1 mL of *0.1 M perchloric acid* is equivalent to 30.07 mg of $C_{16}H_{13}ClN_2O_2$.

STORAGE
Protected from light.

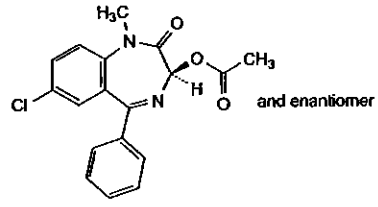
IMPURITIES
Specified impurities *A, B, C, D, E, F, G.*



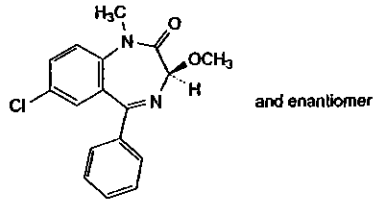
A. [5-chloro-2-(methylamino)phenyl]phenylmethanone,



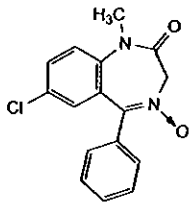
B. (3*RS*)-7-chloro-3-hydroxy-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (oxazepam),



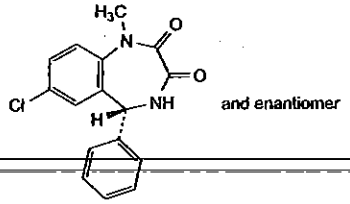
C. (3*RS*)-7-chloro-1-methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl acetate,



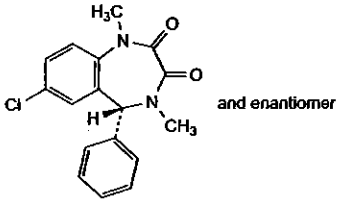
D. (3*RS*)-7-chloro-3-methoxy-1-methyl-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one,



E. 7-chloro-1-methyl-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one 4-oxide,



F. (5*RS*)-7-chloro-1-methyl-5-phenyl-4,5-dihydro-1*H*-1,4-benzodiazepine-2,3-dione,

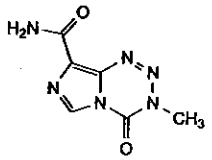


G. (5*RS*)-7-chloro-1,4-dimethyl-5-phenyl-4,5-dihydro-1*H*-1,4-benzodiazepine-2,3-dione.

Ph Eur

Temozolomide

(Ph. Eur. monograph 2780)



$C_6H_6N_6O_2$ 194.2 *85622-93-1*

Action and use
Antineoplastic alkylating agent.

Preparations
Temozolomide Capsules
Temozolomide for Injection

Ph Eur

DEFINITION

3-Methyl-4-oxo-3,4-dihydroimidazo[5,1-*d*][1,2,3,5]tetrazine-8-carboxamide.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or slightly brown or slightly pink powder.

Solubility
Sparingly soluble in water, soluble in dimethyl sulfoxide, very slightly soluble in ethanol (96 per cent), practically insoluble in toluene.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *temozolomide CRS*.

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (d).

TESTS

Related substances
Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 25.0 mg of the substance to be examined in *dimethyl sulfoxide R* and dilute to 25.0 mL with the same solvent.

Test solution (b) Dilute 1.0 mL of test solution (a) to 10.0 mL with *dimethyl sulfoxide R*.

Reference solution (a) Dilute 1.0 mL of test solution (a) to 100.0 mL with *dimethyl sulfoxide R*. Dilute 1.0 mL of this solution to 10.0 mL with *dimethyl sulfoxide R*.

Reference solution (b) In order to prepare impurities A, B and E *in situ*, mix 5 mL of a 10.3 g/L solution of *hydrochloric acid R* and 5 mL of test solution (a). Heat the mixture in a water-bath for 1 h.

Reference solution (c) Dissolve 2 mg of *temozolomide for peak identification CRS* (containing impurity D) in 2 mL of *dimethyl sulfoxide R*.

Reference solution (d) Dissolve 25.0 mg of *temozolomide CRS* in *dimethyl sulfoxide R* and dilute to 25.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with *dimethyl sulfoxide R*.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase 0.94 g/L solution of *sodium hexanesulfonate R* in a mixture of 4 volumes of *methanol R* and 96 volumes of a 0.5 per cent *V/V* solution of *glacial acetic acid R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 270 nm.

Injection 10 µL of test solution (a) and reference solutions (a), (b) and (c).

Run time 3 times the retention time of temozolomide.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B and E; use the chromatogram supplied with *temozolomide for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peak due to impurity D.

Relative retention With reference to temozolomide (retention time = about 11 min): impurity E = about 0.4; impurity D = about 0.5; impurity B = about 0.9; impurity A = about 1.7. The peak due to impurity A in the chromatogram obtained with test solution (a) may be split.

System suitability Reference solution (b):
— *resolution*: minimum 1.5 between the peaks due to impurity B and temozolomide.

Calculation of percentage contents:
— *correction factors*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.4; impurity E = 0.6;
— for each impurity, use the concentration of temozolomide in reference solution (a).

Limits:
— *impurity D*: maximum 0.5 per cent;
— *impurity A (sum of the peaks)*: maximum 0.15 per cent;
— *impurities B, E*: for each impurity, maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.8 per cent;
— *reporting threshold*: 0.05 per cent.

Water (*2.5.32*)
Maximum 0.4 per cent, determined on 50.0 mg.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

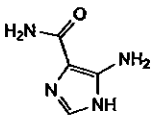
ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (d).

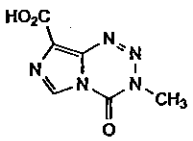
Calculate the percentage content of $C_6H_6N_6O_2$ taking into account the assigned content of *temozolomide CRS*.

IMPURITIES
Specified impurities *A, B, D, E.*

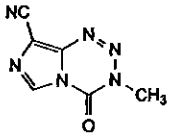
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C.*



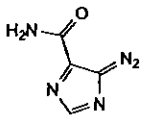
A. 5-amino-1*H*-imidazole-4-carboxamide,



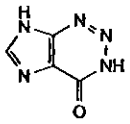
B. 3-methyl-4-oxo-3,4-dihydroimidazo[5,1-*d*][1,2,3,5]tetrazine-8-carboxylic acid,



C. 3-methyl-4-oxo-3,4-dihydroimidazo[5,1-*d*][1,2,3,5]tetrazine-8-carbonitrile,



D. 4-diazo-4*H*-imidazole-5-carboxamide,

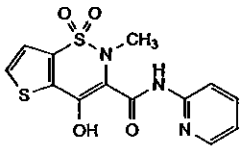


E. 3,7-dihydro-4*H*-imidazo[4,5-*d*][1,2,3]triazin-4-one (2-azahypoxanthine).

Ph Eur

Tenoxicam

(Ph. Eur. monograph 1156)



$C_{13}H_{11}N_3O_4S_2$ 337.4 *59804-37-4*

Action and use
Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Preparations
Tenoxicam Injection
Tenoxicam Tablets

Ph Eur

DEFINITION
4-Hydroxy-2-methyl-*N*-(pyridin-2-yl)-2*H*-thieno[2,3-*e*]1,2-thiazine-3-carboxamide 1,1-dioxide.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS
Appearance
Yellow, crystalline powder.

Solubility
Practically insoluble in water, sparingly soluble in methylene chloride, very slightly soluble in anhydrous ethanol.
It dissolves in solutions of acids and alkalis.

It shows polymorphism (*5.9*).

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *tenoxicam CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *methylene chloride R*, evaporate to dryness and record new spectra using the residues.

TESTS
Appearance of solution
The solution is clear (*2.2.1*).

Dissolve 0.10 g in *methylene chloride R* and dilute to 20 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Solvent mixture Mix equal volumes of *acetonitrile R* and *water R*. Adjust to apparent pH 3.2 with *dilute phosphoric acid R1*.

Test solution Dissolve 35 mg of the substance to be examined in the solvent mixture, sonicate and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 7 mg of pyridin-2-amine R (impurity A) in the solvent mixture and dilute to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 100.0 mL with the solvent mixture.

Reference solution (c) Dissolve the contents of a vial of *tenoxicam impurity mixture CRS* (impurities B, G and H) in 1.0 mL of the test solution.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: diisopropylcyanosilyl silica gel for chromatography R (3.5 µm);
- *temperature*: 35 °C.

Mobile phase:
- *mobile phase A*: mix 25 volumes of *methanol R2* and 75 volumes of *water for chromatography R* and adjust to apparent pH 3.2 with *dilute phosphoric acid R1*;
- *mobile phase B*: mix 25 volumes of *water for chromatography R* and 75 volumes of *methanol R2* and adjust to apparent pH 3.2 with *dilute phosphoric acid R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	96	4
5 - 16	96 → 76	4 → 24
16 - 25	76	24

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µL.

Identification of impurities:
- use the chromatogram obtained with reference solution (b) to identify the peak due to impurity A;
- use the chromatogram supplied with *tenoxicam impurity mixture CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities B, G and H; for identification of impurities G and H, which may be inverted in the elution order, take into account the heights of the corresponding peaks in the chromatogram supplied with *tenoxicam impurity mixture CRS*.

Relative retention With reference to tenoxicam (retention time = about 12 min): impurity A = about 0.1; impurity G = about 0.85; impurity H = about 0.9; impurity B = about 1.3.

System suitability Reference solution (c):
- *resolution*: minimum 1.3 between the peaks due to impurity H (or G if peaks are inverted) and tenoxicam, and between the peaks due to impurities G and H; if necessary, optimise the apparent pH of the mobile phases within the range 3.0-3.4.

Limits:
- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.2; impurity B = 2.0;
- *impurities A, B*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)

Maximum 0.5 per cent, determined on 1.000 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 5 mL of *anhydrous formic acid R*. Add 70 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 33.74 mg of $C_{13}H_{11}N_3O_4S_2$.

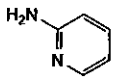
STORAGE

Protected from light.

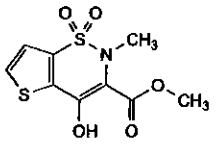
IMPURITIES

Specified impurities *A, B.*

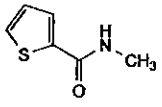
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C, D, E, F, G, H.*



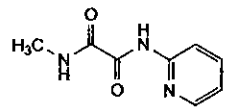
A. pyridin-2-amine,



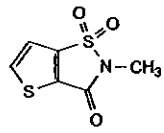
B. methyl 4-hydroxy-2-methyl-2*H*-thieno[2,3-*e*]1,2-thiazine-3-carboxylate 1,1-dioxide,



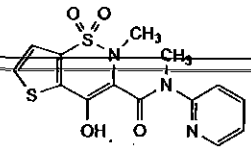
C. *N*-methylthiophene-2-carboxamide,



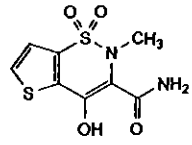
D. *N*-methyl-*N'*-(pyridin-2-yl)-ethanediamide,



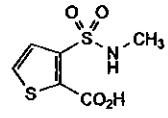
E. 2-methylthieno[2,3-*d*]isothiazol-3(2*H*)-one 1,1-dioxide,



F. 4-hydroxy-*N*,2-dimethyl-*N*-(pyridin-2-yl)-2*H*-thieno[2,3-*e*] 1,2-thiazine-3-carboxamide 1,1-dioxide,



G. 4-hydroxy-2-methyl-2*H*-thieno[2,3-*e*]1,2-thiazine-3-carboxamide 1,1-dioxide,

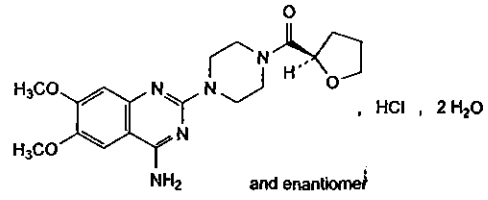


H. 3-[(methylamino)sulfonyl]thiophene-2-carboxylic acid.

Ph Eur

Terazosin Hydrochloride Dihydrate

(Ph. Eur. monograph 2021)



$C_{19}H_{26}ClN_5O_4,2H_2O$ 459.9 *70024-40-7*

Action and use

Alpha1-adrenoceptor antagonist.

Ph Eur

DEFINITION

1-(4-Amino-6,7-dimethoxyquinazolin-2-yl)-4-[[(2*RS*)-tetrahydrofuran-2-yl]carbonyl]piperazine hydrochloride dihydrate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or slightly yellow, crystalline powder.

Solubility

Sparingly soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (96 per cent), practically insoluble in acetone.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *terazosin hydrochloride dihydrate CRS.*

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 1.00 g in *carbon dioxide-free water R* and dilute to 50.0 mL with the same solvent.

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

Dilute 10 mL of solution S to 20 mL with *water R*.

pH (*2.2.3*)

3.0 to 5.0 for solution S.

Impurities N and O

Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R1, water R* (20:80 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dissolve 5 mg of *terazosin impurity A CRS* and 5.0 mg of *terazosin impurity N CRS* in *acetonitrile R1* using sonication, add 5.0 mL of the test solution and dilute to 50.0 mL with *acetonitrile R1*. Dilute 10.0 mL of this solution to 100.0 mL with the solvent mixture.

Reference solution (b) Dilute 10.0 mL of reference solution (a) to 100.0 mL with the solvent mixture.

Column:

— *size*: *l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 25 °C.

Mobile phase Dissolve 2.80 g of *sodium laurilsulfate R* in 1000.0 mL of *water R* and add 11.0 mL of a solution containing 202.4 g/L of *triethylamine R* and 230.0 g/L of *phosphoric acid R*; adjust to pH 2.5 with *phosphoric acid R*; mix 600 volumes of this solution with 400 volumes of *acetonitrile R1*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µL.

Run time 4 times the retention time of terazosin.

Relative retention With reference to terazosin (retention time = about 10 min): impurity O = about 0.2; impurity N = about 0.3; impurity A = about 0.4.

System suitability Reference solution (a):

— *resolution*: minimum 1.5 between the peaks due to impurities A and N.

Limits:

— *impurity N*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *impurity O*: not more than the area of the peak due to terazosin in the chromatogram obtained with reference solution (b) (0.1 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dilute 2.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve the contents of a vial of *terazosin for system suitability CRS* (containing impurities A, B, C, J, K and M) in the mobile phase and dilute to 10 mL with the mobile phase.

Reference solution (c) Dissolve 5.0 mg of *terazosin impurity L CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 100.0 mL with the mobile phase.

Reference solution (d) To 5 mg of *terazosin impurity E CRS*, add 70 mL of *methanol R* and 30 mL of *water R*. Allow to stand for at least 1 h to dissolve the substance. Use sonication if necessary.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 30 °C.

Mobile phase Mix 2 volumes of *triethylamine R*, 350 volumes of *acetonitrile R*, and 1650 volumes of a solution containing 6 g/L of *sodium citrate R* and 14.25 g/L of *anhydrous citric acid R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 245 nm.

Injection 20 µL.

Run time 4 times the retention time of terazosin.

Identification of impurities Use the chromatogram supplied with *terazosin for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C, J, K and M; use the chromatograms obtained with reference solutions (c) and (d) to identify the peaks due to impurities L and E respectively.

Retention time Terazosin = about 11 min.

System suitability Reference solution (b):
— *resolution*: minimum 1.5 between the peaks due to impurities B and J; if necessary, adjust the proportion of the aqueous component in the mobile phase (an increase in the proportion of the aqueous component increases the retention times);
— the chromatogram obtained is similar to the chromatogram supplied with *terazosin for system suitability CRS*; in case of insufficient separation of the impurities, reduce the amount of triethylamine in the mobile phase.

Limits:
— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity C = 0.7; impurity M = 1.6;
— *impurities A, C, E, K*: for each impurity, not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *impurity L*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.1 per cent);
— *impurities B, J, M*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)

7.0 per cent to 8.6 per cent, determined on 0.200 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in a mixture of 5.0 mL of *0.01 M hydrochloric acid* and 50 mL of *methanol R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 42.39 mg of $C_{19}H_{26}ClN_5O_4$.

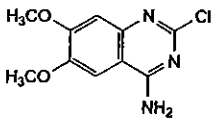
STORAGE

Protected from light.

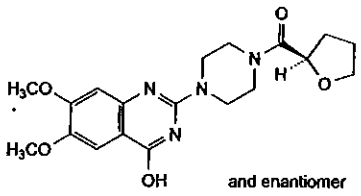
IMPURITIES

Specified impurities *A, B, C, E, J, K, L, M, N, O.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *D, F, G, H, I.*

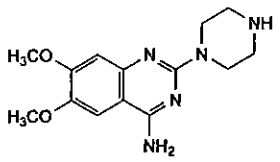


A. 2-chloro-6,7-dimethoxyquinazolin-4-amine,

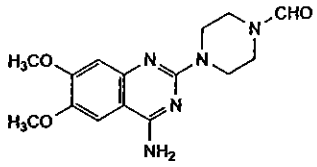


B. 1-(4-hydroxy-6,7-dimethoxyquinazolin-2-yl)-4-[[(2*RS*)-tetrahydrofuran-2-yl]carbonyl]piperazine,

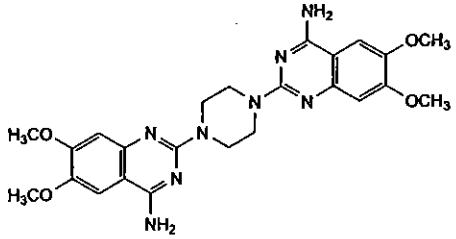
and enantiomer



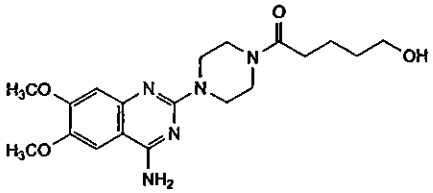
C. 6,7-dimethoxy-2-(piperazin-1-yl)quinazolin-4-amine,



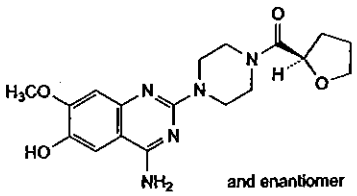
D. 1-(4-amino-6,7-dimethoxyquinazolin-2-yl)-4-formylpiperazine,



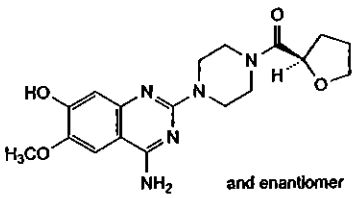
E. 2,2′-(piperazine-1,4-diyl)bis(6,7-dimethoxyquinazolin-4-amine),



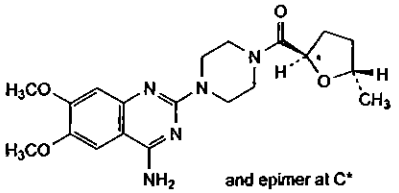
F. 1-(4-amino-6,7-dimethoxyquinazolin-2-yl)-4-(5-hydroxypentanoyl)piperazine,



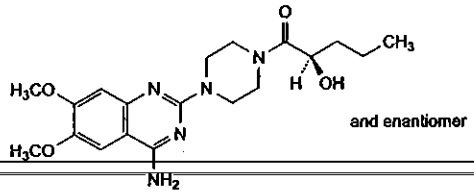
G. 1-(4-amino-6-hydroxy-7-methoxyquinazolin-2-yl)-4-[[(2*RS*)-tetrahydrofuran-2-yl]carbonyl]piperazine,



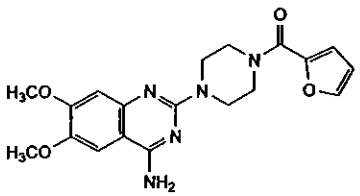
H. 1-(4-amino-7-hydroxy-6-methoxyquinazolin-2-yl)-4-[[(2*RS*)-tetrahydrofuran-2-yl]carbonyl]piperazine,



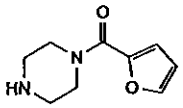
I. 1-(4-amino-6,7-dimethoxyquinazolin-2-yl)-4-[[(2*RS*,5*S*)-5-methyltetrahydrofuran-2-yl]carbonyl]piperazine,



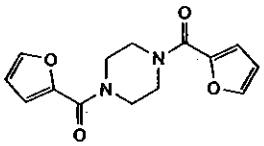
J. 1-(4-amino-6,7-dimethoxyquinazolin-2-yl)-4-[(2*RS*)-2-hydroxypentanoyl]piperazine,



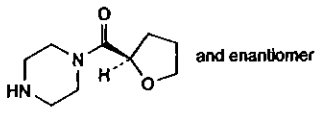
K. 1-(4-amino-6,7-dimethoxyquinazolin-2-yl)-4-(furan-2-ylcarbonyl)piperazine (prazosin),



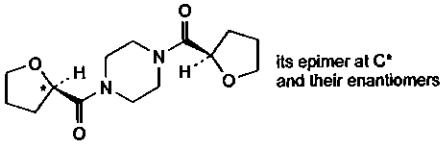
L. 1-(furan-2-ylcarbonyl)piperazine,



M. 1,4-bis(furan-2-ylcarbonyl)piperazine,



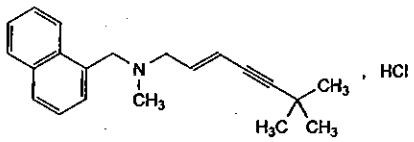
N. 1-[[(2*RS*)-tetrahydrofuran-2-yl]carbonyl]piperazine,



O. 1,4-bis[(tetrahydrofuran-2-yl)carbonyl]piperazine.

Terbinafine Hydrochloride

(Ph. Eur. monograph 1734)



$C_{21}H_{26}ClN$ 327.9 *78628-80-5*

Action and use
Antifungal.

Preparation
Terbinafine Tablets

Ph Eur

DEFINITION

(2*E*)-*N*,6,6-Trimethyl-*N*-(naphthalen-1-ylmethyl)hept-2-en-4-yn-1-amine hydrochloride.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Very slightly or slightly soluble in water, freely soluble in anhydrous ethanol and in methanol, slightly soluble in acetone.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *terbinafine hydrochloride CRS.*

B. It gives reaction (a) of chlorides (*2.3.1*) using *anhydrous ethanol R* as solvent.

TESTS

Related substances
Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Solvent mixture A *acetonitrile R, water R* (50:50 *V/V*).

Solvent mixture B *acetonitrile R, methanol R* (40:60 *V/V*).

Buffer solution Dilute 2.0 mL of *triethylamine R1* to 950 mL with *water R*. Adjust to pH 7.5 with a mixture of 5 volumes of *glacial acetic acid R* and 95 volumes of *water R* and dilute to 1000.0 mL with *water R*.

Test solution Dissolve 25 mg of the substance to be examined in solvent mixture A and dilute to 50.0 mL with solvent mixture A.

Reference solution (a) Dissolve 5 mg of *terbinafine for system suitability CRS* (containing impurities B and E) in 10.0 mL of solvent mixture A.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with solvent mixture A. Dilute 1.0 mL of this solution to 10.0 mL with solvent mixture A.

Column:
— *size*: *l* = 0.15 m, Ø = 3.0 mm;
— *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: buffer solution, solvent mixture B (30:70 *V/V*);
— *mobile phase B*: buffer solution, solvent mixture B (5:95 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 4	100	0
4 - 25	100 → 0	0 → 100
25 - 30	0	100

Flow rate 0.8 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram supplied with *terbinafine for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities B and E.

Relative retention With reference to terbinafine (retention time = about 15 min): impurity B = about 0.9; impurity E = about 1.7.

System suitability Reference solution (a):
— *resolution*: minimum 2.0 between the peaks due to impurity B and terbinafine.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity E by 0.5;
— *impurity B*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
— *impurity E*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 50 mL of *ethanol (96 per cent) R*, add 5 mL of *0.01 M hydrochloric acid*. Titrate with *0.1 M sodium hydroxide* determining the end-point potentiometrically (*2.2.20*). Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 32.79 mg of $C_{21}H_{26}ClN$.

STORAGE

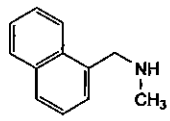
Protected from light.

IMPURITIES

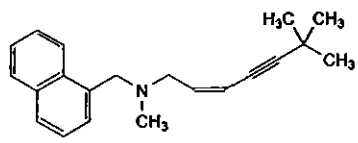
Specified impurities *B, E.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for

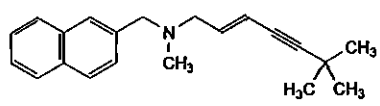
demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, C, D, F.*



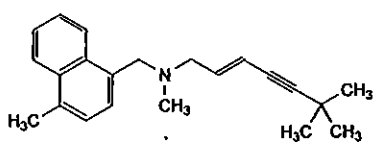
A. *N*-methyl-*C*-(naphthalen-1-yl)methanamine,



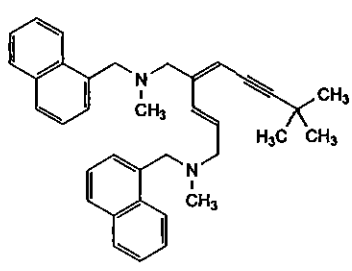
B. (2*Z*)-*N*,6,6-trimethyl-*N*-(naphthalen-1-ylmethyl)hept-2-en-4-yn-1-amine (*cis*-terbinafine),



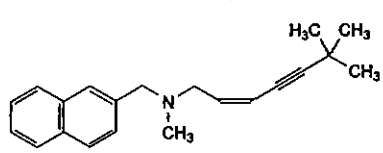
C. (2*E*)-*N*,6,6-trimethyl-*N*-(naphthalen-2-ylmethyl)hept-2-en-4-yn-1-amine (*trans*-isoterbinafine),



D. (2*E*)-*N*,6,6-trimethyl-*N*-[(4-methylnaphthalen-1-yl)methyl]hept-2-en-4-yn-1-amine (4-methylterbinafine),



E. (2*E*,4*E*)-4-(4,4-dimethylpent-2-yn-1-ylidene)-*N*,*N*′-dimethyl-*N*,*N*′-bis(naphthalen-1-ylmethyl)pent-2-ene-1,5-diamine,



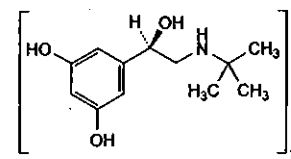
F. (2*Z*)-*N*,6,6-trimethyl-*N*-(naphthalen-2-ylmethyl)hept-2-en-4-yn-1-amine (*cis*-isoterbinafine).

Ph Eur

Terbutaline Sulfate

Terbutaline Sulphate

(Ph. Eur. monograph 0690)



and enantiomer , H_2SO_4

$C_{24}H_{40}N_2O_{10}S$ 548.6 *23031-32-5*

Action and use

~~Beta$_2$-adrenoceptor agonist; bronchodilator.~~

Preparation

Terbutaline Tablets

Ph Eur

DEFINITION

Bis[5-[(1*RS*)-2-(*tert*-butylamino)-1-hydroxyethyl]benzene-1,3-diol] sulfate.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in heptane.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *terbutaline sulfate CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *aldehyde-free methanol R*, evaporate to dryness and record new spectra using the residues.

B. 5 mL of solution S (see Tests) gives reaction (a) of sulfates (*2.3.1*).

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 50.0 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and its absorbance (*2.2.25*) at 400 nm in a 2 cm cell is not greater than 0.11.

Acidity

To 10 mL of solution S add 0.05 mL of *methyl red solution R*. Not more than 1.2 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to yellow.

Optical rotation (*2.2.7*)

−0.10° to + 0.10°, determined on solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 75.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 7.5 mg of *terbutaline impurity C CRS* and 22.5 mg of *terbutaline sulfate CRS* in the

mobile phase and dilute to 50 mL with the mobile phase. Dilute 1 mL of the solution to 100 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:
— *size*: l = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Prepare a 0.050 M ammonium formate solution as follows: dissolve 3.15 g of *ammonium formate R* in about 980 mL of *water for chromatography R*, adjust to pH 3.0 by adding about 8 mL of *anhydrous formic acid R* and dilute to 1000 mL with *water for chromatography R*. Dissolve 4.23 g of *sodium hexanesulfonate R* in 770 mL of the 0.050 M ammonium formate solution, then add 230 mL of *methanol R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 276 nm.

Injection 20 µL.

Run time 6 times the retention time of terbutaline.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity C.

Relative retention With reference to terbutaline (retention time = about 10 min): impurity C = about 0.9.

System suitability Reference solution (a):
— *resolution*: minimum 2.0 between the peaks due to impurity C and terbutaline.

Calculation of percentage contents:
— for each impurity, use the concentration of terbutaline sulfate in reference solution (b).

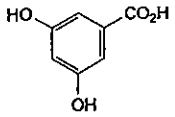
Limits:
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.2 per cent;
— *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

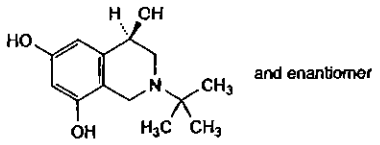
ASSAY
Dissolve 0.400 g in 70 mL of *anhydrous acetic acid R* with heating. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 54.86 mg of $C_{24}H_{40}N_2O_{10}S$.

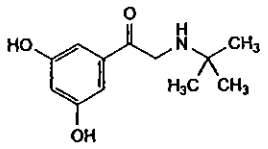
IMPURITIES
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D.*



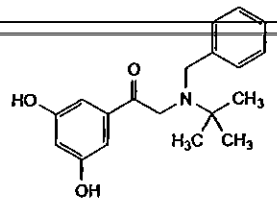
A. 3,5-dihydroxybenzoic acid (α-resorcylic acid),



B. (4*RS*)-2-*tert*-butyl-1,2,3,4-tetrahydroisoquinoline-4,6,8-triol,



C. 2-(*tert*-butylamino)-1-(3,5-dihydroxyphenyl)ethan-1-one,

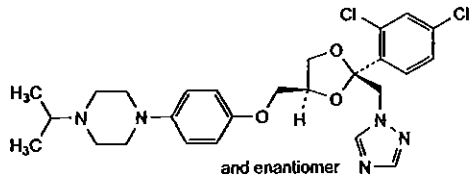


D. 2-[benzyl(*tert*-butyl)amino]-1-(3,5-dihydroxyphenyl)ethan-1-one.

Ph Eur

Terconazole

(Ph. Eur. monograph 1270)



$C_{26}H_{31}Cl_2N_5O_3$ 532.5 *67915-31-5*

Action and use
Antifungal.

Ph Eur

DEFINITION
1-[4-[[(2*RS*,4*SR*)-2-(2,4-Dichlorophenyl)-2-[(1*H*-1,2,4-triazol-1-yl)methyl]-1,3-dioxolan-4-yl]methoxy]phenyl]-4-(1-methylethyl)piperazine.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white powder.

Solubility
Practically insoluble in water, freely soluble in methylene chloride, soluble in acetone, sparingly soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION
First identification: A.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *terconazole CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *acetone R*, evaporate to dryness in a current of air and record new spectra using the residues.

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 30 mg of the substance to be examined in *methanol R* and dilute to 5 mL with the same solvent.

Reference solution (a) Dissolve 30 mg of *terconazole CRS* in *methanol R* and dilute to 5 mL with the same solvent.

Reference solution (b) Dissolve 30 mg of *ketoconazole CRS* and 30 mg of *terconazole CRS* in *methanol R* and dilute to 5 mL with the same solvent.

Plate *TLC octadecylsilyl silica gel plate R*.

Mobile phase *ammonium acetate solution R, dioxan R, methanol R* (20:40:40 *V/V/V*).

Application 5 µL.

Development In an unsaturated tank over half of the plate.

Drying In a current of warm air for 15 min.

Detection Expose to iodine vapour until the spots appear and examine in daylight.

System suitability Reference solution (b):
— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. To 30 mg in a porcelain crucible add 0.3 g of *anhydrous sodium carbonate R*. Heat over an open flame for 10 min. Allow to cool. Take up the residue with 5 mL of *dilute nitric acid R* and filter. To 1 mL of the filtrate add 1 mL of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Optical rotation (*2.2.7*)

−0.10° to + 0.10°.

Dissolve 1.0 g in *methylene chloride R* and dilute to 10 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dissolve 2.0 mg of *ketoconazole CRS* and 2.5 mg of *terconazole CRS* in *methanol R* and dilute to 100.0 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *methanol R*. Dilute 5.0 mL of this solution to 20.0 mL with *methanol R*.

Column:
— *size*: *l* = 0.1 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
— *mobile phase A*: 3.4 g/L solution of *tetrabutylammonium hydrogen sulfate R*;
— *mobile phase B*: *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	95 → 50	5 → 50
10 - 15	50	50

Flow rate 2 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µL.

Relative retention With reference to terconazole (retention time = about 7.5 min): ketoconazole = about 0.8; impurity A = about 0.85; impurity B = about 0.9.

System suitability Reference solution (a):
— *resolution*: minimum 13 between the peaks due to ketoconazole and terconazole.

Limits:
— *impurities A, B*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);
— *unspecified impurities*: for each impurity, not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 70 mL of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically at the 2nd point of inflexion (*2.2.20*).

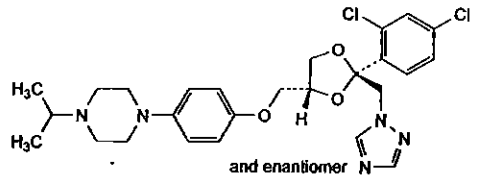
1 mL of *0.1 M perchloric acid* is equivalent to 17.75 mg of $C_{26}H_{31}Cl_2N_5O_3$.

STORAGE

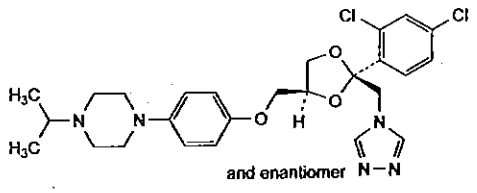
Protected from light.

IMPURITIES

Specified impurities *A, B*.



A. 1-[4-[[(2*RS*,4*RS*)-2-(2,4-dichlorophenyl)-2-[(1*H*-1,2,4-triazol-1-yl)methyl]-1,3-dioxolan-4-yl]methoxy]phenyl]-4-(1-methylethyl)piperazine,

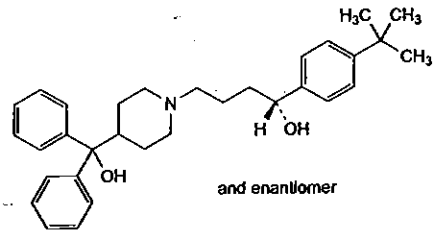


B. 1-[4-[[(2*RS*,4*SR*)-2-(2,4-dichlorophenyl)-2-[(4*H*-1,2,4-triazol-4-yl)methyl]-1,3-dioxolan-4-yl]methoxy]phenyl]-4-(1-methylethyl)piperazine.

Ph Eur

Terfenadine

(Ph. Eur. monograph 0955)



$C_{32}H_{41}NO_2$ 471.7 *50679-08-8*

Action and use
Histamine H_1 receptor antagonist; antihistamine.

Ph Eur

DEFINITION
(1*RS*)-1-[4-(1,1-Dimethylethyl)phenyl]-4-[4-(hydroxydiphenylmethyl)piperidin-1-yl]butan-1-ol.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Very slightly soluble in water, freely soluble in methylene chloride, soluble in methanol. It is very slightly soluble in dilute hydrochloric acid.

It shows polymorphism (*5.9*).

IDENTIFICATION
First identification: C.

Second identification: A, B, D.

A. Melting point (*2.2.14*): 146 °C to 152 °C.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 50.0 mg in *methanol R* and dilute to 100.0 mL with the same solvent.

Spectral range 230-350 nm.

Absorption maximum At 259 nm.

Shoulders At 253 nm and 270 nm.

Specific absorbance at the absorption maximum 13.5 to 14.9.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *terfenadine CRS.*

D. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 50 mg of the substance to be examined in *methylene chloride R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 50 mg of *terfenadine CRS* in *methylene chloride R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *methanol R, methylene chloride R* (10:90 *V/V*).

Application 10 µL.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS
Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 15 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 10.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 20.0 mL with the mobile phase.

Reference solution (b) Dissolve 15 mg of *terfenadine impurity A CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase. To 5.0 mL of this solution, add 5.0 mL of the test solution and dilute to 50.0 mL with the mobile phase.

Reference solution (c) Dilute 10.0 mL of reference solution (a) to 25.0 mL with the mobile phase.

Reference solution (d) Dissolve 0.1 g of *potassium iodide R* in the mobile phase and dilute to 100 mL with the mobile phase. Dilute 1 mL of this solution to 100 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dilute 600 mL of *acetonitrile R1* to 1 L with *diethylammonium phosphate buffer solution pH 6.0 R.*

Flow rate 1 mL/min.

Detection Spectrophotometer at 217 nm.

Injection 20 µL.

Run time 5 times the retention time of terfenadine.

System suitability Reference solution (b):
— *resolution*: minimum 5.0 between the peaks due to terfenadine and impurity A;
— *mass distribution ratio*: minimum 2.0 for the peak due to terfenadine; use *potassium iodide R* as the unretained compound (reference solution (d)).

Limits:
— *impurities A, B, C, D, E, F, G, H, I, J*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);
— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *disregard limit*: 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.005 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying at 60 °C at a pressure not exceeding 0.5 kPa.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.400 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

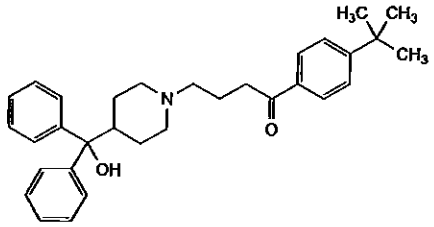
1 mL of *0.1 M perchloric acid* is equivalent to 47.17 mg of $C_{32}H_{41}NO_2$.

STORAGE

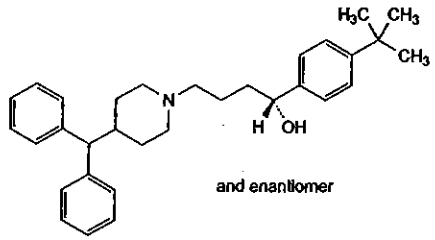
Protected from light.

IMPURITIES

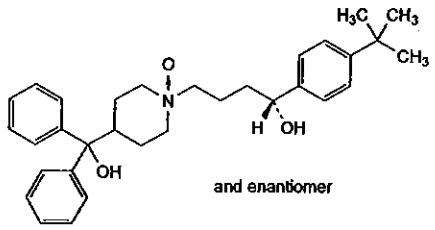
Specified impurities *A, B, C, D, E, F, G, H, I, J.*



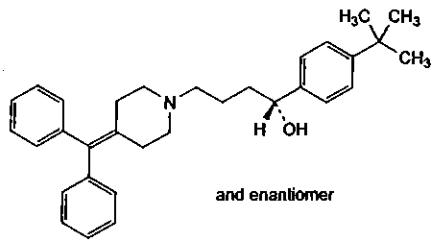
A. 1-[4-(1,1-dimethylethyl)phenyl]-4-[4-(hydroxydiphenylmethyl)piperidin-1-yl]butan-1-one,



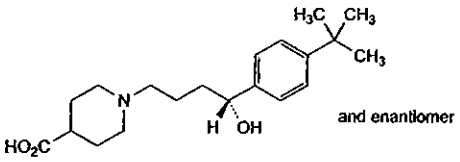
B. (1*RS*)-1-[4-(1,1-dimethylethyl)phenyl]-4-[4-(diphenylmethyl)piperidin-1-yl]butan-1-ol,



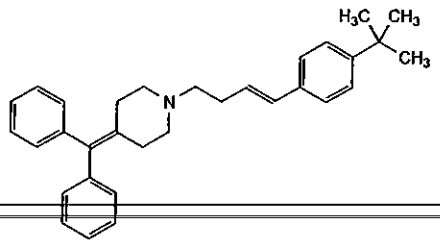
C. 1-[(4*RS*)-4-[4-(1,1-dimethylethyl)phenyl]-4-hydroxybutyl]-4-(hydroxydiphenylmethyl)piperidine 1-oxide,



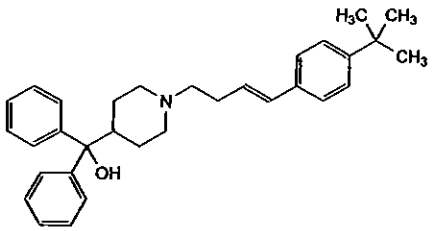
D. (1*RS*)-1-[4-(1,1-dimethylethyl)phenyl]-4-[4-(diphenylmethylene)piperidin-1-yl]butan-1-ol,



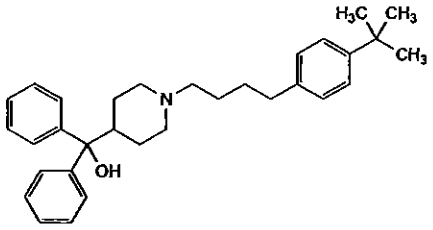
E. 1-[(4*RS*)-4-[4-(1,1-dimethylethyl)phenyl]-4-hydroxybutyl] piperidine-4-carboxylic acid,



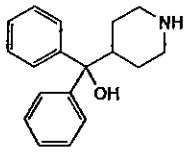
F. 1-[4-[4-(1,1-dimethylethyl)phenyl]but-3-enyl]-4-(diphenylmethylene)piperidine,



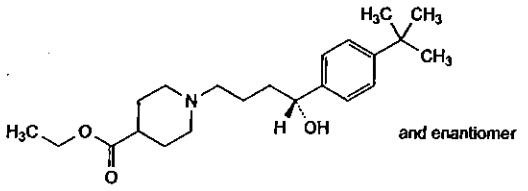
G. [1-[4-[4-(1,1-dimethylethyl)phenyl]but-3-enyl]piperidin-4-yl]diphenylmethanol,



H. [1-[4-[4-(1,1-dimethylethyl)phenyl]butyl]piperidin-4-yl] diphenylmethanol,



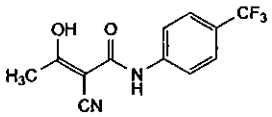
I. diphenyl(piperidin-4-yl)methanol,



J. ethyl 1-[(4*RS*)-4-[4-(1,1-dimethylethyl)phenyl]-4-hydroxybutyl]piperidine-4-carboxylate.

Teriflunomide

(Ph. Eur. monograph 3036)



$C_{12}H_9F_3N_2O_2$ 270.2 *163451-81-8*

Action and use
Immunomodulator; treatment of multiple sclerosis

Ph Eur

DEFINITION

(2*Z*)-2-Cyano-3-hydroxy-*N*-[4-(trifluoromethyl)phenyl]but-2-enamide.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Practically insoluble in water, slightly soluble in anhydrous ethanol, practically insoluble in heptane.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *teriflunomide CRS.*

TESTS

Related substances
Liquid chromatography (*2.2.29*). *Use freshly prepared solutions.*

Buffer solution 3.85 g/L solution of *ammonium acetate R* in *water for chromatography R* adjusted to pH 5.5 with *glacial acetic acid R.*

Solvent mixture Buffer solution, *acetonitrile R* (20:80 *V/V*).

Test solution Dissolve 20.0 mg of the substance to be examined in 40 mL of the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *leflunomide impurity A CRS* (teriflunomide impurity A) in *acetonitrile R* and dilute to 250 mL with the same solvent. Dilute 1 mL of the solution to 200 mL with the solvent mixture.

Reference solution (c) Dissolve 5 mg of *teriflunomide impurity B CRS* in *acetonitrile R* and dilute to 100 mL with the same solvent. Dilute 2 mL of the solution to 50 mL with the solvent mixture. To 1 mL of this solution add 1 mL of reference solution (b) and dilute to 10 mL with the solvent mixture.

Reference solution (d) Dissolve 20.0 mg of *teriflunomide for assay CRS* in 40 mL of the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped solid core octadecylsilyl silica gel for chromatography R* (2.7 µm);
— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: *acetonitrile for chromatography R*, buffer solution (10:90 *V/V*);
— *mobile phase B*: buffer solution, *acetonitrile for chromatography R* (10:90 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	76	24
2 - 12	76 → 23	24 → 77

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 249 nm.

Injection 5 µL of the test solution and reference solutions (a) and (c).

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A and B.

Relative retention With reference to teriflunomide (retention time = about 5 min): impurity B = about 1.5; impurity A = about 1.6.

System suitability Reference solution (c):
— *resolution*: minimum 1.5 between the peaks due to impurities B and A;
— *signal-to-noise ratio*: minimum 10 for the peak due to impurity A.

Calculation of percentage contents:
— for each impurity, use the concentration of teriflunomide in reference solution (a).

Limits:
— *impurity A*: maximum 0.01 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.2 per cent;
— *reporting threshold*: 0.05 per cent; do not disregard the peak due to impurity A.

Water (*2.5.32*)
Maximum 0.5 per cent, determined on 0.100 g by direct sample introduction.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (d).

System suitability Reference solution (d):
— *symmetry factor*: maximum 1.7 for the peak due to teriflunomide;
— *repeatability*: maximum relative standard deviation of 1.2 per cent determined on 6 injections.

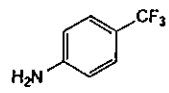
Calculate the percentage content of $C_{12}H_9F_3N_2O_2$ taking into account the assigned content of *teriflunomide for assay CRS.*

IMPURITIES

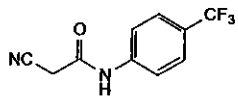
Specified impurities *A.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for

demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) B.



A. 4-(trifluoromethyl)aniline (leflunomide impurity A),



B. 2-cyano-*N*-[4-(trifluoromethyl)phenyl]acetamide.

Ph Eur

Teriparatide

(Ph. Eur. monograph 2829)

H-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-
Leu-Gly-Lys-His-Leu-Asn-Ser-Met-Glu-Arg-
Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-
Val-His-Asn-Phe-OH

$C_{181}H_{291}N_{55}O_{51}S_2$ 4118 *52232-67-4*

Action and use
Parathyroid hormone analogue; treatment of osteoporosis.

Ph Eur

DEFINITION
Tetratriacontapeptide in which the sequence of amino acids is the same as that of the 1-34 *N*-terminal fragment of endogeneous human parathyroid hormone (rhPTH).

Content
95.0 per cent to 105.0 per cent (anhydrous, acetic acid- and chloride-free substance).

PRODUCTION
Teriparatide is produced by a method based on recombinant DNA (rDNA) technology. During the course of product development it must be demonstrated that the manufacturing process produces a biologically active protein using a suitable bioassay as approved by the competent authority.

Prior to release, the following tests are carried out on each batch of teriparatide, unless exemption has been granted by the competent authority.

Host-cell-derived proteins
The limit is approved by the competent authority.

Host-cell- and vector-derived DNA
The limit is approved by the competent authority.

CHARACTERS
Appearance
White or almost white, very hygroscopic powder.

Solubility
Freely soluble in water and in methanol, practically insoluble in acetonitrile.

IDENTIFICATION
A. Peptide mapping (*2.2.55*).

SELECTIVE CLEAVAGE OF THE PEPTIDE BONDS
Solution A Dissolve 230 mg of *anhydrous disodium hydrogen phosphate R* and 60 mg of *sodium dihydrogen phosphate monohydrate R* in 100 mL of *water R* and adjust to pH 7.8 with *sodium hydroxide solution R*.

Test solution Dissolve the substance to be examined in solution A to obtain a concentration of 1.5 mg/mL and transfer 150 µL of the solution to a clean tube. Add 90 µL of a 0.25 mg/mL solution of *glutamyl endopeptidase for peptide mapping R* in solution A. Mix and incubate at 37 °C for 18-24 h. Stop the reaction by adding 660 µL of mobile phase A to reach a final digested protein concentration of about 0.25 mg/mL.

NOTE: if a teriparatide concentration of 1.5 mg/mL is not obtainable, a similar ratio of micrograms of endopeptidase per milligram of teriparatide may be used.

Reference solution Prepare at the same time and in the same manner as for the test solution but using *teriparatide CRS* instead of the substance to be examined.

Blank solution Prepare at the same time and in the same manner as for the test solution but omitting the substance to be examined.

CHROMATOGRAPHIC SEPARATION
Liquid chromatography (*2.2.29*). *Store the solutions at 2-8 °C and use them within 72 h.*

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3.5 µm) with a pore size of 30 nm;
— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: mix 1 mL of *trifluoroacetic acid R* and 1000 mL of *water R*; filter and degas;
— *mobile phase B*: mix 1 mL of *trifluoroacetic acid R*, 400 mL of *water R* and 600 mL of *acetonitrile for chromatography R*; filter and degas;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 6	96	4
6 - 20	96 → 45	4 → 55
20 - 25	45 → 0	55 → 100

Flow rate 1 mL/min.

Detection Spectrophotometer at 214 nm.

Autosampler Set at 2-8 °C.

Injection 20 µL.

System suitability:
— the chromatogram obtained with the reference solution is qualitatively similar to the chromatogram of teriparatide digest supplied with *teriparatide CRS*;
— in the chromatogram obtained with the reference solution, identify the peaks due to digest fragments I, II, III, IV and V:

symmetry factor Maximum 2.3 for the peak due to fragment IV;

resolution Minimum 1.5 between the peaks due to fragments I and III.

Results The profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with the reference solution.

TESTS

Impurities with molecular masses greater than that of teriparatide

Size-exclusion chromatography (*2.2.30*): use the normalisation procedure. *Store the solutions at 2-8 °C and use them within 72 h.*

Test solution Dissolve the substance to be examined in *water R* to obtain a concentration of 1 mg/mL.

Reference solution Dissolve the contents of a vial of *teriparatide CRS* in *water R* to obtain a concentration of 1 mg/mL.

Blank solution *water R.*

Resolution solution Incubate a vial of *teriparatide CRS* at 75 °C for 16-24 h. After incubation, dissolve the contents of the vial in *water R* to obtain a concentration of 1 mg/mL of degraded teriparatide.

Column:
- *size*: *l* = 0.30 m, Ø = 7.8 mm;
- *stationary phase*: *hydrophilic silica gel for chromatography R* (5-10 µm) with a pore size of 12.5 nm, of a grade suitable for fractionation of globular proteins of relative molecular mass up to 150 000.

Mobile phase Add 1 mL of *trifluoroacetic acid R* to 750 mL of *water R*, mix with 250 mL of *acetonitrile for chromatography R* and degas.

Flow rate 0.5 mL/min.

Detection Spectrophotometer at 214 nm.

Autosampler Set at 2-8 °C.

Injection 20 µL.

Run time 1.5 times the retention time of teriparatide monomer.

Retention time Teriparatide monomer = about 17 min.

System suitability:
- the chromatogram obtained with the reference solution is similar to the chromatogram supplied with *teriparatide CRS*;
- *resolution*: minimum 2.0 between the peaks due to teriparatide dimer and monomer in the chromatogram obtained with the resolution solution.

Limit:
- *sum of the peaks eluted before the principal peak*: maximum 0.3 per cent; disregard any peak with a retention time greater than that of the peak due to teriparatide monomer.

Related proteins

Liquid chromatography (*2.2.29*): use the normalisation procedure. *Store the solutions at 2-8 °C and use them within 48 h.*

Buffer solution Dissolve 28.4 g of *anhydrous sodium sulfate R* in 900 mL of *water R* and adjust to pH 2.3 with *phosphoric acid R*. Dilute to 1000 mL with *water for chromatography R* and filter.

Test solution Dissolve the substance to be examined in mobile phase A to obtain a concentration of 0.7 mg/mL.

Reference solution Dissolve the contents of a vial of *teriparatide CRS* in mobile phase A to obtain a concentration of 0.7 mg/mL.

Blank solution Mobile phase A.

Resolution solution Dissolve the contents of a vial of *teriparatide for system suitability CRS* in mobile phase A to obtain a concentration of 1 mg/mL.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3.5 µm) with a pore size of 30 nm;
- *temperature*: 40 °C.

Mobile phase:
- *mobile phase A*: mix 10 volumes of *acetonitrile for chromatography R* and 90 volumes of the buffer solution and degas; apply mild heating at 20-25 °C while stirring continuously during analysis;
- *mobile phase B*: mix equal volumes of *acetonitrile for chromatography R* and the buffer solution and degas; apply mild heating at 20-25 °C while stirring continuously during analysis;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	100 → 65	0 → 35
5 - 35	65 → 60	35 → 40
35 - 45	60 → 0	40 → 100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 214 nm.

Autosampler Set at 2-8 °C.

Injection 20 µL.

Relative retention With reference to teriparatide (retention time = 20-25 min): related protein A ([$MetO^{8}$, $MetO^{18}$]teriparatide) = about 0.40; related protein B ([$MetO^{8}$]teriparatide) = about 0.49; related protein C ([$MetO^{18}$]teriparatide) = about 0.57; related protein D = about 1.06; related protein E = about 1.14.

System suitability Resolution solution:
- the chromatogram obtained is similar to the chromatogram supplied with *teriparatide for system suitability CRS*;
- *resolution*: minimum 1.5 between the peaks due to teriparatide and related protein D;
- *symmetry factor*: 0.8 to 2.0 for the peak due to teriparatide;

Results:
- the profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.

Limits:
- *sum of related proteins A, B and C*: maximum 0.5 per cent;
- *any other related protein*: maximum 0.5 per cent;
- *total*: maximum 2.0 per cent;
- *reporting threshold*: 0.05 per cent.

Water (*2.5.32*)

Maximum 7.0 per cent, determined on 10 mg using the evaporation technique:
- *temperature*: 100 °C;
- *heating time*: 8 min.

Bacterial endotoxins (*2.6.14*)

Less than 50 IU/mg.

ASSAY

Acetate

Liquid chromatography (*2.2.29*): use the normalisation procedure. *Store the solutions at 2-8 °C and use them within 72 h.*

Test solution Dissolve the substance to be examined in the mobile phase to obtain a concentration of 5 mg/mL.

Reference solutions Dissolve separately 100 mg, 200 mg and 300 mg of *anhydrous sodium acetate R* in the mobile phase and dilute to 100 mL with the mobile phase. Further dilute 1.0 mL of each solution to 10.0 mL with the mobile phase to prepare a standard curve with acetate concentrations in the range of 0.072-0.216 mg/mL.

Plot peak areas versus injected acetate content and perform linear regression to create a standard curve.

Column:
— *size*: *l* = 0.25 m, Ø = 9.0 mm;
— *stationary phase*: *ion-exclusion resin for chromatography R* (7.5 µm).

Mobile phase 0.5 per cent *V/V* solution of *dilute sulfuric acid R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 210 nm.

Autosampler Set at 2-8 °C.

Injection 100 µL.

Run time 1.5 times the retention time of acetate.

Retention time Acetate = about 10 min.

System suitability:
— *repeatability*: maximum relative standard deviation of 1.25 per cent for the area of the principal peak, determined on 3 injections of the middle reference solution;
— the correlation coefficient (*r*) calculated for the standard curve is not less than 0.999.

Calculate the acetate content using the standard curve and the area of the peak due to acetate in the chromatogram obtained with the test solution.

Chloride

Liquid chromatography (*2.2.29*): use the normalisation procedure. *Use the solutions within 72 h.*

Test solution Dissolve the substance to be examined in *water R* to obtain a concentration of 1 mg/mL.

Reference solution (a) Dissolve 165.9 mg of *sodium chloride R*, previously dried at 105 °C for 30 min, in *water R* and dilute to 100 mL with the same solvent.

Reference solution (b) Dissolve 150 mg of *sodium nitrite R* in *water R* and dilute to 100 mL with the same solvent. Mix 1.0 mL of the solution and 2.5 mL of reference solution (a) and dilute to 100 mL with *water R*.

Reference solutions Dilute reference solution (a) with *water R* to prepare a standard curve with at least 4 concentrations in the range of 10-40 µg/mL.

Plot peak areas versus injected chloride content and perform linear regression to create a standard curve.

Precolumn:
— *size*: *l* = 0.05 m, Ø = 4.0 mm;
— *stationary phase*: *strongly basic anion-exchange resin for chromatography R* (15 µm).

Column:
— *size*: *l* = 0.25 m, Ø = 4.0 mm;
— *stationary phase*: *strongly basic anion-exchange resin for chromatography R* (15 µm).

Mobile phase Dissolve 285.7 mg of *sodium hydrogen carbonate R* and 381.6 mg of *anhydrous sodium carbonate R* in *water R* and dilute to 2000 mL with the same solvent.

Flow rate 2.0 mL/min.

Detection Conductivity detector; use a self-regenerating anion suppressor at 100 mA.

Injection 50 µL.

Run time 6 times the retention time of chloride.

Retention time Chloride = about 1.6 min; nitrite = about 1.8 min.

System suitability Reference solution (b):
— *resolution*: minimum 1.5 between the peaks due to chloride and nitrite;
— *symmetry factor*: maximum 2.0 for the peaks due to chloride and nitrite;
— *repeatability*: maximum relative standard deviation of 2.0 per cent for the areas of the peaks due to chloride and nitrite, determined on 5 injections;
— the correlation coefficient (*r*) calculated for the standard curve is not less than 0.999.

Calculate the chloride content using the standard curve and the area of the peak due to chloride in the chromatogram obtained with the test solution.

Content

Liquid chromatography (*2.2.29*) as described in the test for related proteins with the following modifications.

Test solution Dissolve the substance to be examined in the mobile phase to obtain a concentration of 0.25 mg/mL.

Reference solution Dissolve the contents of a vial of *teriparatide CRS* in the mobile phase to obtain a concentration of 0.25 mg/mL.

Mobile phase Mobile phase A, mobile phase B (63:37 *V/V*); apply mild heating at 20-25 °C while stirring continuously during analysis.

Run time 1.5 times the retention time of teriparatide.

Retention time Teriparatide = about 10 min.

System suitability Reference solution:
— *symmetry factor*: 0.8 to 1.5 for the peak due to teriparatide;
— *repeatability*: maximum relative standard deviation of 1.25 per cent for the area of the peak due to teriparatide, determined on 3 injections.

Calculate the percentage content of teriparatide ($C_{181}H_{291}N_{55}O_{51}S_2$) taking into account the assigned content of *teriparatide CRS*.

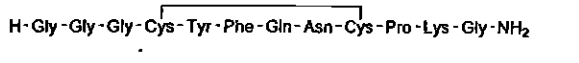
STORAGE

In an airtight container, protected from light, at −10 °C or below.

Ph Eur

Terlipressin

(Ph. Eur. monograph 2646)



$C_{52}H_{74}N_{16}O_{15}S_2$ 1227 *14636-12-5*

Action and use

Vasopressin analogue; treatment of diabetes insipidus, bleeding from varices, dialysis.

Ph Eur

DEFINITION

Glycyl-glycyl-glycyl-L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-L-cysteinyl-L-prolyl-L-lysylglycinamide cyclic (4→9)-disulfide.

Synthetic dodecapeptide analogue of the natural hormone vasopressin. It is available as an acetate.

Content
95.0 per cent to 105.0 per cent (anhydrous, acetic acid-free substance).

CHARACTERS

Appearance
White or almost white fluffy powder, hygroscopic.

IDENTIFICATION

Carry out either tests A, B or tests A, C.

A. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

B. Nuclear magnetic resonance spectrometry *(2.2.64)*.

Preparation 2.9 mg/mL solution in *deuterium oxide R* containing 20 µg/mL of *deuterated sodium trimethylsilylpropionate R*, adjusted to pH 4 with *deuterated acetic acid R*.

Comparison Dissolve the contents of a vial of *terlipressin for NMR identification CRS* in *deuterium oxide R* containing 20 µg/mL of *deuterated sodium trimethylsilylpropionate R*, adjusted to pH 4 with *deuterated acetic acid R*, to obtain a concentration of 2.9 mg/mL.

Operating conditions:
— *field strength*: minimum 300 MHz;
— *temperature*: 23 °C.

Results Examine the ^{1}H NMR spectrum from 0 to 9 ppm. The ^{1}H NMR spectrum obtained is qualitatively similar to the ^{1}H NMR spectrum obtained with *terlipressin for NMR identification CRS*.

C. Amino acid analysis *(2.2.56)*. Method 1 for hydrolysis and method 1 for analysis are suitable.

Express the content of each amino acid in moles. Calculate the relative proportions of the amino acids, taking 1/9 of the sum of the number of moles of aspartic acid, glutamic acid, proline, glycine, phenylalanine and lysine as equal to 1. The values fall within the following limits: glycine 3.6 to 4.4; half-cystine 1.4 to 2.2; tyrosine 0.7 to 1.1; phenylalanine, glutamic acid, aspartic acid, proline and lysine 0.9 to 1.1. Not more than traces of other amino acids are present.

TESTS

Specific optical rotation *(2.2.7)*
−108 to −88 (anhydrous and acetic acid-free substance).

Dissolve 125.0 mg in 25.0 mL of a 1 per cent *V/V* solution of *glacial acetic acid R*.

Related substances
Liquid chromatography *(2.2.29)*.

Buffer solution Dissolve 3.30 g of *ammonium sulfate R* in *water for chromatography R* and dilute to 5000 mL with the same solvent. Add 1.0 mL of *sulfuric acid R* and mix well; filter through a membrane filter (nominal pore size 0.45 µm).

Test solution Dissolve 10.0 mg of the substance to be examined in 50.0 mL of a 9 g/L solution of *sodium chloride R*.

Solution A Dissolve the contents of a vial of *terlipressin impurity mixture CRS* (containing impurities A, D and L) in 1.0 mL of a 9 g/L solution of *sodium chloride R*.

Solution B Dissolve the contents of a vial of *terlipressin CRS* in a 9 g/L solution of *sodium chloride R* and dilute to 2.5 mL with the same solution.

Reference solution (a) Dilute 1.0 mL of solution B to 2.0 mL with a 9 g/L solution of *sodium chloride R*.

Reference solution (b) Mix 20 µL of solution A and 500 µL of solution B, and dilute to 1.0 mL with a 9 g/L solution of *sodium chloride R*.

Reference solution (c) Mix 100 µL of solution A and 500 µL of solution B, and dilute to 1.0 mL with a 9 g/L solution of *sodium chloride R*.

Reference solution (d) Dilute 1.0 mL of the test solution to 100.0 mL with a 9 g/L solution of *sodium chloride R*. Dilute 1.0 mL of this solution to 10.0 mL with a 9 g/L solution of *sodium chloride R*.

Column:
— *size*: *l* = 0.15 m, Ø = 3.0 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 30 °C.

Mobile phase:
— *mobile phase A*: *methanol R*, buffer solution (18.5:81.5 *V/V*);
— *mobile phase B*: *methanol R*, buffer solution (30:70 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 35	100	0
35 - 55	100 → 0	0 → 100
55 - 65	0	100

Flow rate 0.6 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 200 µL of the test solution and reference solutions (a), (b), (c) and (d).

Identification of impurities Use the chromatogram supplied with *terlipressin impurity mixture CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, D and L.

Relative retention With reference to terlipressin (retention time = about 21 min): impurity E = about 0.4; impurity C = about 0.5; impurity B = about 0.7; impurity A = about 0.85; impurity G = about 1.2; impurity H = about 1.4; impurities I and F = about 2.0; impurity L = about 2.2; impurity D = about 2.3; impurity K = about 2.5; impurity J = about 2.8.

System suitability:
— *resolution*: minimum 1.4 between the peak due to impurity A and the principal peak in the chromatogram obtained with reference solution (c); the peaks due to impurities L and D are separated as shown in the chromatogram supplied with *terlipressin impurity mixture CRS*.
— *signal-to-noise ratio*: minimum 50 for the peak due to impurity A in the chromatogram obtained with reference solution (b);
— *symmetry factor*: maximum 2.0 for the peak due to terlipressin in the chromatogram obtained with reference solution (a);
— *repeatability*: maximum relative standard deviation of 2.0 per cent determined on 5 injections of reference solution (a).

Calculation of percentage contents:
— for each impurity, use the concentration of terlipressin in reference solution (d).

Limits:
— *impurity D*: maximum 0.6 per cent;

— *unspecified impurities*: for each impurity, maximum 0.5 per cent;
— *total*: maximum 1.5 per cent;
— *reporting threshold*: 0.1 per cent.

Acetic acid (*2.5.34*)
8.0 per cent to 14.0 per cent.

Test solution Dissolve 20.0 mg of the substance to be examined in a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A, and dilute to 20.0 mL with the same mixture of solvents.

Water (*2.5.32*)
Maximum 10.0 per cent, determined on 10.0 mg using the evaporation technique at 120 °C.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection 200 µL of the test solution and reference solution (a).

Calculate the percentage content of terlipressin ($C_{32}H_{74}N_{16}O_{15}S_2$) taking into account the assigned content of $C_{52}H_{74}N_{16}O_{15}S_2$ in *terlipressin CRS*.

STORAGE
In an airtight container, protected from light, at a temperature of 2 °C to 8 °C.

LABELLING
The label states the terlipressin content ($C_{52}H_{74}N_{16}O_{15}S_2$).

IMPURITIES
Specified impurities *D.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, E, F, G, H, I, J, K, L.*

H-Gly-Gly-Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Lys-Gly-NH_2

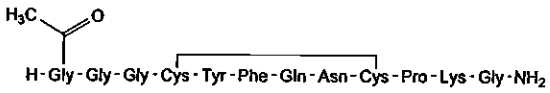
A. des-1-glycine-terlipressin,

H-Gly-Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Lys-Gly-NH_2

B. des-1,2-diglycine-terlipressin,

H-Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Lys-Gly-NH_2

C. des-(1-3)-terlipressin,



D. N^1-acetylterlipressin,

H-Gly-Gly-Gly-Cys-Tyr-D-Phe-Gln-Asn-Cys-Pro-Lys-Gly-NH_2

E. [6-D-phenylalanine]terlipressin,

H-Gly-Gly-Gly-Cys-Tyr-Phe-Glu-Asn-Cys-Pro-Lys-Gly-NH_2

F. [7-L-glutamic acid]terlipressin,

H-Gly-Gly-Gly-Cys-Tyr-Phe-Gln-β-Asp-Cys-Pro-Lys-Gly-NH_2

G. [8-L-β-aspartic acid]terlipressin,

H-Gly-Gly-Gly-Cys-Tyr-Phe-Gln-Asp-Cys-Pro-Lys-Gly-NH_2

H. [8-L-α-aspartic acid]terlipressin,

H-Gly-Gly-Gly-Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Lys-Gly-OH

I. [12-glycine]terlipressin,

H-Gly-Gly-Gly-Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Lys-Gly-OC_2H_5

J. [12-glycine]terlipressin ethyl ester,

H-Gly-Gly-Gly-Cys-Tyr-Phe-Glu-Asn-Cys-Pro-Lys-Gly-OH

K. [7-L-glutamic acid,12-glycine]terlipressin,

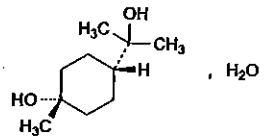
H-Gly-Gly-Gly-Cys-Tyr-Phe-Gln-Asp-Cys-Pro-Lys-Gly-OH

L. [8-L-aspartic acid,12-glycine]terlipressin.

Ph Eur

Terpin Monohydrate

(Ph. Eur. monograph 2940)



$C_{10}H_{20}O_2,H_2O$ 190.3 *2451-01-6*

Action and use
Expectorant.

Ph Eur

DEFINITION
(1*s*,4*s*)-4-(2-Hydroxypropan-2-yl)-1-methylcyclohexan-1-ol monohydrate.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Slightly soluble in water, soluble in ethanol (96 per cent), slightly soluble in methylene chloride.

mp
About 117 °C.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *terpin monohydrate CRS.*
B. Water (see Tests).

TESTS
Solution S
Dissolve 2.50 g in *ethanol (96 per cent) R* and dilute to 50.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity
To 10 mL of solution S add 0.1 mL of *bromothymol blue solution R1*. Not more than 0.2 mL of *0.02 M sodium hydroxide* or *0.02 M hydrochloric acid* is required to change the colour of the indicator.

Related substances
Gas chromatography (*2.2.28*): use the normalisation procedure.

Internal standard solution Dissolve 0.500 g of *biphenyl R* in 1 mL of *methylene chloride R* and dilute to 25.0 mL with *methanol R*.

Test solution (a) Dissolve 0.375 g of the substance to be examined in *methanol R* and dilute to 25.0 mL with the same solvent.

Test solution (b) Dissolve 85.0 mg of the substance to be examined in *methanol R*, add 2.0 mL of the internal standard solution and dilute to 100.0 mL with *methanol R*.

Reference solution (a) Dissolve 15.0 mg of *trans-terpin R* (impurity D) in test solution (a) and dilute to 10.0 mL with test solution (a). Dilute 1.0 mL of the solution to 10.0 mL with test solution (a).

Reference solution (b) Dissolve 85.0 mg of *terpin monohydrate CRS* in *methanol R*, add 2.0 mL of the internal standard solution and dilute to 100.0 mL with *methanol R*.

Column:
— *material*: fused silica;
— *size*: *l* = 30 m, Ø = 0.32 mm;
— *stationary phase*: *methylpolysiloxane R* (film thickness 1.0 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1 mL/min.

Split ratio 1:10.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 11	180
	11 - 14	180 → 270
	14 - 15	270
Injection port		220
Detector		300

Detection Flame ionisation.

Injection 1 µL of test solution (a) and reference solution (a).

Relative retention With reference to terpin (retention time = about 6.3 min): impurity D = about 1.05; internal standard = about 1.2.

System suitability Reference solution (a):
— *peak-to-valley ratio*: minimum 3, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to terpin.

Limits:
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.3 per cent;
— *reporting threshold*: 0.05 per cent.

Water (*2.5.12*)
8.0 per cent to 10.0 per cent, determined on 0.200 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Gas chromatography (*2.2.28*) as described in the test for related substances with the following modification.

Injection 0.5 µL of test solution (b) and reference solution (b).

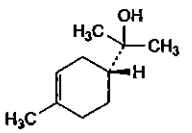
Calculate the percentage content of terpin using the following expression:

$$\frac{A_1 \times m_2 \times A_4 \times 100}{A_2 \times m_1 \times A_3}$$

A_1 = area of the peak due to terpin in the chromatogram obtained with test solution (b);
A_2 = area of the peak due to terpin in the chromatogram obtained with reference solution (b);
A_3 = area of the peak due to the internal standard in the chromatogram obtained with test solution (b);
A_4 = area of the peak due to the internal standard in the chromatogram obtained with reference solution (b);
m_1 = mass of the substance to be examined used to prepare test solution (b), in milligrams;
m_2 = mass of *terpin monohydrate CRS* used to prepare reference solution (b), in milligrams.

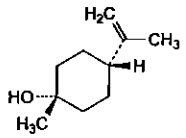
STORAGE
In an airtight container, protected from light.

IMPURITIES
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D.*

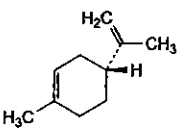


and enantiomer

A. 2-[(1*RS*)-4-methylcyclohex-3-en-1-yl]propan-2-ol (α-terpineol),

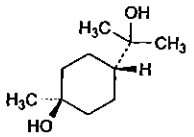


B. (1*s*,4*s*)-1-methyl-4-(prop-1-en-2-yl)cyclohexan-1-ol (*cis*-β-terpineol),



and enantiomer

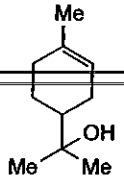
C. (4*RS*)-1-methyl-4-(prop-1-en-2-yl)cyclohex-1-ene (limonene),



D. (1*r*,4*r*)-4-(2-hydroxypropan-2-yl)-1-methylcyclohexan-1-ol (*trans*-terpin).

Ph Eur

Terpineol



$C_{10}H_{18}O$ 154.3 *98-55-5*

DEFINITION

Terpineol is a mixture of structural isomers in which *α-terpineol* predominates.

CHARACTERISTICS

A colourless, slightly viscous, liquid which may deposit crystals; odour, pleasant and characteristic.

Very slightly soluble in *water*; freely soluble in *ethanol (70%)*; soluble in *ether*.

TESTS

Refractive index

1.4800 to 1.4855, Appendix V E.

Weight per mL

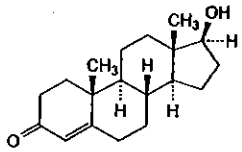
0.931 to 0.935 g, Appendix V G.

Low-boiling substances

Not more than 4.0% v/v distils below 214°, Appendix V C.

Testosterone

(Ph. Eur. monograph 1373)



$C_{19}H_{28}O_2$ 288.4 *58-22-0*

Action and use

Androgen.

Preparation

Testosterone Implants

Ph Eur

DEFINITION

17β-Hydroxyandrost-4-en-3-one.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white crystalline powder, or colourless or yellowish-white crystals.

Solubility

Practically insoluble in water, freely soluble in ethanol (96 per cent) and in methylene chloride, practically insoluble in fatty oils.

mp

About 155 °C.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *testosterone for ID and assay CRS*.

TESTS

Specific optical rotation (*2.2.7*)

~~+ 106 to + 114 (dried substance).~~

Dissolve 0.250 g in *anhydrous ethanol R* and dilute to 25.0 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R*, *water R* (30:70 *V/V*).

Test solution Dissolve 35.0 mg of the substance to be examined in 30 mL of *acetonitrile R* and dilute to 100.0 mL with *water R*.

Reference solution (a) Dissolve 9 mg of *testosterone for system suitability A CRS* (containing impurities C, G and K) in 8 mL of *acetonitrile R* and dilute to 25 mL with *water R*.

Reference solution (b) Dissolve 7.0 mg of *testosterone impurity I CRS* in 30 mL of *acetonitrile R* and dilute to 100.0 mL with *water R*. Dilute 1.0 mL of the solution to 100.0 mL with the solvent mixture.

Reference solution (c) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (d) Dissolve 35.0 mg of *testosterone for ID and assay CRS* in 30 mL of *acetonitrile R* and dilute to 100.0 mL with *water R*.

Column:

— *size*: *l* = 0.10 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped solid core phenylhexylsilyl silica gel for chromatography R* (2.7 µm);

— *temperature*: 30 °C.

Mobile phase:

— *mobile phase A*: *water for chromatography R*;

— *mobile phase B*: *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2.7	65	35
2.7 - 7	65 → 63	35 → 37
7 - 10	63 → 10	37 → 90
10 - 12	10	90

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 242 nm, and for impurity I, at 290 nm.

Injection 20 µL of the test solution and reference solutions (a), (b) and (c).

Identification of impurities Use the chromatogram supplied with *testosterone for system suitability A CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities C, G and K; use the

chromatogram obtained with reference solution (b) to identify the peak due to impurity I.

Relative retention With reference to testosterone (retention time = about 4 min): impurity I = about 0.84; impurity K = about 0.86; impurity G = about 1.1; impurity C = about 1.3.

System suitability Reference solution (a):
— *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to testosterone.

Calculation of percentage contents:
— for impurity I, use the concentration of impurity I in reference solution (b);
— for impurities other than I, use the concentration of testosterone in reference solution (c).

Limits:
— *impurity C*: maximum 0.3 per cent;
— *impurity I at 290 nm*: maximum 0.2 per cent;
— *impurity K*: maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.6 per cent;
— *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 0.500 g by drying in an oven at 105 °C for 2 h.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection 5 µL of the test solution and reference solution (d).

Calculate the percentage content of $C_{19}H_{28}O_2$ taking into account the assigned content of *testosterone for ID and assay CRS*.

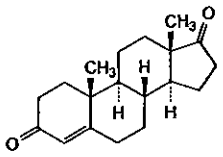
STORAGE

Protected from light.

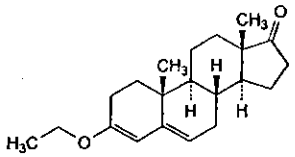
IMPURITIES

Specified impurities *C, I, K.*

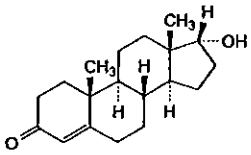
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, E, G, H, J, L, M.*



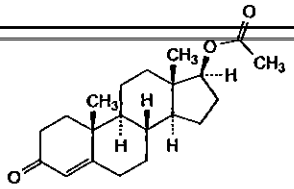
A. androst-4-ene-3,17-dione (androstenedione),



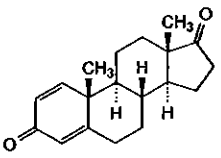
B. 3-ethoxyandrosta-3,5-dien-17-one (androstenedione ethylenolether),



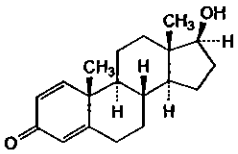
C. 17α-hydroxyandrost-4-en-3-one (17-*epi*-testosterone),



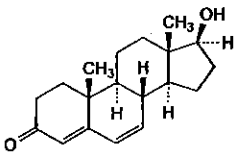
E. 3-oxoandrost-4-en-17β-yl acetate (testosterone acetate),



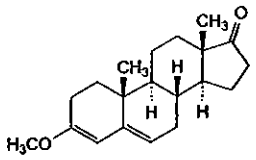
G. androsta-1,4-diene-3,17-dione (androstadienedione),



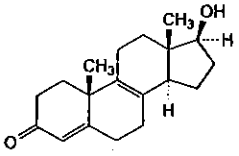
H. 17β-hydroxyandrosta-1,4-dien-3-one (boldenone),



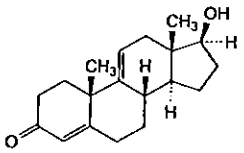
I. 17β-hydroxyandrosta-4,6-dien-3-one (Δ6-testosterone),



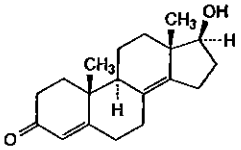
J. 3-methoxyandrosta-3,5-dien-17-one (androstenedione methylenolether),



K. 17β-hydroxyandrosta-4,8-dien-3-one,



L. 17β-hydroxyandrosta-4,9(11)-dien-3-one,

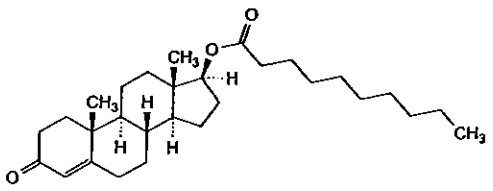


M. 17β-hydroxyandrosta-4,8(14)-dien-3-one.

Ph Eur

Testosterone Decanoate

(Ph. Eur. monograph 1736)



$C_{29}H_{46}O_3$ 442.7

Action and use
Androgen.

Ph Eur

DEFINITION
3-Oxoandrost-4-en-17β-yl decanoate.

Content
97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Practically insoluble in water, very soluble in acetone, in methylene chloride and in anhydrous ethanol, freely soluble in fatty oils.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *testosterone decanoate CRS.*

B. Examine the chromatograms obtained in the assay.
Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (c).

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).
Dissolve 0.20 g in 20 mL of *methanol R.*

Specific optical rotation (*2.2.7*)
+ 75.0 to + 80.0 (dried substance).
Dissolve 0.200 g in *anhydrous ethanol R* and dilute to 20.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).
Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.
Reference solution (a) Dissolve the contents of a vial of *testosterone decanoate for system suitability CRS* (containing impurities A, B, C, D, E and F) in 1 mL of the mobile phase.
Reference solution (b) Dilute 10.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 20.0 mL with the mobile phase.
Reference solution (c) Dissolve 20.0 mg of *testosterone decanoate CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase.
Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature:* 40 °C.
Mobile phase *water R, acetonitrile R* (5:95 *V/V*).
Flow rate 1.0 mL/min.
Detection Spectrophotometer at 240 nm.
Injection 20 µL of the test solution and reference solutions (a) and (b).
Run time Twice the retention time of testosterone decanoate.
Identification of impurities Use the chromatogram supplied with *testosterone decanoate for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D, E and F.
Relative retention With reference to testosterone decanoate (retention time = about 20 min): impurity A = about 0.2; impurity B = about 0.6; impurities C and G = about 0.79; impurity D = about 0.83; impurity E = about 1.3; impurity F = about 1.7.
System suitability Reference solution (a):
— *resolution:* minimum 1.5 between the peaks due to impurities C and D.
Limits:
— *correction factors:* for the calculation of content, multiply the peak area of impurity A by 0.7;
— *impurities A, B, D, E, F:* for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
— *sum of impurities C and G:* not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
— *unspecified impurities:* for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total:* not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
— *disregard limit:* 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Free acid
Dissolve 0.65 g in 10 mL of *ethanol (96 per cent) R,* previously neutralised to *bromothymol blue solution R3,* and

titrate immediately with *0.01 M sodium hydroxide*, using 0.1 mL of *bromothymol blue solution R3* as indicator. Not more than 0.6 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to blue.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at a pressure not exceeding 0.7 kPa.

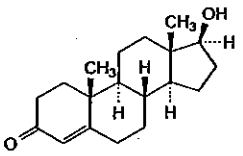
ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection 20 µL of the test solution and reference solution (c).

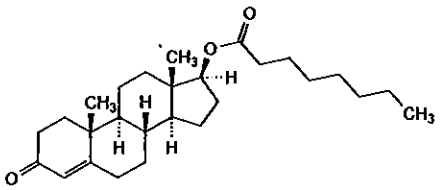
Calculate the percentage content of $C_{29}H_{46}O_3$ from the declared content of *testosterone decanoate CRS*.

STORAGE
~~At a temperature of 2 °C to 8 °C.~~

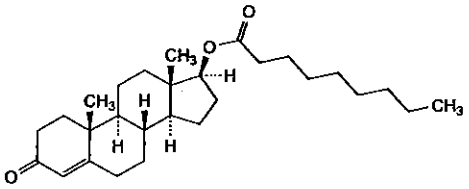
IMPURITIES
Specified impurities *A, B, C, D, E, F, G.*



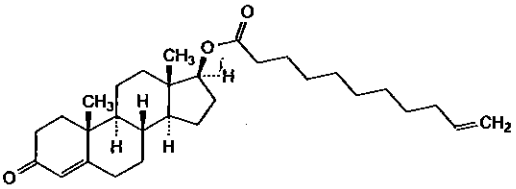
A. testosterone,



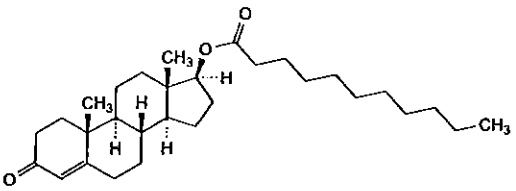
B. 3-oxoandrost-4-en-17β-yl octanoate (testosterone octanoate),



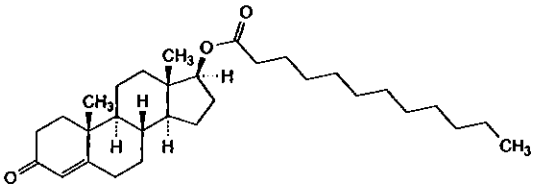
C. 3-oxoandrost-4-en-17β-yl nonanoate (testosterone nonanoate),



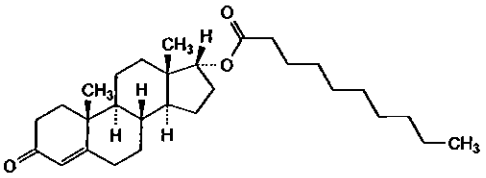
D. 3-oxoandrost-4-en-17β-yl undec-10-enoate (testosterone undecylenate),



E. 3-oxoandrost-4-en-17β-yl undecanoate (testosterone undecanoate),



F. 3-oxoandrost-4-en-17β-yl dodecanoate (testosterone laurate),

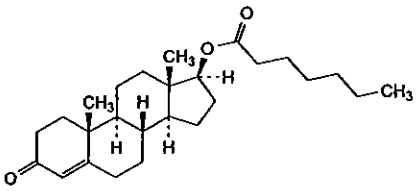


G. 3-oxoandrost-4-en-17α-yl decanoate (epitestosterone decanoate).

Ph Eur

Testosterone Enantate

(Ph. Eur. monograph 1048)



$C_{26}H_{40}O_3$ 400.6 *315-37-7*

Action and use
Androgen.

Ph Eur

DEFINITION
3-Oxoandrost-4-en-17β-yl heptanoate.

Content
97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS
Appearance
White or yellowish-white, crystalline powder.

Solubility
Practically insoluble in water, very soluble in anhydrous ethanol, freely soluble in fatty oils.

IDENTIFICATION
First identification: B.
Second identification: A, C, D.
A. Melting point (*2.2.14*): 34 °C to 39 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *testosterone enantate CRS.*

C. Thin-layer chromatography (*2.2.27*).

Solvent mixture *methanol R, methylene chloride R* (10:90 *V/V*).

Test solution Dissolve 5 mg of the substance to be examined in the solvent mixture and dilute to 10 mL with the solvent mixture.

Reference solution (a) Dissolve 5 mg of *testosterone enantate CRS* in the solvent mixture and dilute to 10 mL with the solvent mixture.

Reference solution (b) Dissolve 5 mg of *testosterone enantate CRS*, 5 mg of *testosterone decanoate CRS* and 5 mg of *testosterone isocaproate CRS* in the solvent mixture and dilute to 10 mL with the solvent mixture.

Plate *TLC octadecylsilyl silica gel F_{254} plate R.*

Mobile phase *water R, acetonitrile R, 2-propanol R* (20:40:60 *V/V/V*).

Application 5 µL.

Development Over 3/4 of the plate.

Drying In air, then at 100 °C for 10 min; allow to cool.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B Spray with *alcoholic solution of sulfuric acid R*; heat at 120 °C for 10 min; allow to cool and examine in daylight.

Results B The principal spot in the chromatogram obtained with the test solution is green and is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability Reference solution (b):
— the chromatogram shows 3 clearly separated principal spots by each method of visualisation.

D. To about 25 mg add 2 mL of a 10 g/L solution of *potassium hydroxide R* in *methanol R* and boil under a reflux condenser for 1 h. Cool. Add 10 mL of *water R*. Acidify with *dilute hydrochloric acid R* until *blue litmus paper R* turns red. Filter and wash the precipitate with a small quantity of *water R*. The residue, after drying at 60 °C at a pressure not exceeding 0.7 kPa for 3 h, melts (*2.2.14*) at 150 °C to 153 °C.

TESTS

Specific optical rotation (*2.2.7*)

+ 81 to + 86 (dried substance).

Dissolve 0.100 g in *anhydrous ethanol R* and dilute to 10.0 mL with the same solvent.

Impurity A

Maximum 0.16 per cent.

Dissolve 0.50 g in 10 mL of *ethanol (96 per cent) R* previously neutralised to *bromothymol blue solution R3*. Titrate immediately with *0.01 M sodium hydroxide* using 0.1 mL of *bromothymol blue solution R3* as indicator. Not more than 0.6 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to blue.

Impurity H

Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.100 g of the substance to be examined in 1.0 mL of *ethanol (96 per cent) R.*

Reference solution Dissolve 3.0 mg of *testosterone enantate impurity H CRS* in 20.0 mL of *ethanol (96 per cent) R.*

Plate *TLC silica gel plate R.*

Mobile phase *ethyl acetate R, cyclohexane R1* (40:60 *V/V*).

Application 1 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with a 200 g/L solution of *toluenesulfonic acid R* in *ethanol (96 per cent) R* and heat at 120 °C for 10 min; examine in ultraviolet light at 366 nm.

System suitability Reference solution:
— the chromatogram shows a clearly visible spot due to impurity H.

Limit:
— *impurity H*: any spot due to impurity H is not more intense than the principal spot in the chromatogram obtained with the reference solution (0.15 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dissolve with the aid of ultrasound the contents of a vial of *testosterone enantate for system suitability CRS* (containing impurities F and G) in the mobile phase and dilute to 1.0 mL with the mobile phase.

Reference solution (b) Dilute 2.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 20.0 mL with the mobile phase.

Reference solution (c) Dissolve 2 mg of *testosterone enantate for peak identification CRS* (containing impurity E) in 1.0 mL of the mobile phase.

Reference solution (d) Dissolve 2 mg of *testosterone caproate CRS* (impurity B) and 2 mg of *testosterone CRS* (impurity D) in the mobile phase and dilute to 5.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 100.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: spherical *end-capped dodecylsilyl silica gel for chromatography R* (4 µm).

Mobile phase *water R, acetonitrile R* (30:70 *V/V*).

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 242 nm.

Injection 10 µL.

Run time 1.5 times the retention time of testosterone enantate.

Identification of impurities Use the chromatogram supplied with *testosterone enantate for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities F and G; use the chromatogram supplied with *testosterone enantate for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peak due to impurity E; use the chromatogram obtained with reference solution (d) to identify the peaks due to impurities B and D.

Relative retention With reference to testosterone enantate (retention time = about 22 min): impurity D = about 0.1; impurity B = about 0.7; impurity E = about 0.8; impurity F = about 0.85; impurity G = about 0.9.

System suitability Reference solution (a):
— *resolution*: minimum 1.3 between the peaks due to impurities F and G.

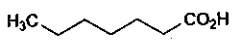
Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity F by 6.3;
— *impurity D*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent);
— *impurities E, F*: for each impurity, not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
— *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
— *impurity G*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than 7 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.7 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at a pressure not exceeding 0.7 kPa.

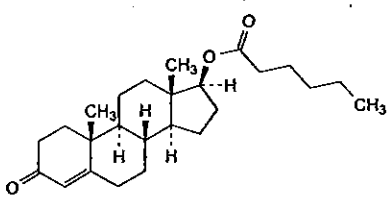
ASSAY
Dissolve 50.0 mg in *anhydrous ethanol R* and dilute to 100.0 mL with the same solvent. Dilute 2.0 mL of the solution to 100.0 mL with *anhydrous ethanol R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 241 nm.
Calculate the content of $C_{26}H_{40}O_3$ taking the specific absorbance to be 422.

STORAGE
Protected from light, at a temperature of 2 °C to 8 °C.

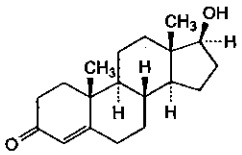
IMPURITIES
Specified impurities *A, B, D, E, F, G, H.*



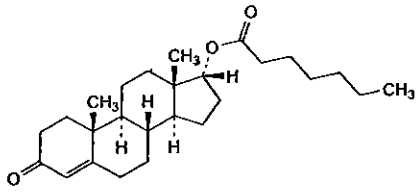
A. heptanoic acid,



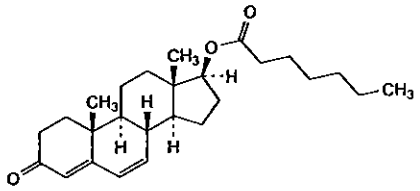
B. 3-oxoandrost-4-en-17β-yl hexanoate (testosterone caproate),



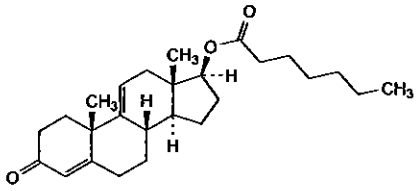
D. 17β-hydroxyandrost-4-en-3-one (testosterone),



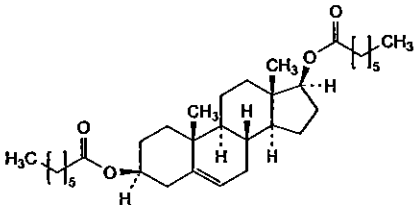
E. 3-oxoandrost-4-en-17α-yl heptanoate (17α-testosterone enantate),



F. 3-oxoandrosta-4,6-dien-17β-yl heptanoate (Δ6-testosterone enantate),



G. 3-oxoandrosta-4,9(11)-dien-17β-yl heptanoate (Δ9(11)-testosterone enantate),

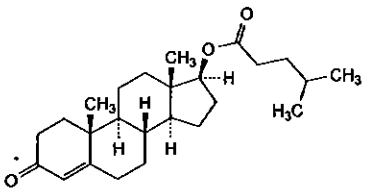


H. androst-5-ene-3β,17β-diyl diheptanoate.

Ph Eur

Testosterone Isocaproate

(Ph. Eur. monograph 1737)



$C_{25}H_{38}O_3$ 386.6

Action and use
Androgen.

Ph Eur

DEFINITION
3-Oxoandrost-4-en-17β-yl 4-methylpentanoate.

Content
97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, very soluble in acetone and in methylene chloride, freely soluble in fatty oils.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *testosterone isocaproate CRS.*

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (c).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Dissolve 0.20 g in 20 mL of *methanol R.*

Specific optical rotation (*2.2.7*)

+ 82.0 to + 88.0 (dried substance).

Dissolve 0.200 g in *anhydrous ethanol R* and dilute to 20.0 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dissolve 2 mg of *testosterone isocaproate for system suitability CRS* (containing impurities A, B, C, D, E, F and G) in 10 mL of the mobile phase.

Reference solution (b) Dilute 10.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 20.0 mL with the mobile phase.

Reference solution (c) Dissolve 20.0 mg of *testosterone isocaproate CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase.

Column:

- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature:* 40 °C.

Mobile phase *water R, acetonitrile R* (15:85 *V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 240 nm.

Injection 20 µL of the test solution and reference solutions (a) and (b).

Run time Twice the retention time of testosterone isocaproate.

Identification of impurities Use the chromatogram supplied with *testosterone isocaproate for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D, E, F and G.

Relative retention With reference to testosterone isocaproate (retention time = about 14 min): impurity A = about 0.2; impurity B = about 0.4; impurity C = about 0.5; impurity D = about 0.7; impurity G = about 0.8; impurity E = about 1.1; impurity F = about 1.4.

System suitability Reference solution (a):

- *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to testosterone isocaproate.

Limits:

- *impurities A, B, C, D, E, F, G*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Free acid

Dissolve 0.44 g in 10 mL of *ethanol (96 per cent) R,* previously neutralised to *bromothymol blue solution R3,* and titrate immediately with *0.01 M sodium hydroxide,* using 0.1 mL of *bromothymol blue solution R3* as indicator. Not more than 0.6 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to blue.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at a pressure not exceeding 0.7 kPa.

ASSAY

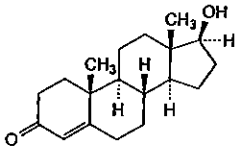
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection 20 µL of the test solution and reference solution (c).

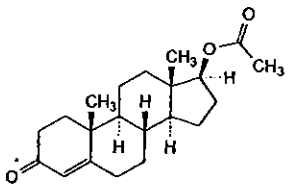
Calculate the percentage content of $C_{25}H_{38}O_3$ from the declared content of *testosterone isocaproate CRS.*

IMPURITIES

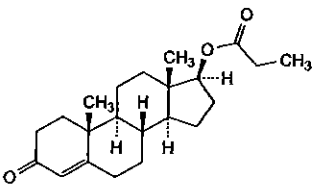
Specified impurities *A, B, C, D, E, F, G.*



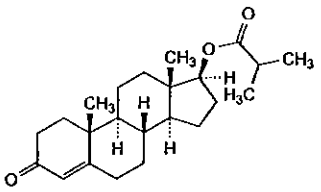
A. testosterone,



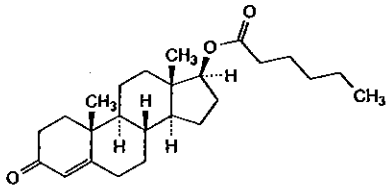
B. 3-oxoandrost-4-en-17β-yl acetate (testosterone acetate),



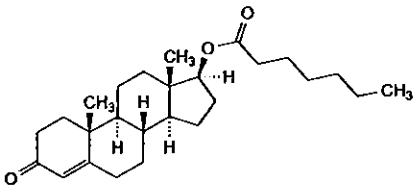
C. testosterone propionate,



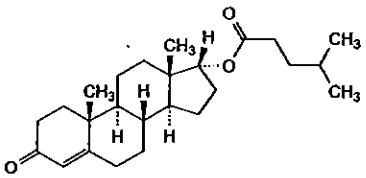
D. 3-oxoandrost-4-en-17β-yl 2-methylpropanoate (testosterone isobutyrate),



E. 3-oxoandrost-4-en-17β-yl hexanoate (testosterone caproate),



F. testosterone enantate,

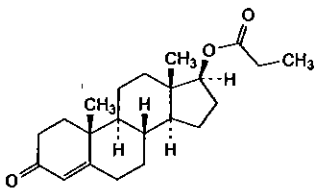


G. 3-oxoandrost-4-en-17α-yl 4-methylpentanoate (epitestosterone isocaproate).

Ph Eur

Testosterone Propionate

(Ph. Eur. monograph 0297)



$C_{22}H_{32}O_3$ 344.5 *57-85-2*

Action and use
Androgen.

Preparation
Testosterone Propionate Injection

Ph Eur

DEFINITION
3-Oxoandrost-4-en-17β-yl propanoate.

Content
97.5 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white powder or colourless crystals.

Solubility
Practically insoluble in water, freely soluble in acetone and in ethanol (96 per cent), soluble in fatty oils.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *testosterone propionate CRS.*

TESTS

Specific optical rotation (*2.2.7*)
+ 84 to + 90 (dried substance).

Dissolve 0.250 g in *ethanol R* and dilute to 25.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in *methanol R* and dilute to 50.0 mL with the same solvent.

Reference solution (a) Dissolve 2 mg of *testosterone propionate for system suitability CRS* (containing impurities A, B and C) in 5.0 mL of *methanol R.*

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *methanol R.* Dilute 1.0 mL of this solution to 10.0 mL with *methanol R.*

Reference solution (c) Dissolve 20.0 mg of *testosterone propionate CRS* in 50.0 mL of *methanol R.*

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *water R, methanol R* (20:80 *V/V*).

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL of the test solution and reference solutions (a) and (b).

Run time Twice the retention time of testosterone propionate.

Identification of impurities Use the chromatogram supplied with *testosterone propionate for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B and C.

Relative retention With reference to testosterone propionate (retention time = about 8 min): impurity C = about 0.4; impurity A = about 0.7; impurity B = about 1.4.

System suitability Reference solution (a):
— *peak-to-valley ratio*: minimum 3.0, where H_p = height above the baseline of the peak due to impurity B and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to testosterone propionate.

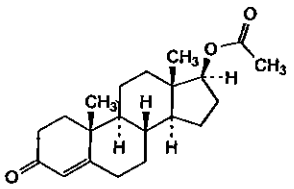
Limits:
— *impurity A*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
— *impurity C*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 7 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.7 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

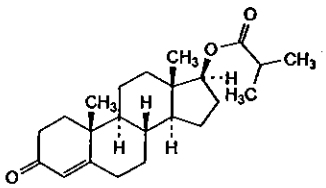
Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.
Injection Test solution and reference solution (c).
Calculate the percentage content of $C_{22}H_{32}O_3$ taking into account the assigned content of *testosterone propionate CRS*.

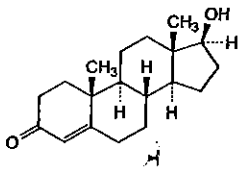
IMPURITIES
Specified impurities *A, C.*
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, D, E.*



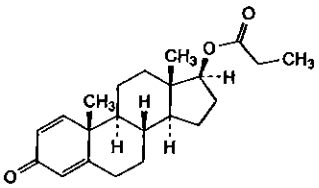
A. 3-oxoandrost-4-en-17β-yl acetate (testosterone acetate),



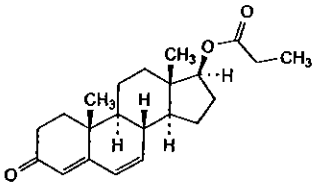
B. 3-oxoandrost-4-en-17β-yl 2-methylpropanoate (testosterone isobutyrate),



C. 17β-hydroxyandrost-4-en-3-one (testosterone),



D. 3-oxoandrosta-1,4-dien-17β-yl propanoate,

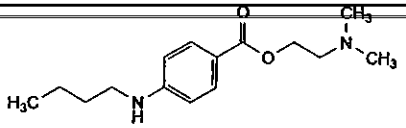


E. 3-oxoandrosta-4,6-dien-17β-yl propanoate.

Ph Eur

Tetracaine

(Ph. Eur. monograph 2909)



$C_{15}H_{24}N_2O_2$ 264.4 *94-24-6*

Action and use
Local anaesthetic.

Ph Eur

DEFINITION
2-(Dimethylamino)ethyl 4-(butylamino)benzoate.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Very slightly soluble in water, freely soluble in methylene chloride, soluble in ethanol (96 per cent).

mp
About 44 °C.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *tetracaine CRS.*

TESTS
Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 2.0 g in *ethanol (96 per cent) R* and dilute to 10 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in *acetonitrile R* and dilute to 50.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *acetonitrile R*. Dilute 1.0 mL of this solution to 10.0 mL with *acetonitrile R*.

Reference solution (b) Dissolve 10 mg of *4-aminobenzoic acid R* (impurity A), 10 mg of *4-(butylamino)benzoic acid R* (impurity B) and 10 mg of *methyl 4-(butylamino)benzoate R* (impurity C) in *acetonitrile R* and dilute to 50 mL with the same solvent. Dilute 1 mL of the solution to 10 mL with the test solution.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl amorphous organosilica polymer for chromatography R* (5 µm);
— *temperature*: 30 °C.

Mobile phase:
— *mobile phase A*: dissolve 1.36 g of *potassium dihydrogen phosphate R* in *water for chromatography R*, add 0.5 mL of *phosphoric acid R* and dilute to 1000 mL with *water for chromatography R*;
— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	80	20
3 - 18	80 → 40	20 → 60
18 - 23	40	60

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 300 nm.

Injection 5 µL.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B and C.

Relative retention With reference to tetracaine (retention time = about 7 min): impurity A = about 0.4; impurity B = about 1.6; impurity C = about 2.2.

System suitability Reference solution (b):
— *resolution*: minimum 5.0 between the peaks due to tetracaine and impurity B.

Calculation of percentage contents:
— *correction factors*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.7; impurity C = 0.7;
— for each impurity, use the concentration of tetracaine in reference solution (a).

Limits:
— *impurities B, C*: for each impurity, maximum 0.15 per cent;
— *impurity A*: maximum 0.05 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.3 per cent;
— *reporting threshold*: 0.05 per cent, except for impurity A.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 30 °C for 2 h.

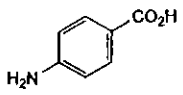
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.300 g in 50 mL of a mixture of 25 volumes of *water R* and 75 volumes of *ethanol (96 per cent) R*. Titrate with *0.1 M hydrochloric acid*, determining the end-point potentiometrically (*2.2.20*).

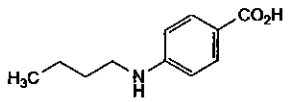
1 mL of *0.1 M hydrochloric acid* is equivalent to 26.44 mg of $C_{15}H_{24}N_2O_2$.

STORAGE
In an airtight container, protected from light.

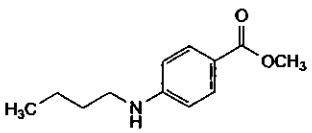
IMPURITIES
Specified impurities *A, B, C.*



A. 4-aminobenzoic acid,



B. 4-(butylamino)benzoic acid,

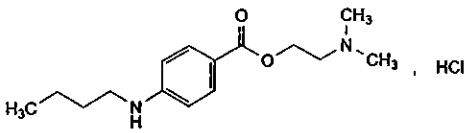


C. methyl 4-(butylamino)benzoate.

Ph Eur

Tetracaine Hydrochloride

(Ph. Eur. monograph 0057)



$C_{15}H_{25}ClN_2O_2$ 300.8 *136-47-0*

Action and use
Local anaesthetic.

Preparation
Tetracaine Eye Drops

Ph Eur

DEFINITION
2-(Dimethylamino)ethyl 4-(butylamino)benzoate hydrochloride.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, slightly hygroscopic, crystalline powder.

Solubility
Freely soluble in water, soluble in ethanol (96 per cent).

It melts at about 148 °C or it may occur in either of 2 other crystalline forms which melt respectively at about 134 °C and 139 °C. Mixtures of these forms melt within the range 134 °C to 147 °C.

IDENTIFICATION
First identification: A, B, D.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *tetracaine hydrochloride CRS.*

B. To 10 mL of solution S (see Tests) add 1 mL of *ammonium thiocyanate solution R*. A white, crystalline precipitate is formed which, after recrystallisation from

water R and drying at 80 °C for 2 h, melts (*2.2.14*) at about 131 °C.

C. To about 5 mg add 0.5 mL of *fuming nitric acid R*. Evaporate to dryness on a water-bath, allow to cool and dissolve the residue in 5 mL of *acetone R*. Add 1 mL of *0.1 M alcoholic potassium hydroxide*. A violet colour develops.

D. Solution S gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Appearance of solution

The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dilute 2 mL of solution S to 10 mL with *water R*.

pH (*2.2.3*)

4.5 to 6.5.

Dilute 1 mL of solution S to 10 mL with *carbon dioxide-free water R*.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use or store them at 2-8 °C.*

Solvent mixture *acetonitrile R, water R* (20:80 *V/V*).

Test solution Dissolve 50 mg of the substance to be examined in the solvent mixture and dilute to 50 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve the contents of a vial of *tetracaine for system suitability CRS* (containing impurities A, B and C) in 2 mL of the solvent mixture.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 30 °C.

Mobile phase:
- *mobile phase A*: dissolve 1.36 g of *potassium dihydrogen phosphate R* in *water R*, add 0.5 mL of *phosphoric acid R* and dilute to 1000 mL with *water R*;
- *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	80	20
3 - 18	80 → 40	20 → 60
18 - 23	40	60

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 300 nm.

Injection 10 µL.

Identification of impurities Use the chromatogram supplied with *tetracaine for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B and C.

Relative retention With reference to tetracaine (retention time = about 8 min): impurity A = about 0.3; impurity B = about 1.7; impurity C = about 2.1.

System suitability Reference solution (b):
- *resolution*: minimum 5.0 between the peaks due to tetracaine and impurity B.

Limits:
- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.6; impurity C = 0.7;
- *impurity A*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent);
- *impurities B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 50 mL of *ethanol (96 per cent) R* and add 5.0 mL of *0.01 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

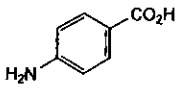
1 mL of *0.1 M sodium hydroxide* is equivalent to 30.08 mg of $C_{15}H_{25}ClN_2O_2$.

STORAGE

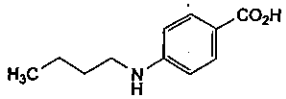
In an airtight container, protected from light.

IMPURITIES

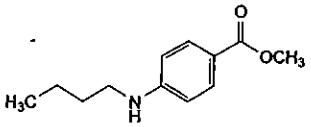
Specified impurities *A, B, C.*



A. 4-aminobenzoic acid,



B. 4-(butylamino)benzoic acid,



C. methyl 4-(butylamino)benzoate.

Tetracosactide

(Ph. Eur. monograph 0644)

H-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro-OH

$C_{136}H_{210}N_{40}O_{31}S$ 2933 *16960-16-0*

Action and use
Corticotropic peptide.

Preparations
Tetracosactide Injection
Tetracosactide Zinc Injection

Ph Eur

DEFINITION

Synthetic tetracosapeptide, in which the sequence of amino acids is the same as that of the first 24 residues of human corticotropin. It increases the rate at which corticoid hormones are secreted by the adrenal glands. It is available as an acetate.

Content
90 per cent to 102 per cent (anhydrous and acetic acid-free substance). By convention, 1 µg of tetracosactide is equivalent to 1 IU of tetracosactide.

CHARACTERS

Appearance
White or yellow, amorphous powder.

Solubility
Sparingly soluble in water.

IDENTIFICATION

A. Examine the chromatograms obtained in the test for related peptides.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with the reference solution.

B. Amino acid analysis (*2.2.56*). For hydrolysis use Method 1 and for analysis use Method 1.

Express the content of each amino acid in moles. Calculate the relative proportions of the amino acids, taking that of valine to be equivalent to 3. The values fall within the following limits: lysine 3.5 to 4.7; histidine 0.9 to 1.1; arginine 2.7 to 3.3; serine 1.1 to 2.2; glutamic acid 0.9 to 1.1; proline 2.5 to 3.5; glycine 1.8 to 2.2; methionine 0.9 to 1.1; tyrosine 1.7 to 2.2; phenylalanine 0.9 to 1.1. Not more than traces of other amino acids are present.

TESTS

Specific optical rotation (*2.2.7*)
−99 to −109 (anhydrous and acetic acid-free substance).

Dissolve 10.0 mg in 1.0 mL of a mixture of 1 volume of *glacial acetic acid R* and 99 volumes of *water R*.

Absorbance (*2.2.25*)
0.51 to 0.61 (anhydrous and acetic acid-free substance), determined at the absorption maximum between 240 nm and 280 nm, at 276 nm. The ratio of the absorbance at the maximum at 276 nm to the absorbance at 248 nm is 2.4 to 2.9.

Dissolve 1.0 mg in *0.1 M hydrochloric acid* and dilute to 5.0 mL with the same acid.

Related peptides
Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution Dissolve an accurately weighed quantity of the substance to be examined in *water R* to obtain the same concentration as in reference solution (a).

Reference solution (a) Dissolve the contents of a vial of *tetracosactide CRS* in *water R* to obtain a concentration of about 1 mg/mL, as indicated in the leaflet provided with the reference standard.

Reference solution (b) In order to prepare impurity A *in situ*, dissolve 1.0 mg of the substance to be examined in 1 mL of a 1 per cent *V/V* solution of *glacial acetic acid R*, add 50 µL of a mixture of 1 volume of *strong hydrogen peroxide solution R* and 999 volumes of *water R*, and allow to stand for 2 h.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm);
— *temperature*: 25 °C.

Mobile phase:
— *mobile phase A*: mix 5.0 mL of *glacial acetic acid R*, 60 mL of *acetonitrile R* and 5.0 g of *ammonium sulfate R* and dilute to 1000 mL with *water R*;
— *mobile phase B*: mix 5.0 mL of *glacial acetic acid R*, 310 mL of *acetonitrile R* and 5.0 g of *ammonium sulfate R* and dilute to 1000 mL with *water R*;
— *mobile phase C*: *acetonitrile R*.

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Mobile phase C (per cent *V/V*)
0 - 50	55 → 40	45 → 60	0
50 - 50.1	40 → 0	60 → 15	0 → 85
50.1 - 55	0	15	85
55 - 55.1	0 → 55	15 → 45	85 → 0
55.1 - 60	55	45	0

Flow rate 0.8 mL/min.

Detection Spectrophotometer at 275 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram supplied with *tetracosactide CRS* and the chromatogram obtained with reference solution (a) to identify the peak due to impurity B; use the chromatogram obtained with reference solution (b) to identify the peak due to impurity A.

Relative retention With reference to tetracosactide (retention time = about 26 min): impurity A = about 0.3; impurity B = about 0.95.

System suitability Reference solution (a):
— *peak-to-valley ratio*: minimum 3, where H_p = height above the baseline of the peak due to impurity B and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to tetracosactide.

Limits:
— *impurity A*: maximum 3 per cent;
— *impurity B*: maximum 4 per cent;
— *unspecified impurities*: for each impurity, maximum 2.5 per cent;
— *sum of impurities other than A*: maximum 9 per cent.

Acetic acid (*2.5.34*)
8.0 per cent to 13.0 per cent.

Test solution Dissolve 10.0 mg of the substance to be examined in a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A and dilute to 10.0 mL with the same mixture of mobile phases.

Water (*2.5.32*)
Maximum 14.0 per cent, determined on 20.0-50.0 mg.

Bacterial endotoxins (*2.6.14*)
Less than 10 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related peptides.

Calculate the content of $C_{136}H_{210}N_{40}O_{31}S$ using the declared content of *tetracosactide CRS*.

STORAGE
Protected from light, at a temperature of 2 °C to 8 °C.

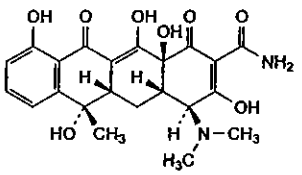
LABELLING
The label states:
— the mass of peptide in the container;
— where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

IMPURITIES
Specified impurities *A, B.*
A. tetracosactide sulfoxide,
B. unknown structure.

Ph Eur

Tetracycline

(Ph. Eur. monograph 0211)



$C_{22}H_{24}N_2O_8$ 444.4 *60-54-8*

Action and use
Tetracycline antibacterial.

Ph Eur

DEFINITION
(4*S*,4a*S*,5a*S*,6*S*,12a*S*)-4-(Dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide.

Substance produced by certain strains of *Streptomyces aerofaciens* or obtained by any other means.

Content
88.0 per cent to 102.0 per cent (dried substance).

CHARACTERS
Appearance
Yellow, crystalline powder.

Solubility
Very slightly soluble in water, soluble in ethanol (96 per cent) and in methanol, sparingly soluble in acetone. It dissolves in dilute acid and alkaline solutions.

IDENTIFICATION
A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 5 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dissolve 5 mg of *tetracycline hydrochloride CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 5 mg of *tetracycline hydrochloride CRS*, 5 mg of *demeclocycline hydrochloride R* and 5 mg of *oxytetracycline hydrochloride R* in *methanol R* and dilute to 10 mL with the same solvent.

Plate *TLC octadecylsilyl silica gel F_{254} plate R.*

Mobile phase Mix 20 volumes of *acetonitrile R*, 20 volumes of *methanol R* and 60 volumes of a 63 g/L solution of *oxalic acid R* previously adjusted to pH 2 with *concentrated ammonia R*.

Application 1 µL.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability The chromatogram obtained with reference solution (b) shows 3 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

B. To about 2 mg add 5 mL of *sulfuric acid R*. A violet-red colour develops. Add the solution to 2.5 mL of *water R*. The colour becomes yellow.

C. Dissolve about 10 mg in a mixture of 1 mL of *dilute nitric acid R* and 5 mL of *water R*. Shake and add 1 mL of *silver nitrate solution R2*. Any opalescence in the solution is not more intense than that in a mixture of 1 mL of *dilute nitric acid R*, 5 mL of *water R* and 1 mL of *silver nitrate solution R2*.

TESTS
pH (*2.2.3*)
3.5 to 6.0.

Suspend 0.1 g in 10 mL of *carbon dioxide-free water R*.

Specific optical rotation (*2.2.7*)
−260 to −280 (dried substance).

Dissolve 0.250 g in *0.1 M hydrochloric acid* and dilute to 50.0 mL with the same acid.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 25.0 mg of the substance to be examined in *0.01 M hydrochloric acid* and dilute to 25.0 mL with the same acid.

Reference solution (a) Dissolve 25.0 mg of *tetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 mL with the same acid.

Reference solution (b) Dissolve 12.5 mg of *4-epitetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 50.0 mL with the same acid.

Reference solution (c) Dissolve 10.0 mg of *anhydrotetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 100.0 mL with the same acid.

Reference solution (d) Dissolve 10.0 mg of *4-epianhydrotetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 50.0 mL with the same acid.

Reference solution (e) Mix 1.0 mL of reference solution (a), 2.0 mL of reference solution (b) and 5.0 mL of reference solution (d) and dilute to 25.0 mL with *0.01 M hydrochloric acid.*

Reference solution (f) Mix 40.0 mL of reference solution (b), 20.0 mL of reference solution (c) and 5.0 mL of reference solution (d) and dilute to 200.0 mL with *0.01 M hydrochloric acid.*

Reference solution (g) Dilute 1.0 mL of reference solution (c) to 50.0 mL with *0.01 M hydrochloric acid.*

Column:
— *size*: l = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *styrene-divinylbenzene copolymer R* (8 µm);
— *temperature*: 60 °C.

Mobile phase Weigh 80.0 g of *2-methyl-2-propanol R* and transfer to a 1000 mL volumetric flask with the aid of 200 mL of *water R*; add 100 mL of a 35 g/L solution of *dipotassium hydrogen phosphate R* adjusted to pH 9.0 with *dilute phosphoric acid R*, 200 mL of a 10 g/L solution of *tetrabutylammonium hydrogen sulfate R* adjusted to pH 9.0 with *dilute sodium hydroxide solution R* and 10 mL of a 40 g/L solution of *sodium edetate R* adjusted to pH 9.0 with *dilute sodium hydroxide solution R*; dilute to 1000.0 mL with *water R.*

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL; inject the test solution and reference solutions (e), (f) and (g).

System suitability:
— *resolution*: minimum 2.5 between the peaks due to impurity A (1st peak) and tetracycline (2nd peak) and minimum 8.0 between the peaks due to tetracycline and impurity D (3rd peak) in the chromatogram obtained with reference solution (e); if necessary, adjust the concentration of 2-methyl-2-propanol in the mobile phase;
— *signal-to-noise ratio*: minimum 3 for the principal peak in the chromatogram obtained with reference solution (g);
— *symmetry factor*: maximum 1.25 for the peak due to tetracycline in the chromatogram obtained with reference solution (e).

Limits:
— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (5.0 per cent);
— *impurity B* (eluting on the tail of the principal peak): not more than 0.4 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (f) (2.0 per cent);
— *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (1.0 per cent);
— *impurity D*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (0.5 per cent).

Loss on drying *(2.2.32)*
Maximum 13.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

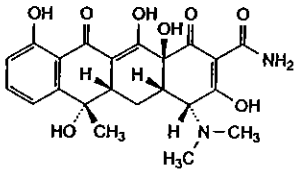
Sulfated ash *(2.4.14)*
Maximum 0.5 per cent, determined on 1.0 g.

ASSAY
Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

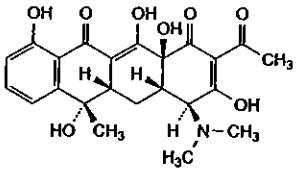
Injection Test solution and reference solution (a).
Calculate the percentage content of $C_{22}H_{24}N_2O_8$.

STORAGE
Protected from light.

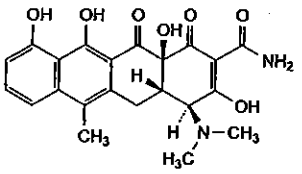
IMPURITIES



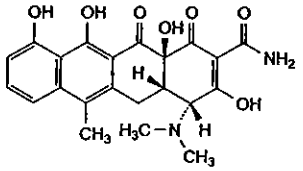
A. (4*R*,4a*S*,5a*S*,6*S*,12a*S*)-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epitetracycline),



B. (4*S*,4a*S*,5a*S*,6*S*,12a*S*)-2-acetyl-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-4a,5a,6,12a-tetrahydrotetracene-1,11(4*H*,5*H*)-dione (2-acetyl-2-decarbamoyltetracycline),



C. (4*S*,4a*S*,12a*S*)-4-(dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide (anhydrotetracycline),

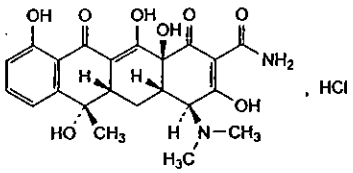


D. (4*R*,4a*S*,12a*S*)-4-(dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide (4-epianhydrotetracycline).

Ph Eur

Tetracycline Hydrochloride

(Ph. Eur. monograph 0210)



$C_{22}H_{25}ClN_2O_8$ 480.9 *64-75-5*

Action and use
Tetracycline antibacterial.

Preparations
Tetracycline Capsules
Tetracycline Tablets

Ph Eur

DEFINITION
(4*S*,4a*S*,5a*S*,6*S*,12a*S*)-4-(Dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide hydrochloride.

Substance produced by certain strains of *Streptomyces aerofaciens* or obtained by any other means.

Content
95.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
Yellow, crystalline powder.

Solubility
Soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in acetone. It dissolves in solutions of alkali hydroxides and carbonates. Solutions in water become turbid on standing, owing to the precipitation of tetracycline.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 5 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dissolve 5 mg of *tetracycline hydrochloride CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 5 mg of *tetracycline hydrochloride CRS*, 5 mg of *demeclocycline hydrochloride R* and 5 mg of *oxytetracycline hydrochloride R* in *methanol R* and dilute to 10 mL with the same solvent.

Plate *TLC octadecylsilyl silica gel F_{254} plate R.*

Mobile phase Mix 20 volumes of *acetonitrile R*, 20 volumes of *methanol R* and 60 volumes of a 63 g/L solution of *oxalic acid R* previously adjusted to pH 2 with *concentrated ammonia R*.

Application 1 µL.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability The chromatogram obtained with reference solution (b) shows 3 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

B. To about 2 mg add 5 mL of *sulfuric acid R*. A violet-red colour develops. Add the solution to 2.5 mL of *water R*. The colour becomes yellow.

C. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

pH (*2.2.3*)
1.8 to 2.8.

Dissolve 0.1 g in 10 mL of *carbon dioxide-free water R*.

Specific optical rotation (*2.2.7*)
−240 to −255 (dried substance).

Dissolve 0.250 g in *0.1 M hydrochloric acid* and dilute to 25.0 mL with the same acid.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 25.0 mg of the substance to be examined in *0.01 M hydrochloric acid* and dilute to 25.0 mL with the same acid.

Reference solution (a) Dissolve 25.0 mg of *tetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 mL with the same acid.

Reference solution (b) Dissolve 15.0 mg of *4-epitetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 50.0 mL with the same acid.

Reference solution (c) Dissolve 10.0 mg of *anhydrotetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 100.0 mL with the same acid.

Reference solution (d) Dissolve 10.0 mg of *4-epianhydrotetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 50.0 mL with the same acid.

Reference solution (e) Mix 1.0 mL of reference solution (a), 2.0 mL of reference solution (b) and 5.0 mL of reference solution (d) and dilute to 25.0 mL with *0.01 M hydrochloric acid*.

Reference solution (f) Mix 20.0 mL of reference solution (b), 10.0 mL of reference solution (c) and 5.0 mL of reference solution (d) and dilute to 200.0 mL using *0.01 M hydrochloric acid*.

Reference solution (g) Dilute 1.0 mL of reference solution (c) to 50.0 mL with *0.01 M hydrochloric acid*.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *styrene-divinylbenzene copolymer R* (8 µm);
— *temperature*: 60 °C.

Mobile phase Weigh 80.0 g of *2-methyl-2-propanol R* and transfer to a 1000 mL volumetric flask with the aid of 200 mL of *water R*; add 100 mL of a 35 g/L solution of *dipotassium hydrogen phosphate R* adjusted to pH 9.0 with *dilute phosphoric acid R*, 200 mL of a 10 g/L solution of *tetrabutylammonium hydrogen sulfate R* adjusted to pH 9.0 with *dilute sodium hydroxide solution R* and 10 mL of a 40 g/L solution of *sodium edetate R* adjusted to pH 9.0 with *dilute sodium hydroxide solution R*; dilute to 1000.0 mL with *water R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL; inject the test solution and reference solutions (e), (f) and (g).

System suitability:
— *resolution*: minimum 2.5 between the peaks due to impurity A (1st peak) and tetracycline (2nd peak) and minimum 8.0 between the peaks due to tetracycline and

impurity D (3rd peak) in the chromatogram obtained with reference solution (e); if necessary, adjust the concentration of 2-methyl-2-propanol in the mobile phase;
— *signal-to-noise ratio*: minimum 3 for the principal peak in the chromatogram obtained with reference solution (g);
— *symmetry factor*: maximum 1.25 for the peak due to tetracycline in the chromatogram obtained with reference solution (e).

Limits:
— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (3.0 per cent);
— *impurity B* (eluting on the tail of the principal peak): not more than 0.5 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (f) (1.5 per cent);
— *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (0.5 per cent);
— *impurity D*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (0.5 per cent).

Loss on drying (*2.2.32*)
Maximum 2.0 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C at a pressure not exceeding 0.7 kPa for 3 h.

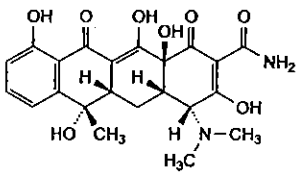
Sulfated ash (*2.4.14*)
Maximum 0.5 per cent, determined on 1.0 g.

Bacterial endotoxins (*2.6.14*)
Less than 0.5 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

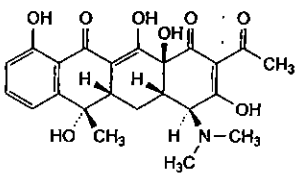
ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.
Injection Test solution and reference solution (a).
Calculate the percentage content of $C_{22}H_{25}ClN_2O_8$.

STORAGE
Protected from light. If the substance is sterile, store in a sterile, tamper-evident container.

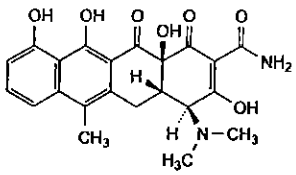
IMPURITIES



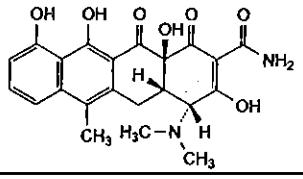
A. (4*R*,4a*S*,5a*S*,6*S*,12a*S*)-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epitetracycline),



B. (4*S*,4a*S*,5a*S*,6*S*,12a*S*)-2-acetyl-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-4a,5a,6,12a-tetrahydrotetracene-1,11(4*H*,5*H*)-dione (2-acetyl-2-decarbamoyltetracycline),



C. (4*S*,4a*S*,12a*S*)-4-(dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide (anhydrotetracycline),

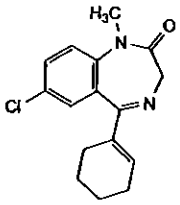


D. (4*R*,4a*S*,12a*S*)-4-(dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide (4-epianhydrotetracycline).

Ph Eur

Tetrazepam

(Ph. Eur. monograph 1738)



$C_{16}H_{17}ClN_2O$ 288.8 *10379-14-3*

Action and use
Benzodiazepine; hypnotic.

Ph Eur

DEFINITION
7-Chloro-5-(cyclohex-1-enyl)-1-methyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
Light yellow or yellow crystalline powder.
Solubility
Practically insoluble in water, freely soluble in methylene chloride, soluble in acetonitrile.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *Ph. Eur. reference spectrum of tetrazepam.*

TESTS
Related substances
Liquid chromatography (*2.2.29*).
Test solution Dissolve 25.0 mg of the substance to be examined in *acetonitrile R* and dilute to 25.0 mL with the same solvent.

Reference solution (a) Dissolve 5.0 mg of the substance to be examined and 5.0 mg of *tetrazepam impurity C CRS* in *acetonitrile R* and dilute to 10.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with *acetonitrile R*.

Reference solution (b) Dilute 1.0 mL of the test solution to 50.0 mL with *acetonitrile R*. Dilute 1.0 mL of this solution to 10.0 mL with *acetonitrile R*.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: mix 40 volumes of *acetonitrile R* and 60 volumes of a 3.4 g/L solution of *potassium dihydrogen phosphate R*;
— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 35	100	0
35 - 40	100 → 55	0 → 45
40 - 50	55	45

Flow rate 1.5 mL/min.

Detection A spectrophotometer at 229 nm.

Injection 20 µL.

System suitability Reference solution (a):
— *resolution*: minimum 2.0 between the peaks due to tetrazepam and to impurity C.

Limits:
— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Chlorides (*2.4.4*)
Maximum 100 ppm.

Dissolve 0.750 g in 10 mL of *methylene chloride R* and add 15 mL of *water R*. Shake and separate the 2 layers. Dilute 10 mL of the aqueous layer to 15 mL with *water R*.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

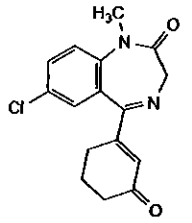
Dissolve 0.230 g in 50.0 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 28.88 mg of $C_{16}H_{17}ClN_2O$.

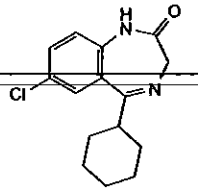
STORAGE

Protected from light.

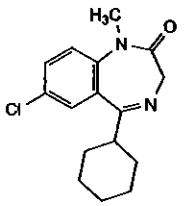
IMPURITIES



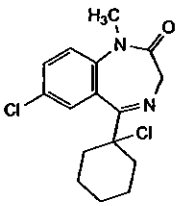
A. 7-chloro-1-methyl-5-(3-oxocyclohex-1-enyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one,



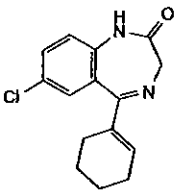
B. 7-chloro-5-cyclohexyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one,



C. 7-chloro-5-cyclohexyl-1-methyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one,



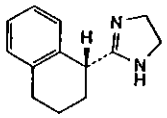
D. 7-chloro-5-(1-chlorocyclohexyl)-1-methyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one,



E. 7-chloro-5-(cyclohex-1-enyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.

Tetryzoline Hydrochloride

(Ph. Eur. monograph 2101)



and enantiomer, HCl

$C_{13}H_{17}ClN_2$ 236.7 *522-48-5*

Action and use
Adrenoceptor agonist; decongestant.

Ph Eur

DEFINITION

2-[(1*RS*)-1,2,3,4-Tetrahydronaphthalen-1-yl]-4,5-dihydro-1*H*-imidazole hydrochloride.

Content
98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water, in anhydrous ethanol and in ethanol (96 per cent), practically insoluble in acetone.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *tetryzoline hydrochloride CRS.*

B. Dissolve 50 mg in 10 mL of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 1.0 g in *water R* and dilute to 10 mL with the same solvent.

Related substances
Gas chromatography (*2.2.28*).

Test solution Dissolve 1.0 g of the substance to be examined in a mixture of 25 volumes of *1 M sodium hydroxide* and 75 volumes of *methanol R* and dilute to 10 mL with the same mixture of solvents.

Reference solution Dilute 1.0 mL of the test solution to 100.0 mL with a mixture of 25 volumes of *1 M sodium hydroxide* and 75 volumes of *methanol R*. Dilute 1.0 mL of this solution to 10.0 mL with a mixture of 25 volumes of *1 M sodium hydroxide* and 75 volumes of *methanol R*.

Column:
— *material*: fused silica,
— *size*: *l* = 25 m, Ø = 0.32 mm,
— *stationary phase*: *methylpolysiloxane R* (1 µm).

Carrier gas *helium for chromatography R.*

Split ratio 1:40.

Flow rate 2.5 mL/min.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 8	160
	8 - 11	160 → 220
	11 - 15	220
Injection port		220
Detector		220

Detection Flame ionisation.

Injection 1 µL.

Relative retention With reference to tetryzoline (retention time = about 12 min): impurity A = about 0.5.

System suitability Reference solution:
— *signal-to-noise ratio*: minimum 50 for the principal peak.

Limits:
— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent),
— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent),
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent),
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

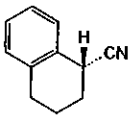
ASSAY

Dissolve 0.200 g in 100 mL of a mixture of 3 volumes of *anhydrous acetic acid R* and 7 volumes of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 mL of *0.1 M perchloric acid* is equivalent to 23.67 mg of $C_{13}H_{17}ClN_2$.

IMPURITIES

Specified impurities *A.*



and enantiomer

A. (1*RS*)-1,2,3,4-tetrahydronaphthalene-1-carbonitrile (α-cyanotetraline).

Ph Eur

Theophylline

(Ph. Eur. monograph 0299)

$C_7H_8N_4O_2$ 180.2 *58-55-9*

Action and use
Non-selective phosphodiesterase inhibitor (xanthine); treatment of reversible airways obstruction.

Preparations
Aminophylline Injection
Theophylline Prolonged-release Tablets

Ph Eur

DEFINITION
1,3-Dimethyl-3,7-dihydro-1*H*-purine-2,6-dione.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Slightly soluble in water, sparingly soluble in ethanol (96 per cent). It dissolves in solutions of alkali hydroxides, in ammonia and in mineral acids.

IDENTIFICATION
First identification: B, D.

Second identification: A, C, D, E.

A. Melting point (*2.2.14*): 270 °C to 274 °C, determined after drying at 100-105 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of theophylline.*

C. Heat 10 mg with 1.0 mL of a 360 g/L solution of *potassium hydroxide R* in a water-bath at 90 °C for 3 min, then add 1.0 mL of *diazotised sulfanilic acid solution R*. A red colour slowly develops. Carry out a blank test.

D. Loss on drying (see Tests).

E. It gives the reaction of xanthines (*2.3.1*).

TESTS
Solution S
Dissolve 0.5 g with heating in *carbon dioxide-free water R*, cool and dilute to 75 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity
To 50 mL of solution S add 0.1 mL of *methyl red solution R*. The solution is red. Not more than 1.0 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to yellow.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 40.0 mg of the substance to be examined in the mobile phase and dilute to 20.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 10 mg of *theobromine R* in the mobile phase, add 5 mL of the test solution and dilute to 100 mL with the mobile phase. Dilute 5 mL of this solution to 50 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (7 µm).

Mobile phase Mix 7 volumes of *acetonitrile for chromatography R* and 93 volumes of a 1.36 g/L solution of *sodium acetate R* containing 5.0 mL/L of *glacial acetic acid R*.

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 272 nm.

Injection 20 µL.

Run time 3.5 times the retention time of theophylline.

Relative retention With reference to theophylline (retention time = about 6 min): impurity C = about 0.3; impurity B = about 0.4; impurity D = about 0.5; impurity A = about 2.5.

System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the peaks due to theobromine and theophylline.

Limits:
— *impurities A, B, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

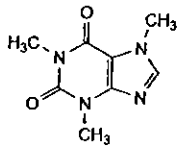
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.150 g in 100 mL of *water R*, add 20 mL of *0.1 M silver nitrate* and shake. Add 1 mL of *bromothymol blue solution R1*. Titrate with *0.1 M sodium hydroxide*.

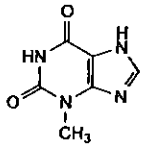
1 mL of *0.1 M sodium hydroxide* is equivalent to 18.02 mg of $C_7H_8N_4O_2$.

IMPURITIES
Specified impurities *A, B, C, D.*

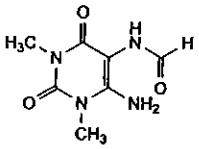
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *E, F.*



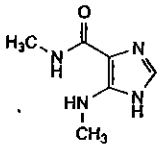
A. 1,3,7-trimethyl-3,7-dihydro-1*H*-purine-2,6-dione (caffeine),



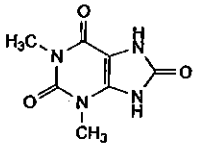
B. 3-methyl-3,7-dihydro-1*H*-purine-2,6-dione,



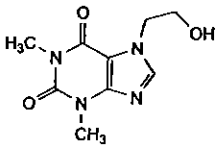
C. *N*-(6-amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)formamide,



D. *N*-methyl-5-(methylamino)-1*H*-imidazole-4-carboxamide (theophyllidine),



E. 1,3-dimethyl-7,9-dihydro-1*H*-purine-2,6,8(3*H*)-trione,

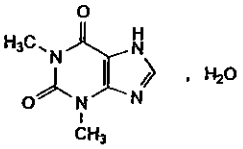


F. 7-(2-hydroxyethyl)-1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione (etofylline).

Ph Eur

Theophylline Hydrate

(Theophylline Monohydrate, Ph. Eur. monograph 0302)



$C_7H_8N_4O_2,H_2O$ 198.2 *5967-84-0*

Action and use
Non-selective phosphodiesterase inhibitor (xanthine); treatment of reversible airways obstruction.

Preparations
Aminophylline Injection
Theophylline Prolonged-release Tablets

Ph Eur

DEFINITION

1,3-Dimethyl-3,7-dihydro-1*H*-purine-2,6-dione monohydrate.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Slightly soluble in water, sparingly soluble in ethanol (96 per cent). It dissolves in solutions of alkali hydroxides, in ammonia and in mineral acids.

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D, E.

A. Melting point (*2.2.14*): 270 °C to 274 °C, determined after drying at 100-105 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Dry the substance to be examined at 100-105 °C before use.

Comparison *Ph. Eur. reference spectrum of theophylline.*

C. Heat 10 mg with 1.0 mL of a 360 g/L solution of *potassium hydroxide R* in a water-bath at 90 °C for 3 min, then add 1.0 mL of *diazotised sulfanilic acid solution R*. A red colour slowly develops. Carry out a blank test.

D. Water (see Tests).

E. It gives the reaction of xanthines (*2.3.1*).

TESTS

Solution S
Dissolve 0.5 g with heating in *carbon dioxide-free water R*, cool and dilute to 75 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity
To 50 mL of solution S add 0.1 mL of *methyl red solution R*. The solution is red. Not more than 1.0 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to yellow.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 40.0 mg of the substance to be examined in the mobile phase and dilute to 20.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 10 mg of *theobromine R* in the mobile phase, add 5 mL of the test solution and dilute to 100 mL with the mobile phase. Dilute 5 mL of this solution to 50 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (7 µm).

Mobile phase Mix 7 volumes of *acetonitrile for chromatography R* and 93 volumes of a 1.36 g/L solution of *sodium acetate R* containing 5.0 mL/L of *glacial acetic acid R*.

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 272 nm.

Injection 20 µL.

Run time 3.5 times the retention time of theophylline.

Relative retention With reference to theophylline (retention time = about 6 min): impurity C = about 0.3; impurity B = about 0.4; impurity D = about 0.5; impurity A = about 2.5.

System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the peaks due to theobromine and theophylline.

Limits:
— *impurities A, B, C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)

8.0 per cent to 9.5 per cent, determined on 0.20 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

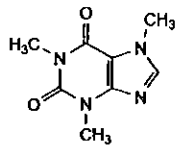
Dissolve 0.160 g in 100 mL of *water R*, add 20 mL of *0.1 M silver nitrate* and shake. Add 1 mL of *bromothymol blue solution R1*. Titrate with *0.1 M sodium hydroxide*.

1 mL of *0.1 M sodium hydroxide* is equivalent to 18.02 mg of $C_7H_8N_4O_2$.

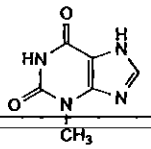
IMPURITIES

Specified impurities *A, B, C, D.*

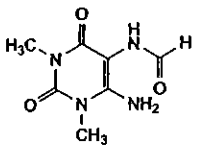
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *E, F.*



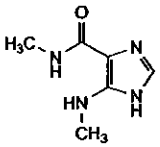
A. 1,3,7-trimethyl-3,7-dihydro-1*H*-purine-2,6-dione (caffeine),



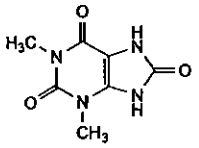
B. 3-methyl-3,7-dihydro-1*H*-purine-2,6-dione,



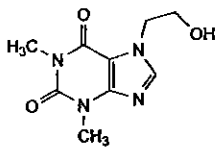
C. *N*-(6-amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)formamide,



D. *N*-methyl-5-(methylamino)-1*H*-imidazole-4-carboxamide (theophyllidine),



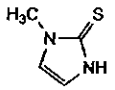
E. 1,3-dimethyl-7,9-dihydro-1*H*-purine-2,6,8(3*H*)-trione,



F. 7-(2-hydroxyethyl)-1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione (etofylline).

Thiamazole

(Ph. Eur. monograph 1706)



$C_4H_6N_2S$ 114.2 *60-56-0*

Action and use
Thionamide antithyroid.

Ph Eur

DEFINITION
1-Methyl-1,3-dihydro-2*H*-imidazole-2-thione.

Content
98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or pale brown, crystalline powder.

Solubility
Freely soluble in water, freely soluble in methylene chloride, freely soluble or soluble in ethanol (96 per cent).

IDENTIFICATION
First identification: A, C.

Second identification: A, B, D.

A. Melting point (*2.2.14*): 143 °C to 146 °C.

B. Dissolve 25 mg in 10 mL of a 0.28 per cent *V/V* solution of *sulfuric acid R* and dilute to 50.0 mL with the same solution. Dilute 1.0 mL of this solution to 100.0 mL with a 0.28 per cent *V/V* solution of *sulfuric acid R*. Examined between 200 nm and 300 nm (*2.2.25*), the solution shows 2 absorption maxima, at 211 nm and 251 nm. The ratio of the absorbance measured at the absorption maximum at 251 nm to that measured at the absorption maximum at 211 nm is 2.5 to 2.7.

C. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *thiamazole CRS*.

D. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 5.0 mg of the substance to be examined in *methanol R* and dilute to 5.0 mL with the same solvent.

Reference solution (a) Dissolve 5.0 mg of *thiamazole CRS* in *methanol R* and dilute to 5.0 mL with the same solvent.

Reference solution (b) Dissolve 5.0 mg of *2-methylimidazole R* in *methanol R* and dilute to 5.0 mL with the same solvent. Dilute 1.0 mL of this solution to 2.0 mL with the test solution.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *concentrated ammonia R1, 2-propanol R, toluene R* (1:24:75 *V/V/V*).

Application 10 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):
— expose the plate to iodine vapour for 30 min; the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS
Solution S
Dissolve 2.0 g in *water R* and dilute to 20.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_6 (*2.2.2, Method II*).

Related substances
Gas chromatography (*2.2.28*).

Test solution Dissolve 0.100 g of the substance to be examined in *chloroform R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *chloroform R*. Dilute 1.0 mL of this solution to 10.0 mL with *chloroform R*.

Reference solution (b) Dissolve 5.0 mg of *thiamazole impurity A CRS*, 5.0 mg of *1-methylimidazole R1* and 5.0 mg of *thiamazole impurity C CRS* in *chloroform R* and dilute to 50.0 mL with the same solvent. Dilute 1.0 mL of this solution to 10.0 mL with *chloroform R*.

Column:
— *material*: fused silica,
— *size*: *l* = 30.0 m, Ø = 0.25 mm,
— *stationary phase*: *base-deactivated phenyl(5)methyl(95) polysiloxane R* (film thickness 0.5 µm).

Carrier gas *helium for chromatography R.*

Flow rate 1.5 mL/min.

Split ratio 3:20.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 2	100
	2 - 7	100 → 250
	7 - 22	250
Injection port		150
Detector		250

Detection Flame ionisation.

Injection 1 µL.

Relative retention With reference to thiamazole (retention time = about 6.5 min): impurity A = about 0.3; impurity B = about 0.4; impurity C = about 0.7.

System suitability Reference solution (b):
— *resolution*: minimum 1.5 between the peaks due to impurity A and impurity B.

Limits:
— *impurities A, B, C*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent),
— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),
— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

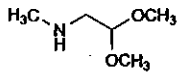
Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

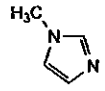
ASSAY
Dissolve 0.250 g in 75 mL of *water R*. Add 15.0 mL of *0.1 M sodium hydroxide*, mix and add with stirring, about 30 mL of *0.1 M silver nitrate*. Continue the titration with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M sodium hydroxide* is equivalent to 11.42 mg of $C_4H_6N_2S$.

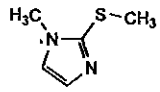
IMPURITIES
Specified impurities *A, B, C.*



A. 2,2-dimethoxy-*N*-methylethanamine,



B. 1-methyl-1*H*-imidazole,

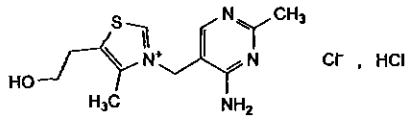


C. 1-methyl-2-(methylsulfanyl)-1*H*-imidazole.

Ph Eur

Thiamine Hydrochloride

(Ph. Eur. monograph 0303)



$C_{12}H_{18}Cl_2N_4OS$ 337.3 *67-03-8*

Action and use
Vitamin B_1.

Preparations
Thiamine Injection
Thiamine Tablets
Vitamins B and C Injection

Ph Eur

DEFINITION
3-[(4-Amino-2-methylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methyl-1,3-thiazol-3-ium chloride hydrochloride.

Content
98.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white, crystalline powder or colourless crystals.

Solubility
Freely soluble in water, soluble in glycerol, slightly soluble in ethanol (96 per cent), practically insoluble in heptane.

IDENTIFICATION
First identification: A, C.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *thiamine hydrochloride CRS.*

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *water R*, evaporate to dryness and record new spectra using the residues.

B. Dissolve about 20 mg in 10 mL of *water R*, add 1 mL of *dilute acetic acid R* and 1.6 mL of *1 M sodium hydroxide*, heat on a water-bath for 30 min and allow to cool. Add 5 mL of *dilute sodium hydroxide solution R*, 10 mL of *potassium ferricyanide solution R* and 10 mL of *butanol R* and shake vigorously for 2 min. The upper alcoholic layer shows an intense light-blue fluorescence, especially in ultraviolet light at 365 nm. Repeat the test using 0.9 mL of *1 M sodium hydroxide* and 0.1 g of *anhydrous sodium sulfite R* instead of 1.6 mL of *1 M sodium hydroxide*. Practically no fluorescence is seen.

C. It gives reaction (a) of chlorides (*2.3.1*).

TESTS
Solution S
Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent.

Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 or GY_7 (*2.2.2, Method II*).

Dilute 2.5 mL of solution S to 5 mL with *water R*.

pH (*2.2.3*)
2.7 to 3.3.

Dilute 2.5 mL of solution S to 10 mL with *water R*.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.35 g of the substance to be examined in 15.0 mL of a 5 per cent *V/V* solution of *glacial acetic acid R* and dilute to 100.0 mL with *water R*.

Reference solution (a) Dissolve the contents of a vial of *thiamine for system suitability CRS* (containing impurities A, B and C) in 1 mL of a 0.75 per cent *V/V* solution of *glacial acetic acid R*.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *water R*. Dilute 1.0 mL of this solution to 10.0 mL with *water R*.

Column:
— *size: l* = 0.25 m, Ø = 4.0 mm;
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 45 °C.

Mobile phase:
— *mobile phase A*: 3.764 g/L solution of *sodium hexanesulfonate R* adjusted to pH 3.1 with *phosphoric acid R*;
— *mobile phase B*: *methanol R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	90	10
2 - 27	90 → 70	10 → 30
27 - 35	70 → 50	30 → 50
35 - 42	50	50

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 248 nm.

Injection 25 µL.

Identification of impurities Use the chromatogram supplied with *thiamine for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B and C.

Relative retention With reference to thiamine (retention time = about 30 min): impurity A = about 0.3; impurity B = about 0.9; impurity C = about 1.2.

System suitability Reference solution (a):
— *resolution*: minimum 3.0 between the peaks due to impurity B and thiamine; minimum 2.0 between the peaks due to thiamine and impurity C.

Calculation of percentage contents:
— for each impurity, use the concentration of thiamine hydrochloride in reference solution (b).

Limits:
— *impurity B*: maximum 0.3 per cent;
— *impurities A, C*: for each impurity, maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.5 per cent;
— *reporting threshold*: 0.05 per cent.

Sulfates (*2.4.13*)
Maximum 300 ppm.

Dilute 5 mL of solution S to 15 mL with *distilled water R*.

Water (*2.5.12*)
Maximum 5.0 per cent, determined on 0.400 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.110 g in 5 mL of *anhydrous formic acid R* and add 50 mL of *acetic anhydride R*. Titrate immediately with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*) and carrying out the titration within 2 min. Carry out a blank titration.

1 mL of *0.1 M perchloric acid* is equivalent to 16.86 mg of $C_{12}H_{18}Cl_2N_4OS$.

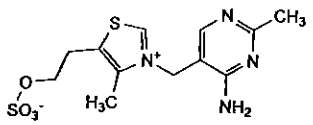
STORAGE

In a non-metallic container, protected from light.

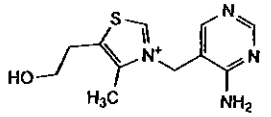
IMPURITIES

Specified impurities *A, B, C.*

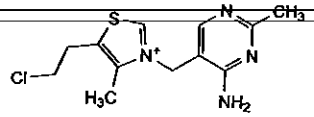
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *D, E, F, G, H.*



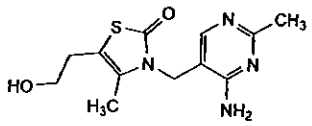
A. 2-[3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-4-methyl-1,3-thiazol-3-ium-5-yl]ethyl sulfate (thiamine sulfate ester),



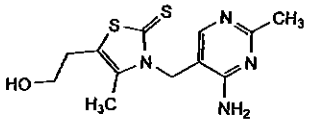
B. 3-[(4-aminopyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methyl-1,3-thiazol-3-ium (desmethylthiamine),



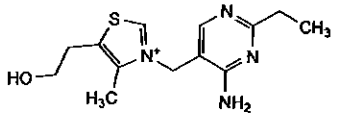
C. 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-5-(2-chloroethyl)-4-methyl-1,3-thiazol-3-ium (chlorothiamine),



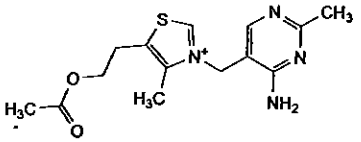
D. 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methyl-1,3-thiazol-2(3*H*)-one (oxothiamine),



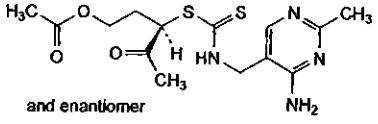
E. 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methyl-1,3-thiazol-2(3*H*)-thione (thioxothiamine),



F. 3-[(4-amino-2-ethylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methyl-1,3-thiazol-3-ium (ethylthiamine),



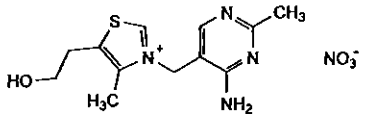
G. 5-[2-(acetyloxy)ethyl]-3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-4-methyl-1,3-thiazol-3-ium (acetylthiamine),



H. (3*RS*)-3-[[[(4-amino-2-methylpyrimidin-5-yl)methyl]thiocarbamoyl]sulfanyl]-4-oxopentyl acetate (ketodithiocarbamate).

Thiamine Nitrate

(Ph. Eur. monograph 0531)



$C_{12}H_{17}N_5O_4S$ 327.4 *532-43-4*

Action and use
Vitamin B_1.

Ph Eur

DEFINITION
3-[(4-Amino-2-methylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methyl-1,3-thiazol-3-ium nitrate.

Content
98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder or small, colourless crystals.

Solubility
Sparingly soluble in water, freely soluble in boiling water, slightly soluble in ethanol (96 per cent), practically insoluble in heptane.

IDENTIFICATION
First identification: A, C.
Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *thiamine nitrate CRS.*

B. Dissolve about 20 mg in 10 mL of *water R*, add 1 mL of *dilute acetic acid R* and 1.6 mL of *1 M sodium hydroxide*, heat on a water-bath for 30 min and allow to cool. Add 5 mL of *dilute sodium hydroxide solution R*, 10 mL of *potassium ferricyanide solution R* and 10 mL of *butanol R* and shake vigorously for 2 min. The upper alcoholic layer shows an intense light-blue fluorescence, especially in ultraviolet light at 365 nm. Repeat the test using 0.9 mL of *1 M sodium hydroxide* and 0.2 g of *sodium sulfite heptahydrate R* instead of 1.6 mL of *1 M sodium hydroxide*. Practically no fluorescence is produced.

C. About 5 mg gives the reaction of nitrates (*2.3.1*).

TESTS

Solution S
Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

pH (*2.2.3*)
6.8 to 7.6 for solution S.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.35 g of the substance to be examined in 15.0 mL of a 5 per cent *V/V* solution of *glacial acetic acid R* and dilute to 100.0 mL with *water R*.

Reference solution (a) Dissolve the contents of a vial of *thiamine for system suitability CRS* (containing impurities A, B and C) in 1 mL of a 0.75 per cent *V/V* solution of *glacial acetic acid R*.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *water R*. Dilute 1.0 mL of this solution to 10.0 mL with *water R*.

Column:
— *size*: *l* = 0.25 m, Ø = 4.0 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 45 °C.

Mobile phase:
— *mobile phase A*: 3.764 g/L solution of *sodium hexanesulfonate R* adjusted to pH 3.1 with *phosphoric acid R*;
— *mobile phase B*: *methanol R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	90	10
2 - 27	90 → 70	10 → 30
27 - 35	70 → 50	30 → 50
35 - 42	50	50

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 248 nm.

Injection 25 µL.

Identification of impurities Use the chromatogram supplied with *thiamine for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B and C.

Relative retention With reference to thiamine (retention time = about 30 min): impurity A = about 0.3; impurity B = about 0.9; impurity C = about 1.2.

System suitability Reference solution (a):
— *resolution*: minimum 3.0 between the peaks due to impurity B and thiamine; minimum 2.0 between the peaks due to thiamine and impurity C.

Calculation of percentage contents:
— for each impurity, use the concentration of thiamine nitrate in reference solution (b).

Limits:
— *impurity B*: maximum 0.6 per cent;
— *impurity C*: maximum 0.4 per cent;
— *impurity A*: maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 1.0 per cent;
— *reporting threshold*: 0.05 per cent; disregard the peak due to the nitrate ion at the beginning of the chromatogram.

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.140 g in 5 mL of *anhydrous formic acid R* and add 50 mL of *acetic anhydride R*. Titrate immediately with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*) and carrying out the titration within 2 min. Carry out a blank titration.

1.0 mL of *0.1 M perchloric acid* is equivalent to 16.37 mg of $C_{12}H_{17}N_5O_4S$.

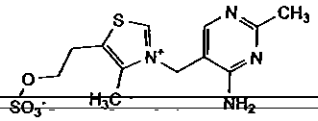
STORAGE

In a non-metallic container, protected from light.

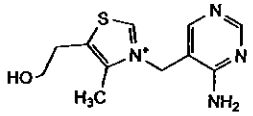
IMPURITIES

Specified impurities *A, B, C.*

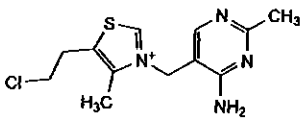
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *D, E, F, G, H.*



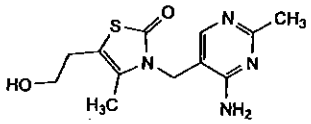
A. 2-[3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-4-methyl-1,3-thiazol-3-ium-5-yl]ethyl sulfate (thiamine sulfate ester),



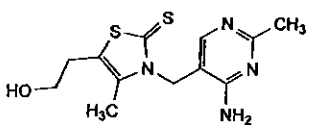
B. 3-[(4-aminopyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methyl-1,3-thiazol-3-ium (desmethylthiamine),



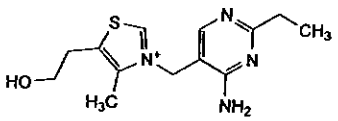
C. 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-5-(2-chloroethyl)-4-methyl-1,3-thiazol-3-ium (chlorothiamine),



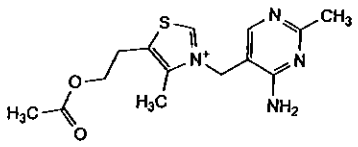
D. 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methyl-1,3-thiazol-2(3*H*)-one (oxothiamine),



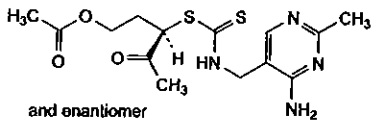
E. 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methyl-1,3-thiazol-2(3*H*)-thione (thioxothiamine),



F. 3-[(4-amino-2-ethylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methyl-1,3-thiazol-3-ium (ethylthiamine),



G. 5-[2-(acetyloxy)ethyl]-3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-4-methyl-1,3-thiazol-3-ium (acetylthiamine),

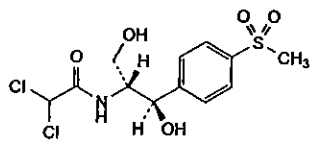


H. (3*RS*)-3-[[[(4-amino-2-methylpyrimidin-5-yl)methyl]thiocarbamoyl]sulfanyl]-4-oxopentyl acetate (ketodithiocarbamate).

Ph Eur

Thiamphenicol

(Ph. Eur. monograph 0109)



$C_{12}H_{15}Cl_2NO_5S$ 356.2 *15318-45-3*

Action and use

Antibacterial.

Ph Eur

DEFINITION

2,2-Dichloro-*N*-[(1*R*,2*R*)-2-hydroxy-1-(hydroxymethyl)-2-[4-(methylsulfonyl)phenyl]ethyl]acetamide.

Content

98.0 per cent to 100.5 per cent (dried substance).

CHARACTERS

Appearance

Fine, white or yellowish-white, crystalline powder or crystals.

Solubility

Slightly soluble in water, very soluble in dimethylacetamide, freely soluble in acetonitrile and in dimethylformamide, soluble in methanol, sparingly soluble in acetone and in anhydrous ethanol, slightly soluble in ethyl acetate.

A solution in anhydrous ethanol is dextrorotatory and a solution in dimethylformamide is laevorotatory.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Dry the substance to be examined and the reference substance at 100-105 °C for 2 h; examine as discs of *potassium bromide R*.

Comparison *thiamphenicol CRS*.

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.1 g of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 0.1 g of *thiamphenicol CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Plate *silica gel GF_{254} R* as the coating substance.

Mobile phase *methanol R, ethyl acetate R* (3:97 *V/V*).

Application 5 µL.

Development Over a path of 10 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the spot in the chromatogram obtained with the reference solution.

C. To 50 mg in a porcelain crucible add 0.5 g of *anhydrous sodium carbonate R*. Heat over an open flame for 10 min. Allow to cool. Take up the residue with 5 mL of *dilute nitric acid R* and filter. To 1 mL of the filtrate add 1 mL of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Acidity or alkalinity
Shake 0.1 g with 20 mL of *carbon dioxide-free water R* and add 0.1 mL of *bromothymol blue solution R1*. Not more than 0.1 mL of *0.02 M hydrochloric acid* or *0.02 M sodium hydroxide* is required to change the colour of the indicator.

Specific optical rotation (*2.2.7*)
−24 to −21 (dried substance).

Dissolve 1.25 g in *dimethylformamide R* and dilute to 25.0 mL with the same solvent.

Melting point (*2.2.14*)
163 °C to 167 °C.

Absorbance (*2.2.25*)
Test solution (a) Dissolve 20 mg in *water R*, heating to about 40 °C, and dilute to 100.0 mL with the same solvent.

Test solution (b) Dilute 2.5 mL of test solution (a) to 50.0 mL with *water R*.

Spectral range 240-300 nm for test solution (a); 200-240 nm for test solution (b).

Absorption maxima At 266 nm and 273 nm for test solution (a); at 224 nm for test solution (b).

Specific absorbances at the absorption maxima:
— at 266 nm: 25 to 28 for test solution (a);
— at 273 nm: 21.5 to 23.5 for test solution (a);
— at 224 nm: 370 to 400 for test solution (b).

Chlorides (*2.4.4*)
Maximum 200 ppm.

Shake 0.5 g with 30 mL of *water R* for 5 min and filter.

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 2.0 g.

ASSAY
Dissolve 0.300 g in 30 mL of *ethanol (96 per cent) R*, add 20 mL of a 500 g/L solution of *potassium hydroxide R*, mix and heat under a reflux condenser for 4 h. Cool, add 100 mL of *water R*, neutralise with *dilute nitric acid R* and add 5 mL of the same acid in excess. Titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank test.

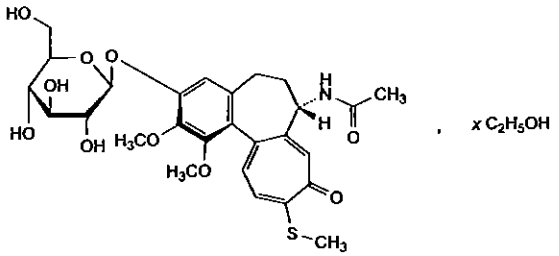
1 mL of *0.1 M silver nitrate* is equivalent to 17.81 mg of $C_{12}H_{15}Cl_2NO_5S$.

STORAGE
In an airtight container, protected from light.

Ph Eur

Thiocolchicoside Crystallised from Ethanol

(Ph. Eur. monograph 2896)



$C_{27}H_{33}NO_{10}S,xC_2H_6O$ 563.6 (ethanol-free substance)

Anhydrous thiocolchicoside *602-41-5*

Action and use
Muscle relaxant.

Ph Eur

DEFINITION
N-[(7*S*,12a*R*$_a$)-3-(β-D-Glucopyranosyloxy)-1,2-dimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide.

Content
97.0 per cent to 102.0 per cent (anhydrous substance).

It contains a variable quantity of ethanol.

CHARACTERS

Appearance
Yellow crystalline powder, hygroscopic.

Solubility
Sparingly soluble in water, practically insoluble in acetone and in ethanol (96 per cent).

mp
About 208 °C.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *thiocolchicoside crystallised from ethanol CRS*.

TESTS

Appearance of solution
The solution is clear (*2.2.1*).

Dissolve 0.100 g in *methanol R* and dilute to 10 mL with the same solvent.

Specific optical rotation (*2.2.7*)
−600 to −570 (anhydrous substance).

Dissolve 0.500 g in *water R* and dilute to 100.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Test solution (a) Dissolve 20.0 mg of the substance to be examined in *methanol R* and dilute to 20.0 mL with the same solvent.

Test solution (b) Dilute 1.0 mL of test solution (a) to 10.0 mL with *methanol R*.

Reference solution (a) Dilute 1.0 mL of test solution (a) to 100.0 mL with *methanol R*. Dilute 2.0 mL of this solution to 10.0 mL with *methanol R*.

Reference solution (b) Dissolve 5 mg of *thiocolchicoside for system suitability CRS* (containing impurities D, E, G, H, K and L) in *methanol R* and dilute to 5 mL with the same solvent.

Reference solution (c) Dissolve 20.0 mg of *thiocolchicoside hydrate CRS* in *methanol R* and dilute to 20.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with *methanol R*.

Column:
- *size*: *l* = 0.10 m, Ø = 2.1 mm;
- *stationary phase*: *end-capped ethylene-bridged phenylsilyl silica gel for chromatography (hybrid material) R* (1.7 µm);
- *temperature*: 25 °C.

Mobile phase:
- *mobile phase A*: dissolve 0.22 g of *ammonium formate R* in 350 mL of *water for chromatography R* and mix with 25 mL of *tetrahydrofuran R*;
- *mobile phase B*: dilute 45 µL of *anhydrous formic acid R* in 900 mL of *acetonitrile R* and mix with 100 mL of *tetrahydrofuran R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	99	1
2 - 6	99 → 92	1 → 8
6 - 7.5	92 → 70	8 → 30
7.5 - 8.8	70 → 50	30 → 50
8.8 - 9.2	50 → 2	50 → 98
9.2 - 10	2	98

Flow rate 0.4 mL/min.

Detection Spectrophotometer at 370 nm.

Injection 1.0 µL of test solution (a) and reference solutions (a) and (b).

Identification of impurities Use the chromatogram supplied with *thiocolchicoside for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities D, E, H and L.

Relative retention With reference to thiocolchicoside (retention time = about 6 min): impurity D = about 0.2; impurity H = about 0.4; impurity E = about 1.05; impurity L = about 1.11.

System suitability:
- *signal-to-noise ratio*: minimum 50 for the principal peak in the chromatogram obtained with reference solution (a);
- *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to thiocolchicoside in the chromatogram obtained with reference solution (b).

Calculation of percentage contents:
- *correction factor*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity D = 1.7; impurity H = 2.8;
- for each impurity, use the concentration of the substance to be examined in reference solution (a).

Limits:
- *impurity D*: maximum 0.4 per cent;
- *impurities E, H*: for each impurity, maximum 0.3 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 1.0 per cent;
- *reporting threshold*: 0.05 per cent; disregard the peak due to impurity L.

Ethanol (*2.4.24*)
Maximum 1.5 per cent.

Water (*2.5.12*)
Maximum 2.5 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (c).

Calculate the percentage content of $C_{27}H_{33}NO_{10}S$ taking into account the assigned content of *thiocolchicoside hydrate CRS*.

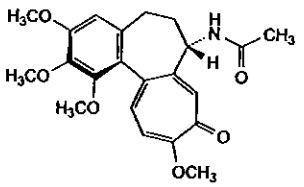
STORAGE

In an airtight container, protected from light.

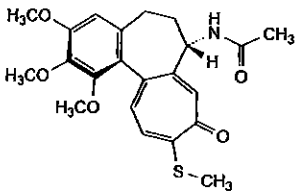
IMPURITIES

Specified impurities *D, E, H.*

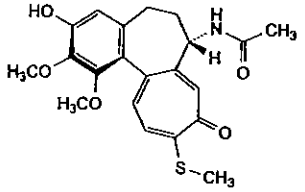
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, G, J, K, L.*



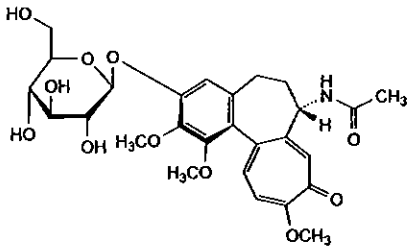
A. *N*-[(7*S*,12aR_a)-1,2,3,10-tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide (colchicine),



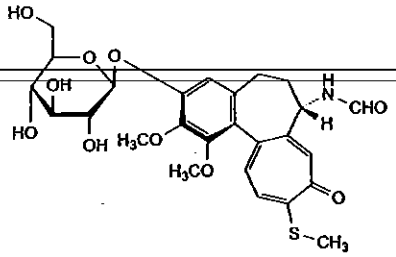
B. *N*-[(7*S*,12aR_a)-1,2,3-trimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide (thiocolchicine),



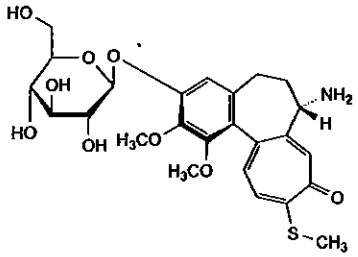
C. *N*-[(7*S*,12aR_a)-3-hydroxy-1,2-dimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide (3-*O*-demethylthiocolchicine),



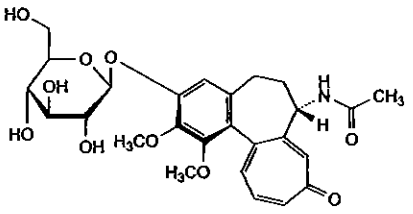
D. *N*-[(7*S*,12aR_a)-3-(β-D-glucopyranosyloxy)-1,2,10-trimethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl] acetamide (colchicoside),



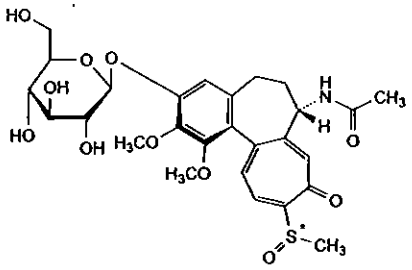
E. *N*-[(7*S*,12aR_a)-3-(β-D-glucopyranosyloxy)-1,2-dimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*] heptalen-7-yl]formamide (*N*-deacetyl-*N*-formylthiocolchicoside),



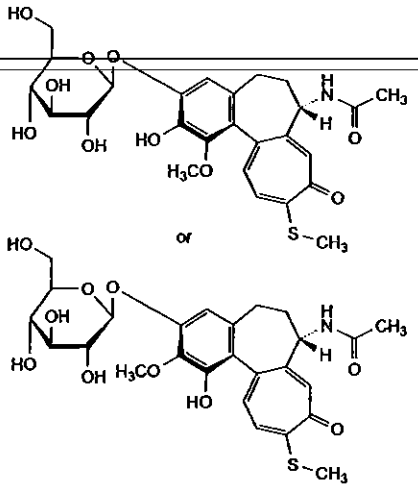
G. (7*S*,12aR_a)-7-amino-3(β-D-glucopyranosyloxy)-1,2-dimethoxy-10-(methylsulfanyl)-6,7-dihydrobenzo[*a*] heptalen-9(5*H*)-one (*N*-deacetylthiocolchicoside),



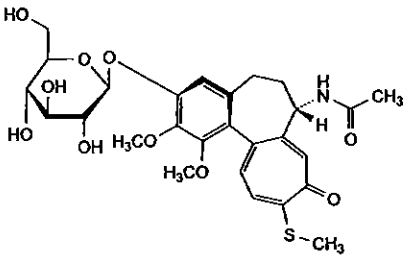
H. *N*-[(7*S*,12aR_a)-3-(β-D-glucopyranosyloxy)-1,2-dimethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide (10-demethoxycolchicoside),



J. *N*-[(7*S*,12aR_a)-3-(β-D-glucopyranosyloxy)-1,2-dimethoxy-10-(methylsulfinyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*] heptalen-7-yl]acetamide (thiocolchicoside *S*-oxide),



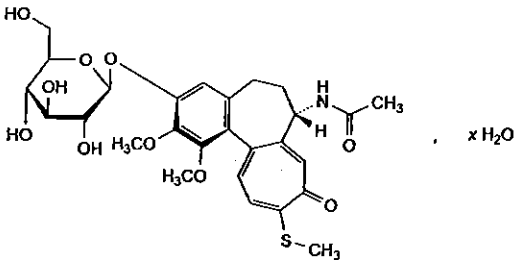
K. *N*-[(7*S*,12aR_a)-3-(β-D-glucopyranosyloxy)-2-hydroxy-1-methoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide or *N*-[(7*S*,12aR_a)-3-(β-D-glucopyranosyloxy)-1-hydroxy-2-methoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide,



L. *N*-[(7*S*,12aS_a)-3-(β-D-glucopyranosyloxy)-1,2-dimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*] heptalen-7-yl]acetamide (12aS_a-thiocolchicoside).

Thiocolchicoside Hydrate

(Ph. Eur. monograph 2814)



$C_{27}H_{33}NO_{10}S,xH_2O$ 563.6 (anhydrous substance) *1622135-03-8*

Action and use
Muscle relaxant.

Ph Eur

DEFINITION

N-[(7*S*,12aR_a)-3-(β-D-Glucopyranosyloxy)-1,2-dimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide.

Content
96.0 per cent to 102.0 per cent (anhydrous substance).
It contains a variable quantity of water.

CHARACTERS

Appearance
Yellow crystalline powder, slightly hygroscopic.

Solubility
Sparingly soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in acetone.

mp
About 272 °C.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).
Comparison *thiocolchicoside hydrate CRS*.

TESTS

Appearance of solution
The solution is clear (*2.2.1*).
Dissolve 0.100 g in *methanol R* and dilute to 10 mL with the same solvent.

Specific optical rotation (*2.2.7*)
−600 to −570 (anhydrous substance).
Dissolve 0.500 g in *water R* and dilute to 100.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Test solution (a) Dissolve 20.0 mg of the substance to be examined in *methanol R* and dilute to 20.0 mL with the same solvent.

Test solution (b) Dilute 1.0 mL of test solution (a) to 10.0 mL with *methanol R*.

Reference solution (a) Dilute 1.0 mL of test solution (a) to 100.0 mL with *methanol R*. Dilute 2.0 mL of this solution to 10.0 mL with *methanol R*.

Reference solution (b) Dissolve 5 mg of *thiocolchicoside for system suitability CRS* (containing impurities D, E, G, H, K and L) in *methanol R* and dilute to 5 mL with the same solvent.

Reference solution (c) Dissolve 20.0 mg of *thiocolchicoside hydrate CRS* in *methanol R* and dilute to 20.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with *methanol R*.

Column:
— *size*: *l* = 0.10 m, Ø = 2.1 mm;
— *stationary phase*: *end-capped ethylene-bridged phenylsilyl silica gel for chromatography (hybrid material) R* (1.7 µm);
— *temperature*: 25 °C.

Mobile phase:
— *mobile phase A*: dissolve 0.22 g of *ammonium formate R* in 350 mL of *water for chromatography R* and mix with 25 mL of *tetrahydrofuran R*;
— *mobile phase B*: dilute 45 µL of *anhydrous formic acid R* in 900 mL of *acetonitrile R* and mix with 100 mL of *tetrahydrofuran R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	99	1
2 - 6	99 → 92	1 → 8
6 - 7.5	92 → 70	8 → 30
7.5 - 8.8	70 → 50	30 → 50
8.8 - 9.2	50 → 2	50 → 98
9.2 - 10	2	98

Flow rate 0.4 mL/min.

Detection Spectrophotometer at 370 nm.

Injection 1.0 µL of test solution (a) and reference solutions (a) and (b).

Identification of impurities Use the chromatogram supplied with *thiocolchicoside for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities D, E, G, H, K and L.

Relative retention With reference to thiocolchicoside (retention time = about 6 min): impurity D = about 0.2; impurity H = about 0.4; impurity G = about 0.7; impurity K = about 0.8; impurity E = about 1.05; impurity L = about 1.11.

System suitability:
— *signal-to-noise ratio*: minimum 50 for the principal peak in the chromatogram obtained with reference solution (a);
— *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to thiocolchicoside in the chromatogram obtained with reference solution (b).

Calculation of percentage contents:
— *correction factors*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity D = 1.7; impurity H = 2.8;
— for each impurity, use the concentration of thiocolchicoside hydrate in reference solution (a).

Limits:
— *impurity E*: maximum 1.0 per cent;
— *impurity H*: maximum 0.7 per cent;
— *impurity K*: maximum 0.3 per cent;
— *impurities D, G*: for each impurity, maximum 0.2 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 2.5 per cent;

— *reporting threshold*: 0.05 per cent; disregard the peak due to impurity L.

Water (*2.5.12*)
Maximum 2.5 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (c).

Calculate the percentage content of $C_{27}H_{33}NO_{10}S$ taking into account the assigned content of *thiocolchicoside hydrate CRS*.

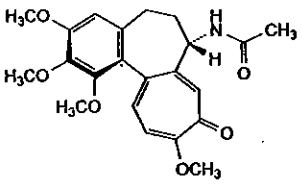
STORAGE

In an airtight container, protected from light.

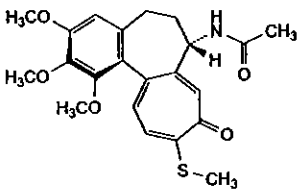
IMPURITIES

Specified impurities *D, E, G, H, K.*

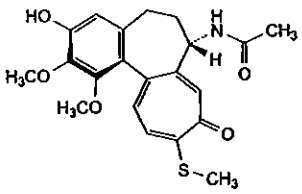
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, J, L.*



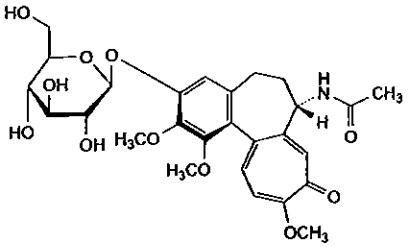
A. *N*-[(7*S*,12aR_a)-1,2,3,10-tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide (colchicine),



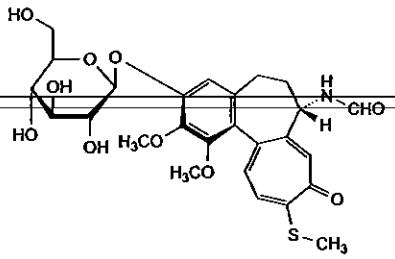
B. *N*-[(7*S*,12aR_a)-1,2,3-trimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide (thiocolchicine),



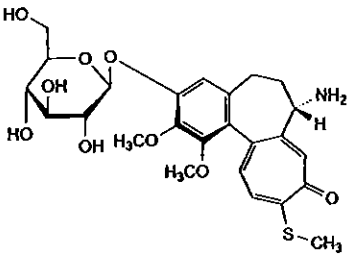
C. *N*-[(7*S*,12aR_a)-3-hydroxy-1,2-dimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide (3-*O*-demethylthiocolchicine),



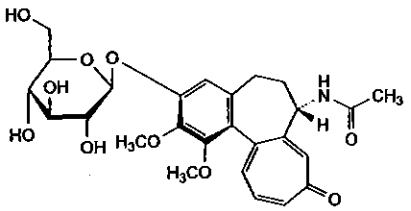
D. *N*-[(7*S*,12aR_a)-3-(β-D-glucopyranosyloxy)-1,2,10-trimethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide (colchicoside),



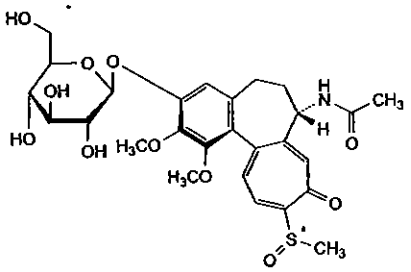
E. *N*-[(7*S*,12aR_a)-3-(β-D-glucopyranosyloxy)-1,2-dimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]formamide (*N*-deacetyl-*N*-formylthiocolchicoside),



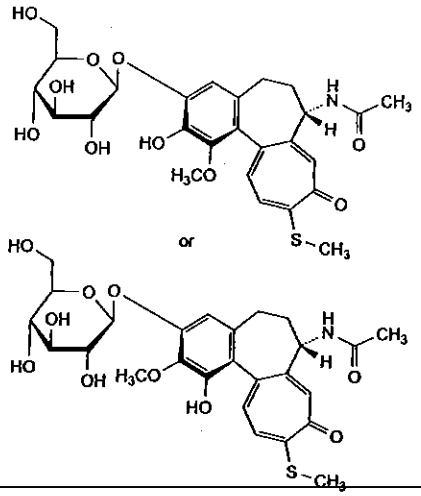
G. (7*S*,12aR_a)-7-amino-3-(β-D-glucopyranosyloxy)-1,2-dimethoxy-10-(methylsulfanyl)-6,7-dihydrobenzo[*a*]heptalen-9(5*H*)-one (*N*-deacetylthiocolchicoside),



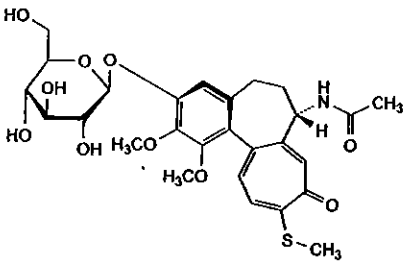
H. *N*-[(7*S*,12aR_a)-3-(β-D-glucopyranosyloxy)-1,2-dimethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide (10-demethoxycolchicoside),



J. *N*-[(7*S*,12aR_a)-3-(β-D-glucopyranosyloxy)-1,2-dimethoxy-10-(methylsulfinyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide (thiocolchicoside *S*-oxide),



K. *N*-[(7*S*,12a*R*$_a$)-3-(β-D-glucopyranosyloxy)-2-hydroxy-1-methoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide or *N*-[(7*S*,12a*R*$_a$)-3-(β-D-glucopyranosyloxy)-1-hydroxy-2-methoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide,

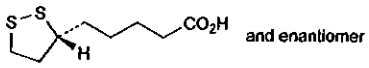


L. *N*-[(7*S*,12a*S*$_a$)-3-(β-D-glucopyranosyloxy)-1,2-dimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide (12a*S*$_a$-thiocolchicoside).

Ph Eur

Thioctic Acid

(Ph. Eur. monograph 1648)



$C_8H_{14}O_2S_2$ 206.3 *1077-28-7*

Action and use
Antioxidant.

Ph Eur

DEFINITION
5-[(3*RS*)-1,2-Dithiolan-3-yl]pentanoic acid.

Content
97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
Yellow, crystalline powder.

Solubility
Very slightly soluble in water, very soluble in dimethylformamide, freely soluble in methanol.

mp
About 61 °C.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *thioctic acid CRS.*

TESTS

Appearance of solution
The solution is clear (*2.2.1*).
Dissolve 0.50 g in a 20 g/L solution of *sodium hydroxide R* and dilute to 10 mL with the same solution.

Impurity B
Thin-layer chromatography (*2.2.27*).
Test solution Dissolve 0.20 g of the substance to be examined in *dimethylformamide R* and dilute to 10 mL with the same solvent.
Reference solution Dissolve 20 mg of *thioctic acid containing impurity B CRS* in *dimethylformamide R* and dilute to 1.0 mL with the same solvent (1.0 per cent impurity B solution).
Plate *TLC silica gel plate R.*
Mobile phase 25 per cent *V/V* solution of *ammonia R, water R, ethyl acetate R, propanol R* (5:10:40:40 *V/V/V/V*).
Application 5 µL.
Development Over 2/3 of the plate.
Drying At 50 °C for 20 min.
Detection Expose to iodine vapour for 30 min or until the spots appear.
System suitability Reference solution:
— the chromatogram shows 2 clearly separated principal spots due to impurity B (R_F = 0.0) and thioctic acid (R_F = about 0.3).
Limit:
— *impurity B*: any spot due to impurity B is not more intense than the corresponding spot in the chromatogram obtained with the reference solution (1.0 per cent).

Related substances
Liquid chromatography (*2.2.29*). *Protect the solutions from light.*
Solvent mixture A mixture of equal volumes of *acetonitrile R1* and a 0.7 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.6 with *phosphoric acid R.*
Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.
Reference solution (a) Dissolve 5 mg of *thioctic acid for system suitability CRS* (containing impurity A) in the solvent mixture and dilute to 5 mL with the solvent mixture.
Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.
Reference solution (c) Dissolve 50.0 mg of *thioctic acid CRS* in the solvent mixture and dilute to 50.0 mL with the solvent mixture.
Blank solution Solvent mixture.
Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 35 °C.
Mobile phase Mix 8 volumes of *acetonitrile R1*, 41 volumes of a 0.7 g/L solution of *potassium dihydrogen phosphate R*

previously adjusted to pH 3.0 with *phosphoric acid R*, and 51 volumes of *methanol R*.

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µL.

Run time 2.5 times the retention time of thioctic acid.

Relative retention With reference to thioctic acid (retention time = about 6 min): impurity A = about 2.2.

System suitability:
- *resolution*: minimum 6.0 between the peaks due to thioctic acid and impurity A in the chromatogram obtained with reference solution (a);
- *symmetry factor*: maximum 2.0 for the peak due to thioctic acid in the chromatogram obtained with reference solution (c).

Limits:
- *correction factor*: for the calculation of content, multiply the peak area of impurity A by 0.6;
- *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.2 per cent, determined on 1.000 g by drying *in vacuo* at 40 °C for 3 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (c).

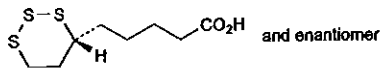
Calculate the percentage content of $C_8H_{14}O_2S_2$ from the peak areas and the declared content of *thioctic acid CRS*.

STORAGE

Protected from light.

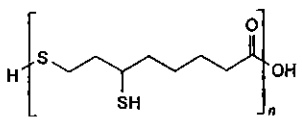
IMPURITIES

Specified impurities *A, B.*



and enantiomer

A. 5-[(4*RS*)-1,2,3-trithian-4-yl]pentanoic acid,



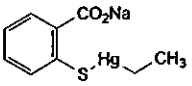
B. α-hydro-ω-hydroxypoly[sulfanediyl(3-sulfanyl-8-oxooctane-1,8-diyl)] (mixture of thioctic acid polymers).

Ph Eur

Thiomersal

Thimerosal

(Ph. Eur. monograph 1625)



$C_9H_9HgNaO_2S$ 404.8 *54-64-8*

Action and use
Antiseptic; adjuvant in vaccine formulations.

Ph Eur

DEFINITION

Sodium ethyl[2-sulfanylbenzoato(2-)-*O*,*S*]mercurate(1-).

Content
97.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water, sparingly soluble or soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 103 °C to 115 °C.

Dissolve 0.5 g in *water R* and dilute to 10 mL with the same solvent. Add 2 mL of *dilute hydrochloric acid R*. A white precipitate is formed. Wash the precipitate with *water R* and dry *in vacuo* (*2.2.32*) at a pressure not exceeding 0.7 kPa.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *thiomersal CRS*.

C. Treat 50 mg by the oxygen-flask method (*2.5.10*). Use a mixture of 1 mL of *strong hydrogen peroxide solution R* and 50 mL of *water R* to absorb the combustion products. To the solution add 5 mL of *dilute nitric acid R*. 0.1 mL of this solution gives reaction (a) of mercury (*2.3.1*). To the remaining part of the solution add 10 mL of *dilute hydrochloric acid R* and filter. 5 mL of the filtrate, without further addition of acid, gives reaction (a) of sulfates (*2.3.1*).

D. Solution S (see Tests) gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S
Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent.

Appearance of solution
Solution S is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution B_6 (*2.2.2, Method II*).

pH (*2.2.3*)
6.0 to 8.0.

Dilute 5 mL of solution S to 50 mL with *carbon dioxide-free water R*.

Inorganic mercury compounds
Maximum 0.70 per cent.

Protect the solutions from light throughout the procedure.

Test solution Dissolve 25 mg of the substance to be examined in *water R* and dilute to 25.0 mL with the same solvent.

Reference solution Dissolve 95.0 mg of *mercuric chloride R* in *water R* and dilute to 50.0 mL with the same solvent. Dilute 1.0 mL of the solution to 20.0 mL with *water R*.

Test, reference and blank preparations Label five 10 mL volumetric flasks A, B, C, D and E. Place 5 mL of the test solution in flasks A, B, C and D. To each of the flasks C and D add 0.5 mL of the reference solution. Dilute the contents of flasks A and C to 10 mL with *water R* (blank preparations A and C). Dilute the contents of flasks B and D to 10 mL with a freshly prepared 332 g/L solution of *potassium iodide R* (test preparation B and reference preparation D). Place 5 mL of a 332 g/L solution of *potassium iodide R* in flask E. Dilute to 10 mL with *water R* (blank preparation E).

Measure the absorbance (*2.2.25*) of each solution (A_a, A_b, A_c, A_d and A_e) at 323 nm using *water R* as the compensation liquid. Calculate the content of inorganic mercury compounds, expressed as Hg from the expression:

$$\frac{(A_b - A_a - A_e) \times m_R \times 0.1847}{(A_d - A_c - A_b + A_a) \times m_T}$$

m_R = mass of mercuric chloride in the reference solution in milligrams,

m_T = mass of the substance to be examined in milligrams.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in a desiccator at a pressure not exceeding 0.7 kPa for 24 h.

ASSAY
Place 0.5 g in a 100 mL long-necked combustion flask, add 5 mL of *sulfuric acid R* and heat gently until charring occurs, continue to heat and add dropwise *strong hydrogen peroxide solution R* until the mixture is colourless. Dilute with *water R*, evaporate until slight fuming occurs, dilute to 10 mL with *water R*, cool down and titrate with *0.1 M ammonium thiocyanate* using *ferric ammonium sulfate solution R2* as indicator.

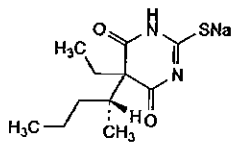
1 mL of *0.1 M ammonium thiocyanate* is equivalent to 20.24 mg of $C_9H_9HgNaO_2S$.

STORAGE
Protected from light.

Ph Eur

Thiopental Sodium

(Thiopental Sodium and Sodium Carbonate, Ph. Eur. monograph 0212)



and enantiomer

$C_{11}H_{17}N_2NaO_2S$ 264.3

Action and use
Intravenous barbiturate; general anaesthetic.

Preparation
Thiopental Injection

Ph Eur

DEFINITION
Mixture of sodium 5-ethyl-5-[(1*RS*)-1-methylbutyl]-4,6-dioxo-1,4,5,6-tetrahydropyrimidine-2-thiolate and anhydrous sodium carbonate.

Content
— *thiopental*: 84.0 per cent to 87.0 per cent (dried substance);
— *sodium*: 10.2 per cent to 11.2 per cent (dried substance).

CHARACTERS
Appearance
Yellowish-white, hygroscopic powder.

Solubility
Freely soluble in water, partly soluble in anhydrous ethanol.

IDENTIFICATION
First identification: A, B, E.

Second identification: A, C, D, E.

A. Acidify 10 mL of solution S (see Tests) with *dilute hydrochloric acid R*. An effervescence is produced. Shake with 20 mL of *1,1-dimethylethyl methyl ether R*. Separate the upper layer, wash with 10 mL of *water R*, dry over *anhydrous sodium sulfate R* and filter. Evaporate the filtrate to dryness and dry the residue at 100-105 °C. Determine the melting point (*2.2.14*) of the residue. Mix equal parts of the residue and *thiopental CRS* and determine the melting point of the mixture. The difference between the melting points (which are about 160 °C) is not greater than 2 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Use the residue obtained in Identification test A.

Comparison *thiopental CRS*.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.1 g of the substance to be examined in *water R* and dilute to 100 mL with the same solvent.

Reference solution Dissolve 85 mg of *thiopental CRS* in 10 mL of *dilute sodium hydroxide solution R* and dilute to 100 mL with *water R*.

Plate *TLC silica gel GF_{254} plate R*.

Mobile phase *concentrated ammonia R, ethanol (96 per cent) R, methylene chloride R* (5:15:80 *V/V/V*); use the lower layer.

Application 10 µL.

Development Over 3/4 of the plate.

Detection Examine immediately in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. It gives the reaction of non-nitrogen substituted barbiturates (*2.3.1*).

E. It gives reaction (a) of sodium (*2.3.1*).

TESTS
Solution S
Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution GY_3 (*2.2.2, Method II*).

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 20.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 2 mg of *thiopental for system suitability CRS* (containing impurities A, B, C and D) in the mobile phase and dilute to 2.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R1*, 1 g/L solution of *phosphoric acid R* (35:65 *V/V*).

Flow rate 1 mL/min.

Detection Spectrophotometer at 225 nm.

Injection 10 µL.

Run time Twice the retention time of thiopental.

Identification of impurities Use the chromatogram supplied with *thiopental for system suitability CRS* (containing impurities A, B, C and D) and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C and D.

Relative retention With reference to thiopental (retention time = about 20 min): impurity A = about 0.3; impurity B = about 0.4; impurity C = about 0.9; impurity D = about 1.3.

System suitability Reference solution (b):
— *resolution*: minimum 1.5 between the peaks due to impurities A and B; minimum 1.5 between the peaks due to impurity C and thiopental.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity B by 1.5;
— *impurity C*: not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.0 per cent);
— *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
— *impurity D*: not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
— *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (5.0 per cent);
— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Chlorides (*2.4.4*)
Maximum 330 ppm.

To 5 mL of solution S add 35 mL of *water R* and 10 mL of *dilute nitric acid R*. Shake with 3 quantities, each of 25 mL, of *1,1-dimethylethyl methyl ether R* and discard the upper layer. Eliminate the organic solvent from the lower layer by heating on a water-bath. 15 mL of the solution complies with the test for chlorides.

Loss on drying (*2.2.32*)
Maximum 2.5 per cent, determined on 0.500 g by drying *in vacuo* at 100 °C for 4 h.

ASSAY

Sodium
Dissolve 0.400 g in 30 mL of *water R*. Add 0.1 mL of *methyl red solution R* and titrate with *0.1 M hydrochloric acid* until a red colour is obtained. Boil gently for 2 min. Allow to cool and, if necessary, continue the titration with *0.1 M hydrochloric acid* until the red colour is again obtained.

1 mL of *0.1 M hydrochloric acid* is equivalent to 2.299 mg of Na.

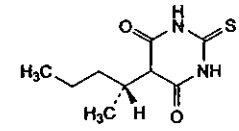
Thiopental
Dissolve 0.150 g in 5 mL of *water R*. Add 2 mL of *dilute sulfuric acid R* and shake with 4 quantities, each of 10 mL, of *chloroform R*. Combine the chloroform layers, filter and evaporate the filtrate to dryness on a water-bath. Dissolve the residue in 30 mL of previously neutralised *dimethylformamide R* and add 0.1 mL of a 2 g/L solution of *thymol blue R* in *methanol R*. Titrate immediately with *0.1 M lithium methoxide* until a blue colour is obtained. Protect the solution from atmospheric carbon dioxide during the titration.

1 mL of *0.1 M lithium methoxide* is equivalent to 24.23 mg of $C_{11}H_{18}N_2O_2S$.

STORAGE
In an airtight container, protected from light.

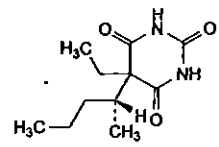
IMPURITIES
Specified impurities *B, C, D.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A.*



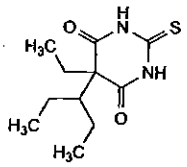
and enantiomer

A. 5-[(1*RS*)-1-methylbutyl]-2-thioxo-2,3-dihydropyrimidine-4,6(1*H*,5*H*)-dione,

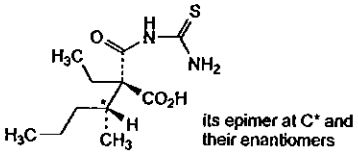


and enantiomer

B. 5-ethyl-5-[(1*RS*)-1-methylbutyl]pyrimidine-2,4,6 (1*H*,3*H*,5*H*)-trione,



C. 5-ethyl-5-(1-ethylpropyl)-2-thioxo-2,3-dihydropyrimidine-4,6(1*H*,5*H*)-dione,

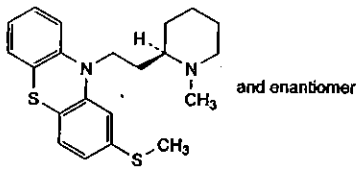


D. mixture of (2*RS*,3*RS*)-2-(carbamothioylcarbamoyl)-2-ethyl-3-methylhexanoic acid and (2*RS*,3*SR*)-2-(carbamothioylcarbamoyl)-2-ethyl-3-methylhexanoic acid.

Ph Eur

Thioridazine

(Ph. Eur. monograph 2005)



$C_{21}H_{26}N_2S_2$ 370.6 *50-52-2*

Action and use
Dopamine receptor antagonist; neuroleptic.

Ph Eur

DEFINITION
10-[2-[(2*RS*)-1-Methylpiperidin-2-yl]ethyl]-2-(methylsulfanyl)-10*H*-phenothiazine

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white powder.

Solubility
Practically insoluble in water, very soluble in methylene chloride, freely soluble in methanol, soluble in ethanol (96 per cent).

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *thioridazine CRS*.

TESTS
Solution S
Dissolve 1.25 g in *methanol R* and dilute to 25 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than intensity 6 of the range of reference solutions of the most appropriate colour (*2.2.2, Method II*).

Related substances
Liquid chromatography (*2.2.29*). *Carry out the test as quickly as possible and protected from light.*

Test solution Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 100 mL with the same solvent.

Reference solution (a) Dilute 5.0 mL of the test solution to 100.0 mL with *methanol R*. Dilute 2.0 mL of this solution to 100.0 mL with *methanol R*.

Reference solution (b) Dissolve the contents of a vial of *thioridazine for system suitability CRS* (containing impurities A, B, C, D and E) in 1.0 mL of *methanol R*.

Column:
— *size*: *l* = 0.25 m, Ø = 4.0 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* resistant to bases up to pH 11.

Mobile phase:
— *mobile phase A*: *triethylamine R1, acetonitrile R, water R* (2:400:600 *V/V/V*);
— *mobile phase B*: *triethylamine R1, acetonitrile R* (2:1000 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	100	0
5 - 35	100 → 5	0 → 95
35 - 40	5	95

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 275 nm.

Injection 25 µL.

Identification of impurities Use the chromatogram supplied with *thioridazine for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C, D and E.

Relative retention With reference to thioridazine (retention time = about 30 min): impurity D = about 0.1; impurity A = about 0.3; impurity C = about 0.4; impurity B = about 0.5; impurity E = about 0.6.

System suitability Reference solution (b):
— *resolution*: minimum 3.5 between the peaks due to impurities C and B.

Limits:
— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.9; impurity B = 2.4; impurity C = 0.5; impurity D = 1.5;
— *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g *in vacuo* at 50 °C for 4 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

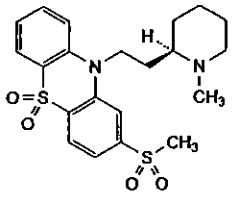
ASSAY
Dissolve 0.300 g in 60 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 37.06 mg of $C_{21}H_{26}N_2S_2$.

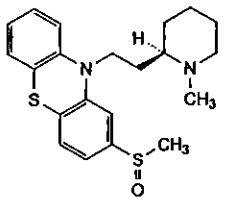
STORAGE
Protected from light.

IMPURITIES
Specified impurities A, B, C, D, E.

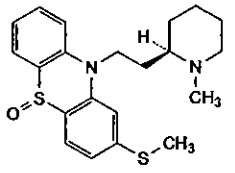
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) F.



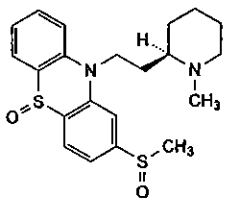
A. 10-[2-[(2*RS*)-1-methylpiperidin-2-yl]ethyl]-2-(methylsulfonyl)-10*H*-phenothiazine 5,5-dioxide,



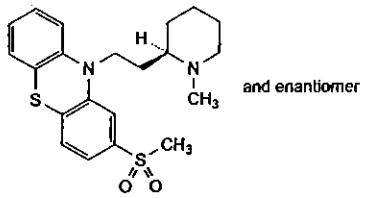
B. 10-[2-[(2*RS*)-1-methylpiperidin-2-yl]ethyl]-2-(methylsulfinyl)-10*H*-phenothiazine (mesoridazine),



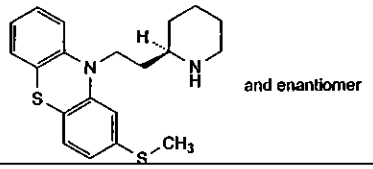
C. 10-[2-[(2*RS*)-1-methylpiperidin-2-yl]ethyl]-2-(methylsulfanyl)-10*H*-phenothiazine 5-oxide,



D. 10-[2-[(2*RS*)-1-methylpiperidin-2-yl]ethyl]-2-(methylsulfinyl)-10*H*-phenothiazine 5-oxide,



E. 10-[2-[(2*RS*)-1-methylpiperidin-2-yl]ethyl]-2-(methylsulfonyl)-10*H*-phenothiazine (sulforidazine),

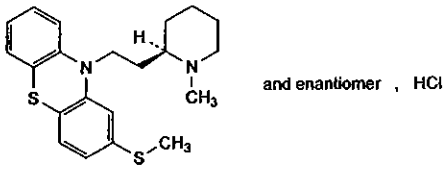


F. 2-(methylsulfanyl)-10-[2-[(2*RS*)-piperidin-2-yl]ethyl]-10*H*-phenothiazine (northioridazine).

Ph Eur

Thioridazine Hydrochloride

(Ph. Eur. monograph 0586)



$C_{21}H_{27}ClN_2S_2$ 407.0 *130-61-0*

Action and use
Dopamine receptor antagonist; neuroleptic.

Ph Eur

DEFINITION
10-[2-[(2*RS*)-1-Methylpiperidin-2-yl]ethyl]-2-(methylsulfanyl)-10*H*-phenothiazine hydrochloride.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water and in methanol, soluble in ethanol 96 per cent.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison thioridazine hydrochloride CRS.

B. 0.2 g gives reaction (b) of chlorides (*2.3.1*).

TESTS
Carry out all operations protected from light.

Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than intensity 6 of the range of reference solutions of the most appropriate colour (*2.2.2, Method II*).

Dissolve 1.0 g in *methanol R* and dilute to 20 mL with the same solvent.

Optical rotation (*2.2.7*)
−0.10° to + 0.10°.

Dissolve 1.0 g in *methanol R* and dilute to 20.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*). *Carry out the test as quickly as possible and protected from light.*

Test solution Dissolve 20.0 mg of the substance to be examined in *methanol R* and dilute to 100.0 mL with the same solvent.

Reference solution (a) Dilute 5.0 mL of the test solution to 100.0 mL with *methanol R*. Dilute 2.0 mL of this solution to 100.0 mL with *methanol R*.

Reference solution (b) Dissolve the contents of a vial of *thioridazine for system suitability CRS* (containing impurities A, B, C, D and E) in 1.0 mL of *methanol R*.

Column:
— *size*: *l* = 0.25 m, Ø = 4.0 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm) resistant to bases up to pH 11.

Mobile phase:
— *mobile phase A*: *triethylamine R1, acetonitrile R, water R* (2:400:600 *V/V/V*);
— *mobile phase B*: *triethylamine R1, acetonitrile R* (2:1000 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	100	0
5 - 35	100 → 5	0 → 95
35 - 40	5	95

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 275 nm.

Injection 25 µL.

Identification of impurities Use the chromatogram supplied with *thioridazine for system suitability CRS* to identify the peaks due to impurities A, B, C, D and E .

Relative retention With reference to thioridazine (retention time = about 30 min): impurity D = about 0.1; impurity A = about 0.3; impurity C = about 0.4; impurity B = about 0.5; impurity E = about 0.6; impurity F = about 0.9.

System suitability Reference solution (b):
— *resolution*: minimum 3.5 between the peaks due to impurities C and B.

Limits:
— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.9; impurity B = 2.4; impurity C = 0.5; impurity D = 1.5;
— *impurities A, B, C, D, E*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

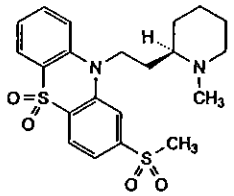
ASSAY
Dissolve 0.300 g in a mixture of 10 mL of *anhydrous acetic acid R* and 60 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 40.70 mg of $C_{21}H_{27}ClN_2S_2$.

STORAGE
Protected from light.

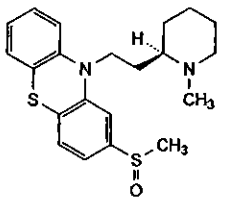
IMPURITIES
Specified impurities A, B, C, D, E.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) F.



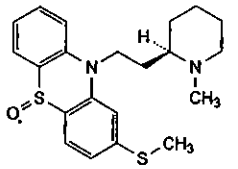
and enantiomer

A. 10-[2-[(2*RS*)-1-methylpiperidin-2-yl]ethyl]-2-(methylsulfonyl)-10*H*-phenothiazine 5,5-dioxide,



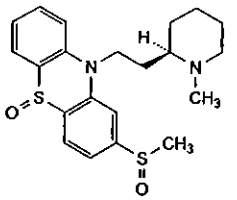
and enantiomer

B. 10-[2-[(2*RS*)-1-methylpiperidin-2-yl]ethyl]-2-(methylsulfinyl)-10*H*-phenothiazine,

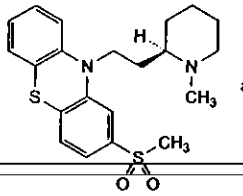


and enantiomer

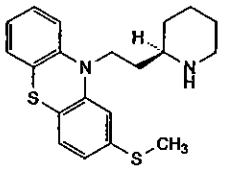
C. 10-[2-[(2*RS*)-1-methylpiperidin-2-yl]ethyl]-2-(methylsulfanyl)-10*H*-phenothiazine 5-oxide,


and enantiomer

D. 10-[2-[(2*RS*)-1-methylpiperidin-2-yl]ethyl]-2-(methylsulfinyl)-10*H*-phenothiazine 5-oxide,


and enantiomer

E. 10-[2-[(2*RS*)-1-methylpiperidin-2-yl]ethyl]-2-(methylsulfonyl)-10*H*-phenothiazine,


and enantiomer

F. 2-(methylsulfanyl)-10-[2-[(2*RS*)-piperidin-2-yl]ethyl]-10*H*-phenothiazine.

Ph Eur

Thiotepa

$C_6H_{12}N_3PS$ 189.2 *52-24-4*

Action and use
Cytotoxic alkylating agent.

Preparation
Thiotepa Injection

DEFINITION
Thiotepa is phosphorothioic tri(ethyleneamide). It contains not less than 97.0% and not more than 102.0% of $C_6H_{12}N_3PS$, calculated with reference to the anhydrous substance.

CHARACTERISTICS
Fine, white crystalline flakes.

Freely soluble in *water*, in *chloroform* and in *ethanol (96%)*.

IDENTIFICATION
A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of thiotepa *(RS 337)*.

B. Burn 20 mg by the method for *oxygen-flask combustion*, Appendix VIII C, using 5 mL of 1.25M *sodium hydroxide* as the absorbing liquid. When the process is complete, dilute to 25 mL with *water*. To 5 mL of the resulting solution add 0.1 mL of *hydrogen peroxide solution (100 vol)* and 1 mL of 1M *hydrochloric acid*, mix and add 0.05 mL of *barium chloride solution*. The solution becomes turbid.

C. To 2 mL of the solution obtained in test B add 40 mL of *water* and 4 mL of *ammonium molybdate-sulfuric acid solution*, mix, add 0.1 g of L-*ascorbic acid* and boil for 1 minute. A blue colour is produced.

TESTS
Melting point
52° to 57°, Appendix V A.

Clarity of solution
A 2.0% w/v solution is *clear*, Appendix IV A.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following freshly prepared solutions. Solutions (1) and (2) are solutions of the substance being examined in *water* containing 0.350% w/v and 0.00035% w/v, respectively. For solution (3) dissolve 10 mg of the substance being examined in 2 mL of *methanol* in a ground-glass-stoppered tube, add 50 µL of a 0.1% v/v solution of *orthophosphoric acid*, stopper the tube and heat in a water bath at 65° for 50 seconds (generation of methoxythiotepa). Allow the solution to cool and add 1 mL of *methanol*. For solution (4) dissolve 15 mg of the substance being examined in 10 mL of *water*, add 1 g of *sodium chloride*, boil in a water bath for 10 minutes and cool (generation of chloro-adduct).

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable), (b) 15 volumes of *acetonitrile* and 85 volumes of 0.1M *mixed phosphate buffer pH 7.0* as the mobile phase with a flow rate of 1 mL per minute and (c) a detection wavelength of 215 nm.

The chromatogram obtained with solution (3) shows a peak corresponding to methoxythiotepa with a retention time relative to thiotepa of about 1.3 and the chromatogram obtained with solution (4) shows a peak due to the chloro-adduct with a retention time relative to thiotepa of about 3.75. The test is not valid unless the *resolution factor* between the two principal peaks in the chromatogram obtained with solution (3) is at least 3.

For solution (1) allow the chromatography to proceed for 4 times the retention time of the principal peak. In the chromatogram obtained with solution (1) the area of any peak corresponding to the 'chloro-adduct' (identified from the peak in the chromatogram obtained with solution (4)) is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15%), the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%) and the sum of the areas of all the *secondary peaks* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).

Water
Not more than 0.5% w/w, Appendix IX C. Cool the reagents and titration vessel in ice throughout the procedure and use 1.2 g. Complete the procedure as quickly as possible.

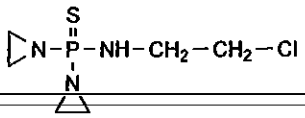
ASSAY
Transfer 0.2 g to an iodine flask with the aid of 50 mL of a 20% w/v solution of *sodium thiosulfate* and titrate immediately with *0.1M hydrochloric acid VS*, using 0.05 mL of *methyl orange solution* as indicator, until a faint red colour persists for 10 seconds. Stopper the flask, allow to stand for 30 minutes

and titrate with *0.1M sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Subtract the volume of *0.1M sodium hydroxide VS* used from the volume of *0.1M hydrochloric acid VS* used. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of hydrochloric acid required. Each mL of *0.1M hydrochloric acid VS* is equivalent to 6.307 mg of $C_6H_{12}N_3PS$.

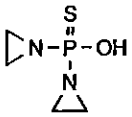
STORAGE

Thiotepa should be stored at a temperature of 2° to 8°. At higher temperatures it polymerises and becomes inactive.

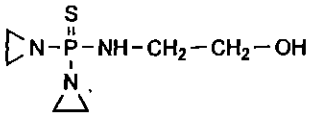
IMPURITIES



A. 'chloro-adduct',



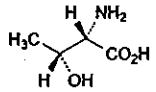
B. 'hydroxythiotepa A',



C. 'hydroxythiotepa B'.

Threonine

(Ph. Eur. monograph 1049)



$C_4H_9NO_3$ 119.1 *72-19-5*

Action and use

Amino acid.

Ph Eur

DEFINITION

(2*S*,3*R*)-2-Amino-3-hydroxybutanoic acid.

Product of fermentation or of protein hydrolysis.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

First identification: A, B.

Second identification: A, C, D.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *threonine CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in a 1 per cent *V/V* solution of *hydrochloric acid R* and dilute to 50 mL with the same solution.

Reference solution Dissolve 10 mg of *threonine CRS* in a 1 per cent *V/V* solution of *hydrochloric acid R* and dilute to 50 mL with the same solution.

Plate *TLC silica gel plate R.*

Mobile phase *glacial acetic acid R, water R, butanol R* (20:20:60 *V/V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *ninhydrin solution R* and heat at 105 °C for 15 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Mix 1 mL of a 2 g/L solution of the substance to be examined and 1 mL of a 20 g/L solution of *sodium periodate R*. Add 0.2 mL of *piperidine R* and 0.1 mL of a 25 g/L solution of *sodium nitroprusside R*. A blue colour develops that changes to yellow after a few minutes.

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 100 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)

5.0 to 6.5 for solution S.

Specific optical rotation (*2.2.7*)

−29.0 to −27.6 (dried substance).

Dissolve 1.50 g in *water R* and dilute to 25.0 mL with the same solvent.

Ninhydrin-positive substances

Amino acid analysis (*2.2.56*). For analysis, use Method 1.

The concentrations of the test solution and the reference solutions may be adapted according to the sensitivity of the equipment used. The concentrations of all solutions are adjusted so that the system suitability requirements described in general chapter *2.2.46* are fulfilled, keeping the ratios of concentrations between all solutions as described.

Solution A *dilute hydrochloric acid R1* or a sample preparation buffer suitable for the apparatus used.

Test solution Dissolve 30.0 mg of the substance to be examined in solution A and dilute to 50.0 mL with solution A.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with solution A. Dilute 2.0 mL of this solution to 10.0 mL with solution A.

Reference solution (b) Dissolve 30.0 mg of *proline R* in solution A and dilute to 100.0 mL with solution A. Dilute 1.0 mL of the solution to 250.0 mL with solution A.

Reference solution (c) Dilute 6.0 mL of *ammonium standard solution (100 ppm NH_4) R* to 50.0 mL with solution A. Dilute 1.0 mL of this solution to 100.0 mL with solution A.

Reference solution (d) Dissolve 30 mg of *isoleucine R* (impurity D) and 30 mg of *leucine R* in solution A and dilute to 50.0 mL with solution A. Dilute 1.0 mL of the solution to 200.0 mL with solution A.

Blank solution Solution A.

Inject suitable, equal amounts of the test, blank and reference solutions into the amino acid analyser. Run a program suitable for the determination of physiological amino acids.

System suitability Reference solution (d):
— *resolution*: minimum 1.5 between the peaks due to impurity D and leucine.

Calculation of percentage contents:
— ~~for any ninhydrin-positive substance detected at 570 nm,~~ use the concentration of threonine in reference solution (a);
— for any ninhydrin-positive substance detected at 440 nm, use the concentration of proline in reference solution (b); if a peak is above the reporting threshold at both wavelengths, use the result obtained at 570 nm for quantification.

Limits:
— *any ninhydrin-positive substance*: for each impurity, maximum 0.2 per cent;
— *total*: maximum 0.5 per cent;
— *reporting threshold*: 0.05 per cent.

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Chlorides (*2.4.4*)
Maximum 200 ppm.

Dilute 10 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 300 ppm.

Dissolve 0.5 g in *distilled water R* and dilute to 15 mL with the same solvent.

Ammonium
Amino acid analysis (*2.2.56*) as described in the test for ninhydrin-positive substances with the following modifications.

Injection Test solution, reference solution (c) and blank solution.

Limit:
— *ammonium at 570 nm*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.02 per cent), taking into account the peak due to ammonium in the chromatogram obtained with the blank solution.

Iron (*2.4.9*)
Maximum 10 ppm.

In a separating funnel, dissolve 1.0 g in 10 mL of *dilute hydrochloric acid R*. Shake with 3 quantities, each of 10 mL, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 mL of *water R* and shake for 3 min. Use the aqueous layer.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

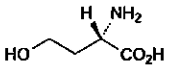
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.100 g in 5 mL of *anhydrous formic acid R*. Add 30 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

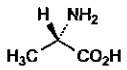
1 mL of *0.1 M perchloric acid* is equivalent to 11.91 mg of $C_4H_9NO_3$.

STORAGE
Protected from light.

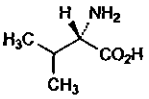
IMPURITIES
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, E.*



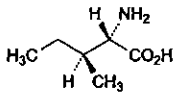
A. (2*S*)-2-amino-4-hydroxybutanoic acid (homoserine),



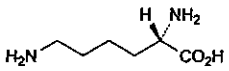
B. (2*S*)-2-aminopropanoic acid (alanine),



C. (2*S*)-2-amino-3-methylbutanoic acid (valine),



D. (2*S*,3*S*)-2-amino-3-methylpentanoic acid (isoleucine),

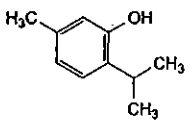


E. (2*S*)-2,6-diaminohexanoic acid (lysine).

Ph Eur

Thymol

(Ph. Eur. monograph 0791)



$C_{10}H_{14}O$ 150.2 *89-83-8*

Ph Eur

DEFINITION
5-Methyl-2-(methylethyl)phenol.

CHARACTERS
Appearance
Colourless crystals.

Solubility
Very slightly soluble in water, very soluble in ethanol (96 per cent), freely soluble in essential oils and in fatty oils, sparingly soluble in glycerol. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION
First identification: B.
Second identification: A, C, D.
A. Melting point (*2.2.14*): 48 °C to 52 °C.
B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *thymol CRS.*
C. Dissolve 0.2 g with heating in 2 mL of *dilute sodium hydroxide solution R* and add 0.2 mL of *chloroform R*. Heat on a water-bath. A violet colour develops.
D. Dissolve about 2 mg in 1 mL of *anhydrous acetic acid R*. Add 0.15 mL of *sulfuric acid R* and 0.05 mL of *nitric acid R*. A bluish-green colour develops.

TESTS
Appearance of solution
The solution is not more opalescent than reference suspension IV (*2.2.1*) and not more intensely coloured than reference solution R_6 (*2.2.2, Method II*).
Dissolve 1.0 g in 10 mL of *dilute sodium hydroxide solution R.*

Acidity
To 1.0 g in a 100 mL glass-stoppered conical flask add 20 mL of *water R*. Boil until dissolution is complete, cool and stopper the flask. Shake vigorously for 1 min. Add a few crystals of the substance to be examined to initiate crystallisation. Shake vigorously for 1 min and filter.
To 5 mL of the filtrate add 0.05 mL of *methyl red solution R* and 0.05 mL of *0.01 M sodium hydroxide*. The solution is yellow.

Related substances
Gas chromatography (*2.2.28*).
Test solution Dissolve 0.100 g of the substance to be examined in *ethanol (96 per cent) R* and dilute to 10.0 mL with the same solvent.
Reference solution (a) Dilute 1 mL of the test solution to 100 mL with *ethanol (96 per cent) R.*
Reference solution (b) Dilute 1 mL of reference solution (a) to 10 mL with *ethanol (96 per cent) R.*
Reference solution (c) Dilute 5 mL of reference solution (b) to 10 mL with *ethanol (96 per cent) R.*
Column:
— *material*: glass or steel;
— *size*: *l* = 4 m, Ø = 2 mm;
— *stationary phase*: diatomaceous earth for gas chromatography R, impregnated with a mixture suitable for the separation of free fatty acids.
Carrier gas *nitrogen for chromatography R.*
Flow rate 30 mL/min.
Temperature:

	Time (min)	Temperature (°C)
Column	0 - 2	80
	2 - 22	80 → 240
	22 - 37	240
Injection port		250
Detector		300

Detection Flame ionisation.
Injection 1 µL.
System suitability Reference solution (b):
— *signal-to-noise ratio*: minimum 5 for the principal peak.
Limits:
— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

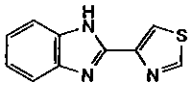
Residue on evaporation
Maximum 0.05 per cent.
Evaporate 2.00 g on a water-bath and heat in an oven at 100-105 °C for 1 h. The residue weighs not more than 1.0 mg.

STORAGE
Protected from light.

Ph Eur

Tiabendazole

(Ph. Eur. monograph 0866)



$C_{10}H_7N_3S$ 201.2 *148-79-8*

Action and use
Benzimidazole antihelminthic.

Ph Eur

DEFINITION
Tiabendazole contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of 2-(thiazol-4-yl)-1*H*-benzimidazole, calculated with reference to the anhydrous substance.

CHARACTERS
A white or almost white, crystalline powder, practically insoluble in water, slightly soluble in alcohol and in methylene chloride. It dissolves in dilute mineral acids. It melts at about 300 °C.

IDENTIFICATION
First identification: B.
Second identification: A, C, D.
A. Dissolve 25 mg in *0.1 M hydrochloric acid* and dilute to 100.0 mL with the same acid. Dilute 2.0 mL of the solution to 100.0 mL with *0.1 M hydrochloric acid*. Examined between 230 nm and 350 nm (*2.2.25*), the solution shows two absorption maxima, at 243 nm and 302 nm. The ratio of the absorbance measured at the maximum at 302 nm to that measured at the maximum at 243 nm is 1.8 to 2.1.
B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *tiabendazole CRS*. Examine the substances prepared as discs.
C. Examine the chromatograms obtained in the test for related substances in ultraviolet light at 254 nm.
The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve about 5 mg in *0.1 M hydrochloric acid* and dilute to 5 mL with the same acid. Add 3 mg of *p-phenylenediamine dihydrochloride R* and shake until dissolved. Add 0.1 g of *zinc powder R*, mix, allow to stand for 2 min and add 5 mL of *ferric ammonium sulfate solution R2*. A bluish-violet colour develops.

TESTS

Related substances

Examine by thin-layer chromatography (*2.2.27*), using *silica gel HF_{254} R* as the coating substance.

Test solution (a) Dissolve 0.10 g of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Test solution (b) Dilute 2 mL of test solution (a) to 20 mL with *methanol R*.

Reference solution (a) Dissolve 20 mg of *tiabendazole CRS* in *methanol R* and dilute to 20 mL with the same solvent.

Reference solution (b) Dilute 1 mL of test solution (b) to 10 mL with *methanol R*.

Reference solution (c) Dilute 1 mL of test solution (b) to 25 mL with *methanol R*.

Apply separately to the plate 20 µL of each solution. Develop over a path of 15 cm using a mixture of 2.5 volumes of *water R*, 10 volumes of *acetone R*, 25 volumes of *glacial acetic acid R* and 62.5 volumes of *toluene R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.0 per cent) and at most one such spot is more intense than the spot in the chromatogram obtained with reference solution (c) (0.4 per cent).

o-Phenylenediamine

To 5.0 g in a flask fitted with a ground-glass stopper, add 25 mL of a mixture of 1 volume of *methanol R* and 2 volumes of *water R*. Shake for 3 min. Filter through a sintered-glass filter (16) (*2.1.2*) under reduced pressure. To 10 mL of the filtrate add 0.5 mL of *hydrochloric acid R* and 0.5 mL of *acetylacetone R* and shake until the solution is clear. The solution is not more intensely coloured than reference solution R_7 (*2.2.2, Method I*) (10 ppm).

Water (*2.5.12*)

Not more than 0.5 per cent, determined on 1.00 g by the semi-micro determination of water.

Sulfated ash (*2.4.14*)

Not more than 0.2 per cent, determined on 1.0 g.

ASSAY

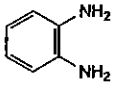
Dissolve 0.150 g in 30 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 20.12 mg of $C_{10}H_7N_3S$.

STORAGE

Store protected from light.

IMPURITIES

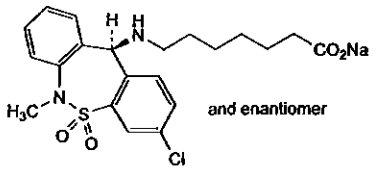


A. benzene-1,2-diamine.

Ph Eur

Tianeptine Sodium

(Ph. Eur. monograph 2022)



$C_{21}H_{24}ClN_2NaO_4S$ 458.9 *30123-17-2*

Action and use

Antidepressant.

Ph Eur

DEFINITION

Sodium 7-[[(11*RS*)-3-chloro-6-methyl-6,11-dihydrodibenzo[*c*,*f*][1,2]thiazepin-11-yl]amino]heptanoate *S*,*S*-dioxide.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or yellowish powder, very hygroscopic.

Solubility

Freely soluble in water, in methanol and in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of tianeptine sodium.*

B. It gives reaction (a) of sodium (*2.3.1*).

TESTS

Impurity A

Gas chromatography (*2.2.28*).

Internal standard solution Dilute 1 mL of *ethyl 5-bromovalerate R* in *anhydrous ethanol R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of the solution to 250.0 mL with *anhydrous ethanol R*.

Test solution Dissolve 0.1000 g of the substance to be examined in the internal standard solution and dilute to 2.0 mL with the same solution.

Reference solution Dissolve 10.0 mg of *tianeptine impurity A CRS* in the internal standard solution and dilute to 200.0 mL with the same solution.

Column:
- *material*: fused silica,
- *size*: *l* = 25 m, Ø = 0.25 mm,
- *stationary phase*: *cyanopropylpolysiloxane R* (film thickness 0.2 µm).

Carrier gas *helium for chromatography R.*

Linear velocity 26 cm/s.

Split ratio 1:100.

Temperature:
- *column*: 150 °C,
- *injection port and detector*: 210 °C.

Detection Flame ionisation.

Injection 1 µL.

Run time Twice the retention time of ethyl 5-bromovalerate.

System suitability Reference solution:
- *elution order*: ethanol, ethyl 5-bromovalerate, impurity A,

— *resolution*: minimum 10 between the peaks due to ethyl 5-bromovalerate and impurity A,
— *signal-to-noise ratio*: minimum 20 for the peak due to impurity A.

Limit:
— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.1 per cent).

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture Mix 50 volumes of *methanol R* and 50 volumes of *water R*.

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 20.0 mL with the solvent mixture.

Reference solution (b) Dissolve 20 mg of *sodium tianeptine for system suitability CRS* in the solvent mixture and dilute to 200 mL with the solvent mixture.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm,
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm) with a pore size of 0.01 µm,
— *temperature*: 30 °C.

Mobile phase:
— *mobile phase A*: mix 21 volumes of *methanol R1*, 31.5 volumes of *acetonitrile R1* and 47.5 volumes of a 2 g/L solution of *sodium laurilsulfate R*, adjusted to pH 2.5 with *phosphoric acid R*,
— *mobile phase B*: mix 20 volumes of *methanol R1*, 20 volumes of a 2 g/L solution of *sodium laurilsulfate R*, adjusted to pH 2.5 with *phosphoric acid R* and 60 volumes of *acetonitrile R1*,

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 35	100	0
35 - 45	100 → 40	0 → 60
45 - 60	40	60
60 - 70	40 → 100	60 → 0

Flow rate 1 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µL.

Relative retention with reference to tianeptine (retention time = about 30 min): impurity C = about 0.4; impurity D1 = about 0.6; impurity D2 = about 0.8; impurity E = about 1.1; impurity B = about 1.7.

System suitability Reference solution (b):
— *resolution*: minimum 2.5 between the peaks due to tianeptine and impurity E.

Limits:
— *any impurity*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
— *total*: not more than 8 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent),
— *disregard limit*: area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

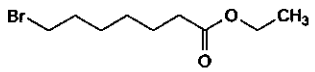
Water (*2.5.12*)
Maximum 5.0 per cent, determined on 0.100 g.

ASSAY
Dissolve 0.165 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

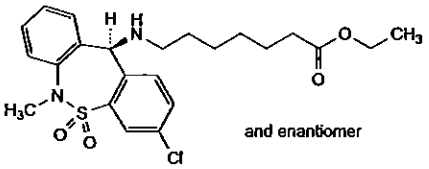
1 mL of *0.1 M perchloric acid* is equivalent to 22.95 mg of $C_{21}H_{24}ClN_2NaO_4S$.

STORAGE
In an airtight container.

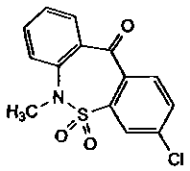
IMPURITIES



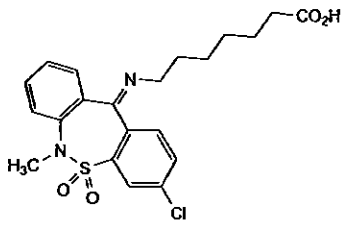
A. ethyl 7-bromoheptanoate,



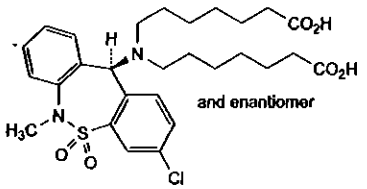
B. ethyl 7-[[(11*RS*)-3-chloro-6-methyl-6,11-dihydrodibenzo[*c*,*f*][1,2]thiazepin-11-yl]amino]heptanoate *S*,*S*-dioxide,



C. 3-chloro-6-methyldibenzo[*c*,*f*][1,2]thiazepin-11(6*H*)-one *S*,*S*-dioxide,



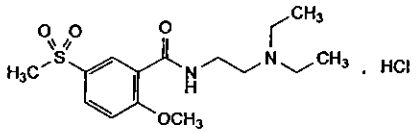
D. 7-[[(11*RS*)-3-chloro-6-methyldibenzo[*c*,*f*][1,2]thiazepin-11(6*H*)-ylidene]amino]heptanoic acid *S*,*S*-dioxide,



E. 7,7′-[[(11*RS*)-3-chloro-6-methyl-6,11-dihydrodibenzo[*c*,*f*][1,2]thiazepin-11-yl]imino]diheptanoic acid *S*,*S*-dioxide.

Tiapride Hydrochloride

(Ph. Eur. monograph 1575)



$C_{15}H_{25}ClN_2O_4S$ 364.9 *51012-33-0*

Action and use
Dopamine receptor antagonist; neuroleptic.

Ph Eur

DEFINITION
N-[2-(Diethylamino)ethyl]-2-methoxy-5-(methylsulfonyl)benzamide hydrochloride.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Very soluble in water, soluble in methanol, slightly soluble in anhydrous ethanol.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *tiapride hydrochloride CRS.*
B. Solution S (see Tests) gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S
Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and its absorbance (*2.2.25*) at 450 nm is not greater than 0.030.

pH (*2.2.3*)
4.0 to 6.0 for solution S.

Impurity C
Thin-layer chromatography (*2.2.27*).
Test solution Dissolve 0.400 g of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.
Reference solution Dissolve 20.0 mg of *metoclopramide impurity E CRS* (impurity C) in *methanol R* and dilute to 50 mL with the same solvent. Dilute 2.0 mL of the solution to 20 mL with *methanol R*.
Plate *TLC silica gel F_{254} plate R.*
Mobile phase *concentrated ammonia R, dioxan R, methanol R, methylene chloride R* (2:10:14:90 *V/V/V/V*).
Application 10 µL.
Development Over 4/5 of the plate.
Drying In air.
Detection Spray with a 2 g/L solution of *ninhydrin R* in *butanol R* and heat at 100 °C for 15 min.
Retardation factors Impurity C = about 0.1; tiapride = about 0.6.
Limit:
— *impurity C*: any spot due to impurity C is not more intense than the corresponding spot in the chromatogram obtained with the reference solution (0.1 per cent).

Related substances
Liquid chromatography (*2.2.29*).
Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.
Reference solution (a) Dilute 1.0 mL of the test solution to 10.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 100.0 mL with the mobile phase.
Reference solution (b) Dissolve 5.0 mg of *tiapride hydrochloride CRS* and 5.0 mg of *tiapride N-oxide CRS* (impurity D) in the mobile phase and dilute to 100.0 mL with the mobile phase.
Column:
— *size:* *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase:* *base-deactivated end-capped octylsilyl silica gel for chromatography R* (5 µm);
— *temperature:* 40 °C.
Mobile phase Dissolve 5.44 g of *potassium dihydrogen phosphate R* and 0.08 g of *sodium octanesulfonate R* in 780 mL of *water R*, adjust to pH 2.7 with *phosphoric acid R* and dilute to 800 mL with *water R*; add 150 mL of *methanol R* and 50 mL of *acetonitrile R* and mix.
Flow rate 1.5 mL/min.
Detection Spectrophotometer at 240 nm.
Injection 10 µL.
Run time 3 times the retention time of tiapride.
Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity D.
Relative retention With reference to tiapride (retention time = about 7 min): impurity D = about 1.2.
System suitability Reference solution (b):
— *resolution:* minimum 4.0 between the peaks due to tiapride and impurity D.
Calculation of percentage contents:
— for each impurity, use the concentration of tiapride hydrochloride in reference solution (a).
Limits:
— *unspecified impurities:* for each impurity, maximum 0.10 per cent;
— *total:* maximum 0.2 per cent;
— *reporting threshold:* 0.05 per cent.

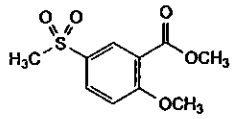
Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

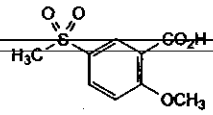
ASSAY
Dissolve 0.300 g in 20 mL of *anhydrous acetic acid R*. Add 20 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).
1 mL of *0.1 M perchloric acid* is equivalent to 36.49 mg of $C_{15}H_{25}ClN_2O_4S$.

IMPURITIES
Specified impurities *C.*

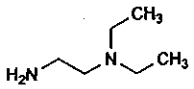
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, D.*



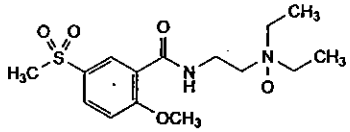
A. methyl 2-methoxy-5-(methylsulfonyl)benzoate,



B. 2-methoxy-5-(methylsulfonyl)benzoic acid,



C. *N*,*N*-diethylethane-1,2-diamine,

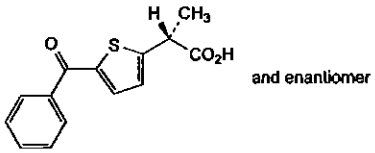


D. *N*-[2-(diethyloxidoaminol)ethyl]-2-methoxy-5-(methylsulfonyl)benzamide (tiapride *N*-oxide).

Ph Eur

Tiaprofenic Acid

(Ph. Eur. monograph 1157)



$C_{14}H_{12}O_3S$ 260.3 *33005-95-7*

Action and use
Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Ph Eur

DEFINITION
(2*RS*)-2-(5-Benzoylthiophen-2-yl)propanoic acid.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, freely soluble in acetone, in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION
First identification: C.

Second identification: A, B, D.

A. Melting point (*2.2.14*): 95 °C to 99 °C.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 25.0 mg in *ethanolic hydrochloric acid R* and dilute to 50.0 mL with the same solvent. Dilute 1.0 mL of this solution to 50.0 mL with *ethanolic hydrochloric acid R*.

Spectral range 220-350 nm.

Absorption maximum 305 nm.

Shoulder 262 nm.

Specific absorbance at the absorption maximum 550 to 590.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *tiaprofenic acid CRS.*

D. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *methylene chloride R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dissolve 10 mg of *tiaprofenic acid CRS* in *methylene chloride R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 10 mg of *ketoprofen CRS* in *methylene chloride R* and dilute to 10 mL with the same solvent. Dilute 1 mL of this solution to 2 mL with reference solution (a).

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *acetic acid R, methylene chloride R, acetone R* (1:20:80 *V/V/V*).

Application 10 µL.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Retardation factors Tiaprofenic acid = about 0.3; ketoprofen = about 0.4.

System suitability Reference solution (b):
- — the chromatogram shows 2 clearly separated principal spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS
Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Dissolve 2.0 g in *ethanol (96 per cent) R* and dilute to 20 mL with the same solvent.

Optical rotation (*2.2.7*)
−0.10° to + 0.10°.

Dissolve 0.50 g in *ethyl acetate R* and dilute to 10.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 20.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 50.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dilute 5.0 mL of reference solution (a) to 10.0 mL with the mobile phase.

Reference solution (c) Dissolve 10.0 mg of *tiaprofenic acid impurity C CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution (d) Dilute 1 mL of reference solution (a) to 2 mL with reference solution (c).

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *silica gel for chromatography R* (5 µm).

Mobile phase *water for chromatography R, glacial acetic acid R, hexane R, methylene chloride R* (0.25:20:500:500 *V/V/V/V*); add the water to the acetic acid, then hexane and methylene chloride; sonicate the mixture for 2 min. Do not degas with helium during analysis.

Flow rate 1 mL/min.

Detection Spectrophotometer at 250 nm.

Injection 20 µL.

Run time Twice the retention time of tiaprofenic acid.

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peak due to impurity C.

Relative retention With reference to tiaprofenic acid (retention time = about 14 min): impurity C = about 0.86.

System suitability Reference solution (d):
— *resolution*: minimum 3.0 between the peaks due to impurity C and tiaprofenic acid.

Limits:
— *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.2 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *sum of impurities other than C*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 3 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

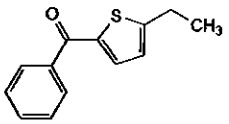
ASSAY
Dissolve 0.250 g in 25 mL of *ethanol (96 per cent) R*. Add 25 mL of *water R* and 0.5 mL of *phenolphthalein solution R*. Titrate with *0.1 M sodium hydroxide*.

1 mL of *0.1 M sodium hydroxide* is equivalent to 26.03 mg of $C_{14}H_{12}O_3S$.

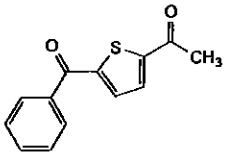
STORAGE
Protected from light.

IMPURITIES
Specified impurities *C*.

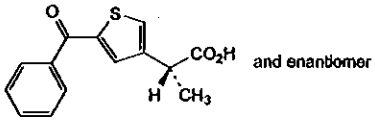
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, D, E, F.*



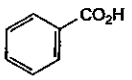
A. (5-ethylthiophen-2-yl)phenylmethanone,



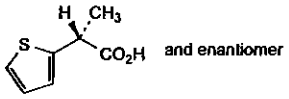
B. 1-(5-benzoylthiophen-2-yl)ethanone,



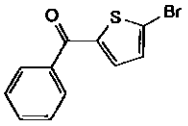
C. (2*RS*)-2-(5-benzoylthiophen-3-yl)propanoic acid,



D. benzoic acid,



E. (2*RS*)-2-(thiophen-2-yl)propanoic acid,

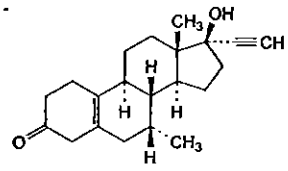


F. (5-bromothiophen-2-yl)phenylmethanone.

Ph Eur

Tibolone

(Ph. Eur. monograph 1739)



$C_{21}H_{28}O_2$ 312.5

Action and use
Steroid with estrogenic and progestogenic properties.

Preparation
Tibolone Tablets

Ph Eur

DEFINITION

17-Hydroxy-7α-methyl-19-nor-17α-pregn-5(10)-en-20-yn-3-one.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or crystals.

Solubility

Practically insoluble in water, soluble in acetone and in methanol.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of tibolone.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined in the minimum volume of *anhydrous ethanol R*, evaporate to dryness on a water-bath and record a new spectrum using the residue.

TESTS

Specific optical rotation (*2.2.7*)

+ 100 to + 106 (dried substance).

Dissolve 0.250 g in *anhydrous ethanol R* and dilute to 25.0 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Select a brand of acetonitrile such that the formation of possible artefact peaks, eluting after impurity C at relative retentions 0.6 to 0.8, is avoided.

Solvent mixture *water R, acetonitrile R1* (25:75 *V/V*).

Test solution Dissolve 40.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (a) Dissolve 4 mg of *tibolone for system suitability CRS* (containing impurities A, B, C, D and E) in 1 mL of the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: spherical *end-capped octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *methanol R, acetonitrile R1, water R* (8:40:52 *V/V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 205 nm.

Injection 5 µL.

Run time 3 times the retention time of tibolone.

Identification of impurities Use the chromatogram supplied with *tibolone for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D and E.

Relative retention With reference to tibolone (retention time = about 14 min): impurity A = about 0.22; impurity B = about 0.24; impurity C = about 0.58; impurity D = about 1.12; impurity E = about 2.24.

System suitability Reference solution (a):

— *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to tibolone.

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.7; impurity B = 1.5; impurity C = 2.1;

— *impurities A, E*: for each impurity, not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *impurity B*: not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent);

— *impurity C*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *impurity D*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 60 mL of *tetrahydrofuran R*. Add 25 mL of a 100 g/L solution of *silver nitrate R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

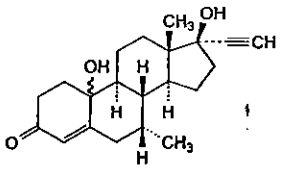
1 mL of *0.1 M sodium hydroxide* is equivalent to 31.25 mg of $C_{21}H_{28}O_2$.

STORAGE

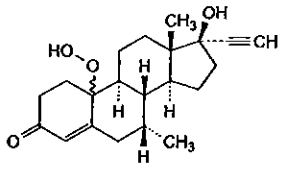
At a temperature of 2 °C to 8 °C.

IMPURITIES

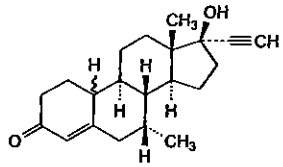
Specified impurities *A, B, C, D, E.*



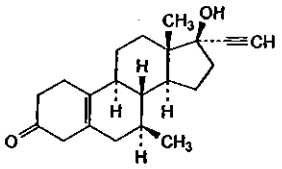
A. 10,17-dihydroxy-7α-methyl-19-nor-10ξ,17α-pregn-4-en-20-yn-3-one,



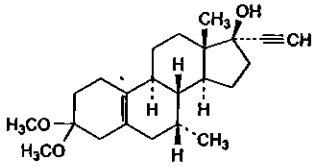
B. 10-hydroperoxy-17-hydroxy-7α-methyl-19-nor-10ξ,17α-pregn-4-en-20-yn-3-one,



C. 17-hydroxy-7α-methyl-19-nor-10ξ,17α-pregn-4-en-20-yn-3-one,



D. 17-hydroxy-7β-methyl-19-nor-17α-pregn-5(10)-en-20-yn-3-one,

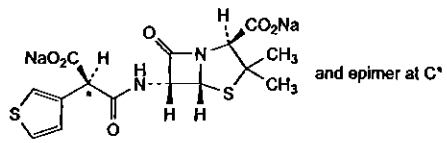


E. 3,3-dimethoxy-7α-methyl-19-nor-17α-pregn-5(10)-en-20-yn-17-ol.

Ph Eur

Ticarcillin Sodium

(Ph. Eur. monograph 0956)



$C_{15}H_{14}N_2Na_2O_6S_2$ 428.4 *4697-14-7*

Action and use
Penicillin antibacterial.

Preparation
Ticarcillin and Clavulanic Acid Infusion

Ph Eur

DEFINITION

Disodium (2*S*,5*R*,6*R*)-6-[[(2*RS*)-2-carboxylato-2-(thiophen-3-yl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate.

Semi-synthetic product derived from a fermentation product.

Content
89.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or slightly yellow, hygroscopic powder.

Solubility
Freely soluble in water, soluble in methanol.

IDENTIFICATION

First identification: A, D, E.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Dissolve 50 mg of the substance to be examined in 1 mL of *water R*, add 0.1 mL of *hydrochloric acid R1*, swirl and allow to stand in iced water for 10 min. Filter the precipitate and rinse with 2 mL of *water R*. Dissolve in a mixture of 1 volume of *water R* and 9 volumes of *acetone R*. Evaporate the solvent almost to dryness, then dry in an oven at 60 °C for 30 min.

Comparison Repeat the operations using *ticarcillin monosodium CRS*.

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 5 mL with the same solvent.

Reference solution (a) Dissolve 25 mg of *ticarcillin monosodium CRS* in *methanol R* and dilute to 5 mL with the same solvent.

Reference solution (b) Dissolve 25 mg of *carbenicillin sodium CRS* and 25 mg of *ticarcillin monosodium CRS* in *methanol R* and dilute to 5 mL with the same solvent.

Plate *TLC silanised silica gel plate R.*

Mobile phase Mix 10 volumes of *acetone R* and 90 volumes of a 154 g/L solution of *ammonium acetate R*, adjusted to pH 5.0 with *glacial acetic acid R*.

Application 1 µL.

Development Over a path of 12 cm.

Drying In a current of hot air.

Detection Expose to iodine vapour.

System suitability Reference solution (b):
— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Place about 2 mg in a test-tube about 15 cm long and 15 mm in diameter. Moisten with 0.05 mL of *water R* and add 2 mL of *sulfuric acid-formaldehyde reagent R*. Mix the contents of the tube by swirling; the solution is brown. Place the test-tube in a water-bath for 1 min; a dark reddish-brown colour develops.

D. It gives reaction (a) of sodium (*2.3.1*).

E. Specific optical rotation (see Tests).

TESTS

Solution S
Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5 (*2.2.2, Method II*).

pH (*2.2.3*)
5.5 to 7.5 for solution S.

Specific optical rotation (*2.2.7*)
+ 172 to + 187 (anhydrous substance).

Dissolve 0.250 g in *water R* and dilute to 25.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in mobile phase A and dilute to 25.0 mL with mobile phase A.

Reference solution (a) Dissolve 20.0 mg of *ticarcillin impurity A CRS* in mobile phase A and dilute to 100.0 mL with mobile phase A. Dilute 5.0 mL of this solution to 50.0 mL with mobile phase A.

Reference solution (b) Dilute 1 mL of the test solution to 50 mL with mobile phase A.

Column:
— *size*: *l* = 0.25 m, Ø = 4 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: 1.3 g/L solution of *ammonium phosphate R* adjusted to pH 7.0 with *phosphoric acid R*;
— *mobile phase B*: *methanol R*, mobile phase A (50:50 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	100 → 30	0 → 70
30 - 40	30	70

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µL.

System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the 2 principal peaks (diastereoisomers).

Limits:
— *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (4 per cent);
— *any other impurity*: for each impurity, not more than 1.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (2.5 per cent).

N,N-Dimethylaniline (*2.4.26, Method B*)
Maximum 20 ppm.

2-Ethylhexanoic acid (*2.4.28*)
Maximum 0.5 per cent *m/m*.

Water (*2.5.12*)
Maximum 5.5 per cent, determined on 0.150 g.

Bacterial endotoxins (*2.6.14*)
Less than 0.05 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 10.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution Dissolve 50.0 mg of *ticarcillin monosodium CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 10.0 mL of this solution to 50.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 20 volumes of *methanol R* and 80 volumes of a 1.3 g/L solution of *ammonium phosphate R* adjusted to pH 7.0 with *phosphoric acid R*.

Flow rate 1 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µL.

System suitability Reference solution:
— *resolution*: minimum 2.5 between the 2 principal peaks;
— *repeatability*: maximum relative standard deviation of 1.0 per cent for the 2 peaks due to ticarcillin after 6 injections.

Calculate the percentage content of ticarcillin sodium as the sum of the areas of the 2 peaks, multiplying the content of ticarcillin monosodium by 1.054.

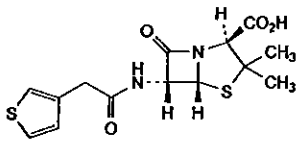
STORAGE

In an airtight container, at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-evident container.

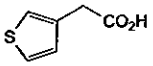
IMPURITIES

Specified impurities *A.*

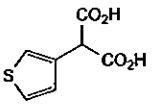
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D, E.*



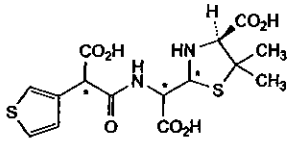
A. (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[[(thiophen-3-yl)acetyl]amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (decarboxyticarcillin),



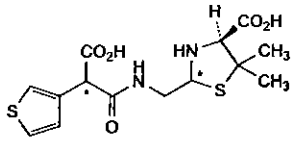
B. (thiophen-3-yl)acetic acid,



C. 2-(thiophen-3-yl)propanedioic acid (3-thienylmalonic acid),



D. (4*S*)-2-[carboxy[[2-carboxy-2-(thiophen-3-yl)acetyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of ticarcillin),

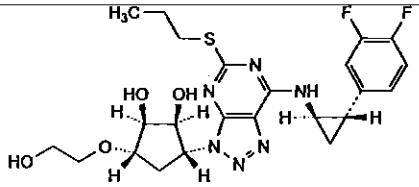


E. (4*S*)-2-[[[2-carboxy-2-(thiophen-3-yl)acetyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of ticarcillin).

Ph Eur

Ticagrelor

(Ph. Eur. monograph 3087)



$C_{23}H_{28}F_2N_6O_4S$ 522.6 *274693-27-5*

Action and use
Platelet aggregation inhibitor.

Ph Eur

DEFINITION
(1*S*,2*S*,3*R*,5*S*)-3-[7-[[(1*R*,2*S*)-2-(3,4-Difluorophenyl)cyclopropyl]amino]-5-(propylsulfanyl)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-5-(2-hydroxyethoxy)cyclopentane-1,2-diol.

Content
97.5 per cent to 102.0 per cent (anhydrous substance).

PRODUCTION
It is produced by highly stereoselective methods of manufacture; consideration must be given to the formation of potential stereoisomeric impurities during the manufacturing process, and procedures must be implemented for the appropriate control of these impurities.

CHARACTERS

Appearance
White or almost white to pale pink powder.

Solubility
Practically insoluble in water, freely soluble in methanol, soluble in anhydrous ethanol, practically insoluble in heptane.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *ticagrelor CRS*.

TESTS

Impurity E
Liquid chromatography (*2.2.29*).

Buffer solution 156 g/L solution of *sodium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*.

Test solution Dissolve 30.0 mg of the substance to be examined in 2 mL of a mixture of equal volumes of *acetonitrile R* and *water R* and dilute to 10.0 mL with *water R*. Centrifuge for 15 min at about 1200 *g* or until a clear solution is obtained. Use the clear supernatant.

Reference solution Dissolve 4.5 mg of *ticagrelor impurity E CRS* ((*R*)-mandelate salt) in *acetonitrile R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of the solution to 100.0 mL with *acetonitrile R*. Dilute 1.0 mL of this solution to 10.0 mL with *water R* (solution equivalent to a 24 ng/mL solution of impurity E).

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (1.8 µm);
— *temperature*: 55 °C.

Mobile phase:
— *mobile phase A*: mix 1 volume of the buffer solution and 89 volumes of *water for chromatography R* and add 10 volumes of *acetonitrile for chromatography R*;
— *mobile phase B*: mix 1 volume of the buffer solution and 29 volumes of *water for chromatography R* and add 70 volumes of *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 8	94	6
8 - 9	94 → 0	6 → 100
9 - 20	0	100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 222 nm.

Injection: 100 µL.

Relative retention With reference to ticagrelor (retention time = about 11.5 min): impurity E = about 0.6.

System suitability Reference solution:
— *repeatability*: maximum relative standard deviation of 10.0 per cent determined on 6 injections.

Limit:
— *impurity E*: not more than the area of the principal peak in the chromatogram obtained with the reference solution (8 ppm).

Related substances
Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Solvent mixture *acetonitrile R*, *water R* (35:65 *V/V*).

Buffer solution 156 g/L solution of *sodium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*.

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Reference solution (a) Dissolve 50.0 mg of *ticagrelor CRS* in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 5 mg of *ticagrelor for system suitability CRS* (containing impurities A, B, C and D) in the solvent mixture and dilute to 10 mL with the solvent mixture.

Column:
— *size*: *l* = 0.15 m, Ø = 3.0 mm;
— *stationary phase*: *base-deactivated end-capped phenylsilyl silica gel for chromatography R* (3 µm);
— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: mix 1 volume of the buffer solution and 89 volumes of *water for chromatography R* and add 10 volumes of *acetonitrile for chromatography R*;

— *mobile phase B*: mix 1 volume of the buffer solution and 29 volumes of *water for chromatography R* and add 70 volumes of *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	80	20
2 - 42	80 → 25	20 → 75
42 - 47	25	75

Flow rate 0.65 mL/min.

Detection Spectrophotometer at 242 nm.

Injection 5 µL of the test solution and reference solutions (b) and (c).

Identification of impurities Use the chromatogram supplied with *ticagrelor for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify impurities A, B, C and D.

Relative retention With reference to ticagrelor (retention time = about 23 min): impurity A = about 0.15; impurity B = about 1.06; impurity C = about 1.23; impurity D = about 1.5.

System suitability Reference solution (c):
— *resolution*: minimum 4.0 between the peaks due to ticagrelor and impurity B.

Calculation of percentage contents:
— *correction factor*: multiply the peak area of impurity A by 0.5;
— for each impurity, use the concentration of ticagrelor in reference solution (b).

Limits:
— *impurity D*: maximum 0.3 percent;
— *impurities A, B*: for each impurity, maximum 0.2 per cent;
— *impurity C*: maximum 0.1 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 1.0 per cent;
— *reporting threshold*: 0.05 per cent.

Water (*2.5.32*)
Maximum 0.5 per cent determined on 0.500 g using the evaporation technique at 130 °C.

Sulfated ash (*2.4.14*)
Maximum 0.6 per cent, determined on 1.0 g in a platinum crucible.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution and reference solutions (a) and (c).

Mobile phase Mix 1 volume of the buffer solution and 56 volumes of *water for chromatography R* and add 43 volumes of *acetonitrile for chromatography R*.

Run time 4.5 times the retention time of ticagrelor.

Relative retention With reference to ticagrelor (retention time = about 6 min): impurity B = about 1.15.

System suitability Reference solution (c):
— *resolution*: minimum 1.5 between the peaks due to ticagrelor and impurity B.

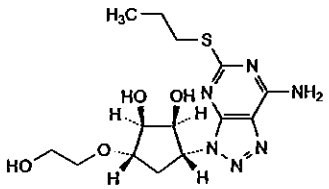
Calculate the percentage content of $C_{23}H_{28}F_2N_6O_4S$ using the chromatogram obtained with reference solution (a) and taking into account the assigned content of *ticagrelor CRS*.

STORAGE

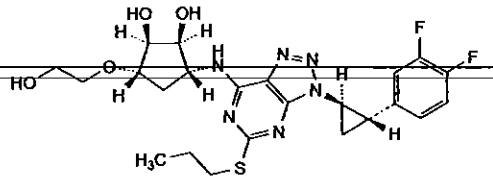
Protected from light.

IMPURITIES

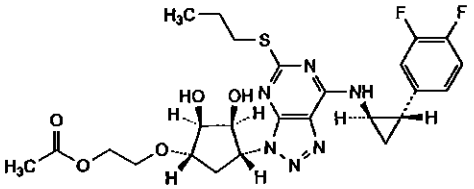
Specified impurities *A, B, C, D, E.*



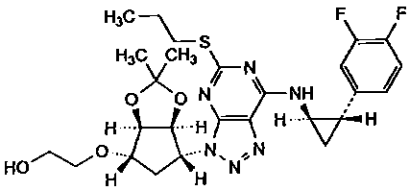
A. (1*S*,2*S*,3*R*,5*S*)-3-[7-amino-5-(propylsulfanyl)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-5-(2-hydroxyethoxy)cyclopentane-1,2-diol,



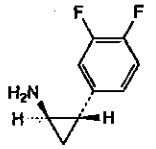
B. (1*S*,2*S*,3*R*,5*S*)-3-[[3-[(1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl]-5-(propylsulfanyl)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-7-yl]amino]-5-(2-hydroxyethoxy)cyclopentane-1,2-diol,



C. 2-[[(1*S*,2*S*,3*S*,4*R*)-4-[7-[[(1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl]amino]-5-(propylsulfanyl)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-2,3-dihydroxycyclopentyl]oxy]ethyl acetate,



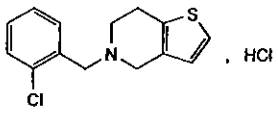
D. 2-[[(3a*R*,4*S*,6*R*,6a*S*)-6-[7-[[(1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl]amino]-5-(propylsulfanyl)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-2,2-dimethyltetrahydro-2*H*,3a*H*-cyclopenta[*d*][1,3]dioxol-4-yl]oxy]ethan-1-ol,



E. (1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropan-1-amine.

Ticlopidine Hydrochloride

(Ph. Eur. monograph 1050)



$C_{14}H_{15}Cl_2NS$ 300.2 *53885-35-1*

Action and use
Inhibition of adenosine diphosphate (ADP)-mediated platelet aggregation; antiplatelet drug.

Ph Eur

DEFINITION
5-(2-Chlorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine hydrochloride.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Sparingly soluble in water and in anhydrous ethanol, very slightly soluble in ethyl acetate.

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution (a) Dissolve 40 mg in *water R* and dilute to 100.0 mL with the same solvent.

Test solution (b) Dilute 5.0 mL of test solution (a) to 100.0 mL with *water R*.

Spectral range 250-350 nm for test solution (a); 200-350 nm for test solution (b).

Absorption maxima At 268 nm and 275 nm for test solution (a); at 214 nm and 232 nm for test solution (b).

Absorption ratio A_{268}/A_{275} = 1.1 to 1.2.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *ticlopidine hydrochloride CRS.*

C. Mix about 6 mg of *citric acid monohydrate R* and 0.3 mL of *acetic anhydride R*. Add about 5 mg of the substance to be examined and heat in a water-bath at 80 °C. A red colour develops.

D. About 20 mg gives reaction (a) of chlorides (*2.3.1*).

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 0.5 g in a 1 per cent *V/V* solution of *hydrochloric acid R* and dilute to 20 mL with the same solution.

pH (*2.2.3*)
3.5 to 4.0.

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 20 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture Mobile phase B, mobile phase A (20:80 *V/V*).

Test solution Dissolve 0.250 g of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution Dissolve 5.0 mg of *ticlopidine impurity F CRS* in the solvent mixture. Add 1.00 mL of the test solution and dilute to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Column:
— *size: l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature:* 40 °C.

Mobile phase:
— *mobile phase A:* 0.95 g/L solution of *sodium pentanesulfonate monohydrate R*, adjusted to pH 3.4 with a 50 per cent *V/V* solution of *phosphoric acid R*;
— *mobile phase B: methanol R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 45	80 → 20	20 → 80
45 - 50	20	80

Flow rate 1.3 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µL; inject the solvent mixture as a blank.

Identification of impurities Use the chromatogram obtained with the reference solution to identify the peak due to impurity F.

Retention time Ticlopidine = about 15 min.

System suitability Reference solution:
— *resolution:* minimum 2.0 between the peaks due to ticlopidine and impurity F; if necessary, adjust the pH of mobile phase A;
— *signal-to-noise ratio:* minimum 50 for the peak due to ticlopidine.

Limits:
— *impurity F:* not more than 0.5 times the area of the corresponding peak in the chromatogram obtained with the reference solution (0.05 per cent);
— *unspecified impurities:* for each impurity, not more than the area of the peak due to ticlopidine in the chromatogram obtained with the reference solution (0.10 per cent);
— *total:* not more than the area of the peak due to ticlopidine in the chromatogram obtained with the reference solution (0.10 per cent);
— *disregard limit:* 0.5 times the area of the peak due to ticlopidine in the chromatogram obtained with the reference solution (0.05 per cent). Do not disregard the peak due to impurity F.

Formaldehyde
Maximum 20 ppm.

Dissolve 0.200 g in 4.0 mL of *water R*. Add 0.4 mL of *dilute sodium hydroxide solution R*. Centrifuge, filter the supernatant through cotton previously impregnated with *water R* and dilute to 5.0 mL with *water R*. Transfer to a test-tube. Add 5.0 mL of *acetylacetone reagent R1*. Place the test-tube in a water-bath at 40 °C for 40 min. The test solution is not more intensely coloured than a standard prepared at the same time and in the same manner using 5.0 mL of a 0.8 ppm solution of formaldehyde (CH_2O), obtained by

dilution of *formaldehyde standard solution (5 ppm CH_2O) R* with *water R*. Examine the tubes down their vertical axis.

Water (*2.5.12*)
Maximum 0.5 per cent, determined on 0.500 g.

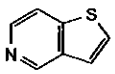
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.150 g in 15 mL of *anhydrous acetic acid R*. Add 35 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

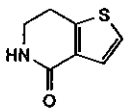
1 mL of *0.1 M perchloric acid* is equivalent to 30.02 mg of $C_{14}H_{15}Cl_2NS$.

IMPURITIES
Specified impurities F.

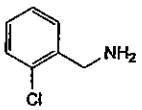
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) A, B, C, D, E, G, H, I, J, K, L.



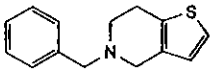
A. thieno[3,2-*c*]pyridine,



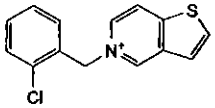
B. 6,7-dihydrothieno[3,2-*c*]pyridin-4(5*H*)-one,



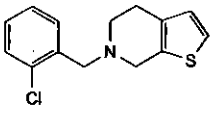
C. (2-chlorophenyl)methanamine,



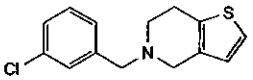
D. 5-benzyl-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine,



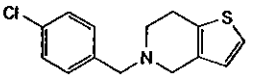
E. 5-(2-chlorobenzyl)thieno[3,2-*c*]pyridinium,



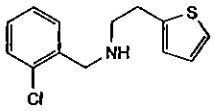
F. 6-(2-chlorobenzyl)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine,



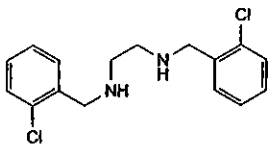
G. 5-(3-chlorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine,



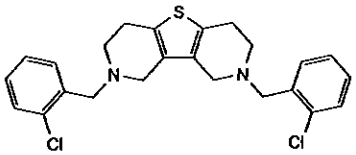
H. 5-(4-chlorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine,



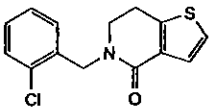
I. *N*-(2-chlorobenzyl)-2-(thiophen-2-yl)ethanamine,



J. *N*,*N*′-bis(2-chlorobenzyl)ethane-1,2-diamine,



K. 2,8-bis(2-chlorobenzyl)-1,2,3,4,6,7,8,9-octahydrothieno[3,2-*c*:4,5-*c*′]dipyridine (bis-ticlopidine),

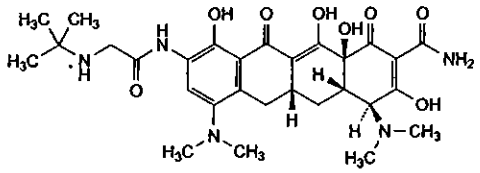


L. 5-(2-chlorobenzyl)-6,7-dihydrothieno[3,2-*c*]pyridin-4(5*H*)-one.

Ph Eur

Tigecycline

(Ph. Eur. monograph 2825)



$C_{29}H_{39}N_5O_8$ 585.7 *220620-09-7*

Action and use
Antibacterial.

Preparation
Tigecycline for Infusion

Ph Eur

DEFINITION

(4*S*,4a*S*,5a*R*,12a*S*)-9-[2-(*tert*-Butylamino)acetamido]-4,7-bis (dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide.

Semi-synthetic product derived from a fermentation product.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Orange, hygroscopic, crystalline powder.

Solubility

Freely soluble in water, slightly soluble in anhydrous ethanol and in heptane.

~~It shows polymorphism (5.9).~~

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *tigecycline CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

TESTS

pH (*2.2.3*)

7.7 to 8.2.

Dissolve 0.500 g in *carbon dioxide-free water R* and dilute to 50.0 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*). *Carry out the test protected from light. Store the solutions at 2-8 °C and use them within 12 h.*

Solution A Solution containing 4.35 g/L of *dipotassium hydrogen phosphate R* and 0.5 g/L of *sodium hydrogensulfite R* adjusted to pH 8.0 with a 56.1 g/L solution of *potassium hydroxide R*.

Test solution (a) Dissolve 50.0 mg of the substance to be examined in solution A and dilute to 100.0 mL with solution A.

Test solution (b) Dilute 10.0 mL of test solution (a) to 50.0 mL with solution A.

Reference solution (a) Dissolve 50.0 mg of *tigecycline CRS* in solution A and dilute to 100.0 mL with solution A. Dilute 10.0 mL of this solution to 50.0 mL with solution A.

Reference solution (b) Dilute 1.0 mL of test solution (a) to 100.0 mL with solution A. Dilute 1.0 mL of this solution to 10.0 mL with solution A.

Reference solution (c) Dissolve 2 mg of *tigecycline impurity B CRS* and 2 mg of *minocycline hydrochloride R* (impurity C) in solution A and dilute to 25 mL with solution A. Dilute 3 mL of the solution to 20 mL with solution A. Dissolve 2.5 mg of *tigecycline for system suitability CRS* (containing impurity A) in 1 mL of this solution and dilute to 5 mL with solution A.

Reference solution (d) Dissolve 2.5 mg of *tigecycline for system suitability CRS* (containing impurity A) in solution A and dilute to 5 mL with solution A.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm);
— *temperature*: 30 °C.

Mobile phase:

— *mobile phase A*: dissolve 4.35 g of *dipotassium hydrogen phosphate R* and 0.93 g of *sodium edetate R* in 940 mL of *water for chromatography R*, and adjust to pH 6.4 with *phosphoric acid R*; make up to 950 mL with *water for chromatography R* and dilute to 1000 mL with *acetonitrile for chromatography R*;
— *mobile phase B*: dissolve 4.35 g of *dipotassium hydrogen phosphate R* and 0.93 g of *sodium edetate R* in 490 mL of *water for chromatography R*, and adjust to pH 6.4 with *phosphoric acid R*; make up to 500 mL with *water for chromatography R* and dilute to 1000 mL with *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	85	15
2 - 42	85 → 57	15 → 43
42 - 57	57 → 0	43 → 100
57 - 60	0	100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 248 nm.

Injection 25 µL of test solution (a) and reference solutions (b) and (c).

Identification of impurities Use the chromatogram supplied with *tigecycline for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B and C.

Relative retention With reference to tigecycline (retention time = about 20 min): impurity B = about 0.6; impurity A = about 0.7; impurity C = about 1.6.

System suitability Reference solution (c):

— *resolution*: minimum 1.6 between the peaks due to impurities B and A.

Calculation of percentage contents:

— for each impurity, use the concentration of tigecycline in reference solution (b).

Limits:

— *impurity A*: maximum 0.6 per cent;
— *impurity C*: maximum 0.2 per cent;
— *impurity B*: maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 1.0 per cent;
— *reporting threshold*: 0.05 per cent.

Water (*2.5.32*)

Maximum 2.5 per cent, determined on 60.0 mg using the evaporation technique at 90 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Column:

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 14 volumes of *acetonitrile for chromatography R* and 86 volumes of a solution containing 4.35 g/L of *dipotassium hydrogen phosphate R* and 0.93 g/L of *sodium edetate R* previously adjusted to pH 6.2 with *phosphoric acid R*.

Injection 20 µL of test solution (b) and reference solutions (a) and (d).

Run time 4.5 times the retention time of tigecycline.

Relative retention With reference to tigecycline (retention time = about 12 min): impurity A = about 0.6.

System suitability Reference solution (d):
— *resolution*: minimum 3.0 between the peaks due to impurity A and tigecycline.

Calculate the percentage content of $C_{29}H_{39}N_5O_8$ using the chromatogram obtained with reference solution (a) and taking into account the assigned content of *tigecycline CRS*.

STORAGE

Under nitrogen, in an airtight container, protected from light, at a temperature of 2 °C to 8 °C. If the substance is sterile, the container is also sterile and tamper-evident.

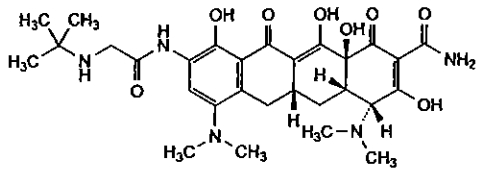
LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

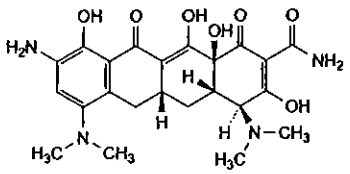
IMPURITIES

Specified impurities *A, B, C.*

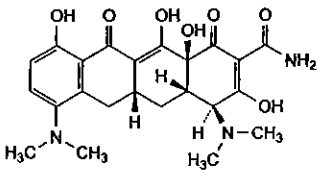
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *D.*



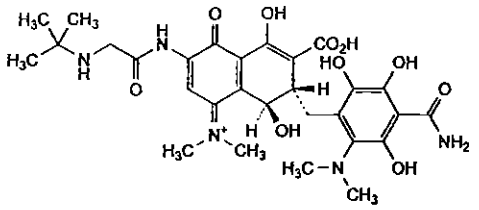
A. (4*R*,4a*S*,5a*R*,12a*S*)-9-[2-(*tert*-butylamino)acetamido]-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide,



B. (4*S*,4a*S*,5a*R*,12a*S*)-9-amino-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (9-aminominocycline),



C. (4*S*,4a*S*,5a*R*,12a*S*)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (minocycline),

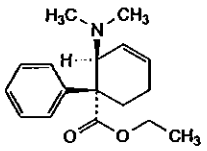


D. (7*S*,8*R*)-3-[2-(*tert*-butylamino)acetamido]-7-[[4-carbamoyl-2-(dimethylamino)-3,5,6-trihydroxyphenyl]methyl]-6-carboxy-5,8-dihydroxy-*N*,*N*-dimethyl-4-oxo-7,8-dihydronaphthalen-1(4*H*)-iminium.

Ph Eur

Tilidine Hydrochloride Hemihydrate

(Ph. Eur. monograph 1767)



and enantiomer , HCl , ½ H_2O

$C_{17}H_{24}ClNO_2,½H_2O$ 318.8 *255733-17-6*

Action and use

Opioid Analgesic.

Ph Eur

DEFINITION

Ethyl (1*RS*,6*SR*)-6-(dimethylamino)-3,6-dihydro[1,1′-biphenyl]-1(2*H*)-carboxylate hydrochloride hemihydrate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

A suitable antioxidant may be added.

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, very soluble in methylene chloride, freely soluble in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *tilidine hydrochloride hemihydrate CRS.*

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

Acidity or alkalinity

To 20.0 mL of solution S add 0.2 mL of *0.01 M sodium hydroxide*. The pH is not less than 4.1. Add 0.4 mL of *0.01 M hydrochloric acid*. The pH is not more than 4.3.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 5 mg of *tilidine for system suitability CRS* (containing impurities D and E) in the mobile phase and dilute to 5 mL with the mobile phase.

Precolumn:
— *size*: *l* = 4 mm, Ø = 4.0 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R1* (5 µm).

Column:
— *size*: *l* = 0.125 m, Ø = 4.0 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R1* (5 µm).

Mobile phase Mix equal volumes of *acetonitrile for chromatography R* and a 0.98 g/L solution of *ammonium carbonate R*.

Flow rate 0.8 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µL.

Run time Twice the retention time of tilidine.

Identification of impurities Use the chromatogram supplied with *tilidine for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities D and E; impurities D and E may be inverted in the elution order, therefore take into account the heights of the corresponding peaks in the chromatogram supplied with *tilidine for system suitabity CRS*.

Relative retention With reference to tilidine (retention time = about 15 min): impurity E = about 0.4; impurity D = about 0.5 (D and E may be inverted).

System suitability Reference solution (b):
— *resolution*: minimum 1.5 between the peaks due to impurities E and D.

Calculation of percentage contents:
— *correction factor*: multiply the peak area of impurity E by 0.5;
— for each impurity, use the concentration of tilidine hydrochloride hemihydrate in reference solution (a).

Limits:
— *impurity E*: maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.5 per cent;
— *reporting threshold*: 0.05 per cent.

Water (*2.5.12*)

2.5 per cent to 3.1 per cent, determined on 0.300 g.

ASSAY

Dissolve 0.250 g in a mixture of 10 mL of *anhydrous acetic acid R* and 50 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 30.98 mg of $C_{17}H_{24}ClNO_2$.

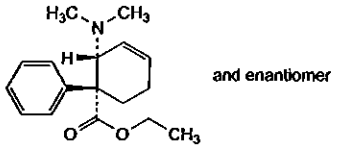
STORAGE

Protected from light.

IMPURITIES

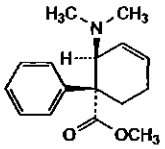
Specified impurities E.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) A, B, C, D.



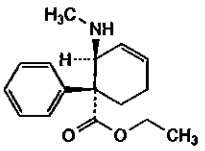
and enantiomer

A. ethyl (1*RS*,6*RS*)-6-(dimethylamino)-3,6-dihydro[1,1′-biphenyl]-1(2*H*)-carboxylate,



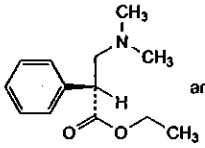
and enantiomer

B. methyl (1*RS*,6*SR*)-6-(dimethylamino)-3,6-dihydro[1,1′-biphenyl]-1(2*H*)-carboxylate,



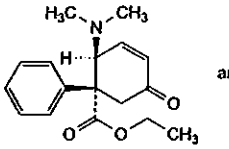
and enantiomer

C. ethyl (1*RS*,6*SR*)-6-(methylamino)-3,6-dihydro[1,1′-biphenyl]-1(2*H*)-carboxylate,



and enantiomer

D. ethyl (2*RS*)-3-(dimethylamino)-2-phenylpropanoate,



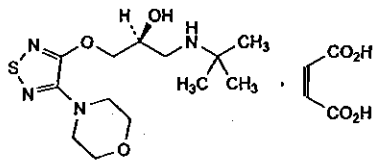
and enantiomer

E. ethyl (1*RS*,6*SR*)-6-(dimethylamino)-3-oxo-3,6-dihydro[1,1′-biphenyl]-1(2*H*)-carboxylate.

Ph Eur

Timolol Maleate

(Ph. Eur. monograph 0572)



$C_{17}H_{28}N_4O_7S$ 432.5 *26921-17-5*

Action and use
Beta-adrenoceptor antagonist.
For Timolol Eye Drops only: treatment of glaucoma.

Preparations
Dorzolamide and Timolol Eye Drops
Timolol Eye Drops
Timolol Tablets

Ph Eur

DEFINITION
(2*S*)-1-[(1,1-Dimethylethyl)amino]-3-[[4-(morpholin-4-yl)-1,2,5-thiadiazol-3-yl]oxy]propan-2-ol (*Z*)-butenedioate.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder or colourless crystals.

Solubility
Soluble in water and in ethanol (96 per cent).

mp
About 199 °C, with decomposition.

IDENTIFICATION
First identification: A, B.
Second identification: A, C, D.

A. Specific optical rotation (*2.2.7*): −6.2 to −5.7.
Dissolve 1.000 g in *1 M hydrochloric acid* and dilute to 10.0 mL with the same acid.

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *timolol maleate CRS.*

C. Thin-layer chromatography (*2.2.27*).
Test solution Dissolve 5 mg of the substance to be examined in *methanol R* and dilute to 5 mL with the same solvent.
Reference solution Dissolve 5 mg of *timolol maleate CRS* in *methanol R* and dilute to 5 mL with the same solvent.
Plate *TLC silica gel GF_{254} plate R.*
Mobile phase *concentrated ammonia R, methanol R, methylene chloride R* (1:20:80 *V/V/V*).
Application 10 µL.
Development Over 2/3 of the plate.
Drying In air.
Detection Expose to iodine vapour for 2 h.
Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Triturate 0.1 g with a mixture of 1 mL of *dilute sodium hydroxide solution R* and 3 mL of *water R*. Shake with 3 quantities, each of 5 mL, of *ether R*. To 0.1 mL of the aqueous layer add a solution containing 10 mg of *resorcinol R* in 3 mL of *sulfuric acid R*. Heat on a water-bath for 15 min; no violet-red colour develops. Neutralise the remainder of the aqueous layer with *dilute sulfuric acid R* and add 1 mL of *bromine water R*. Heat on a water-bath for 15 min, then heat to boiling and cool. To 0.2 mL of this solution add a solution containing 10 mg of *resorcinol R* in 3 mL of *sulfuric acid R*. Heat on a water-bath for 15 min; a violet-red colour develops. Add 0.2 mL of a 100 g/L solution of *potassium bromide R* and heat for 5 min on a water-bath; the colour becomes violet-blue.

TESTS
Solution S
Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_8 (*2.2.2, Method II*).

pH (*2.2.3*)
3.8 to 4.3 for solution S.

Enantiomeric purity
Liquid chromatography (*2.2.29*). *Carry out the test protected from actinic light.*
Solvent mixture *methylene chloride R, 2-propanol R* (10:30 *V/V*).
Test solution Dissolve 30.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.
Reference solution (a) Dissolve 30 mg of *timolol maleate CRS* in the solvent mixture and dilute to 10 mL with the solvent mixture.
Reference solution (b) Dissolve 3 mg of *(R)-timolol CRS* (impurity A) in the solvent mixture and dilute to 10.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 10.0 mL with the solvent mixture.
Reference solution (c) Dilute 1 mL of reference solution (a) to 100 mL with the solvent mixture. Mix 1 mL of this solution with 1 mL of reference solution (b).
Reference solution (d) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture.
Column:
— *size:* l = 0.25 m, Ø = 4.6 mm;
— *stationary phase: cellulose derivative of silica gel for chiral separation R* (5 µm).
Mobile phase *diethylamine R, 2-propanol R, hexane R* (2:40:960 *V/V/V*).
Flow rate 1 mL/min.
Detection Spectrophotometer at 297 nm.
Injection 5 µL.
Elution order Impurity A is eluted first.
System suitability:
— *resolution:* minimum 4.0 between the peaks due to impurity A and the (*S*)-enantiomer in the chromatogram obtained with reference solution (c);
— the retention times of the principal peaks due to the (*S*)-enantiomer in the chromatograms obtained with the test solution and reference solution (a) are identical.
Limit:
— *impurity A:* not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (1.0 per cent).

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in mobile phase A and dilute to 20 mL with mobile phase A.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

Reference solution (b) Dissolve 3 mg of *timolol impurity F CRS* in *methanol R* and dilute to 20.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with mobile phase A (solution A). Dissolve the contents of a vial of *timolol for system suitability CRS* (containing impurities B, C and D) in 1.0 mL of solution A.

Reference solution (c) Dissolve 2 mg of the substance to be examined and 20 mg of *maleic acid R* in 10 mL of *acetonitrile R*. Evaporate 1 mL of the solution to dryness under a stream of *nitrogen R* in an amber glass vial. Heat the open vial at 105 °C for 1 h. Reconstitute the residue with 1.0 mL of mobile phase A (*in situ* preparation of impurity E).

Column:
— *size*: *l* = 0.150 m, Ø = 3.9 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: mixture of equal volumes of *methanol R* and a 4.32 g/L solution of *sodium octanesulfonate R* previously adjusted to pH 3.0 with *glacial acetic acid R*;
— *mobile phase B*: *methanol R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	97.5	2.5
10 - 11	97.5 → 70	2.5 → 30
11 - 20	70	30

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 295 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram supplied with *timolol for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B, C, D and F; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity E.

Relative retention With reference to timolol (retention time = about 7.5 min): maleic acid = about 0.1; impurity D = about 0.3; impurity E = about 0.4; impurity B = about 0.7; impurity F = about 0.8; impurity C = about 2.1.

System suitability Reference solution (b):
— *resolution*: minimum 1.5 between the peaks due to impurities B and F.

Limits:
— *correction factor*: for the calculation of content, multiply the peak area of impurity D by 0.6;
— *impurities B, C, D, E, F*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent); disregard any peak due to maleic acid.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

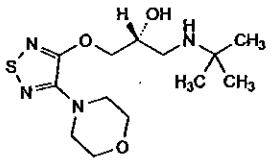
ASSAY
Dissolve 0.350 g in 60 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 43.25 mg of $C_{17}H_{28}N_4O_7S$.

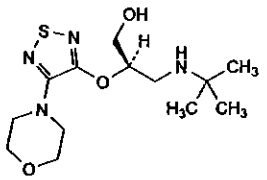
STORAGE
Protected from light.

IMPURITIES
Specified impurities *A, B, C, D, E, F.*

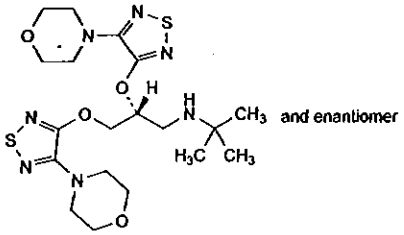
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *G, H, I, J.*



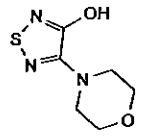
A. (2*R*)-1-[(1,1-dimethylethyl)amino]-3-[[4-(morpholin-4-yl)-1,2,5-thiadiazol-3-yl]oxy]propan-2-ol ((*R*)-timolol),



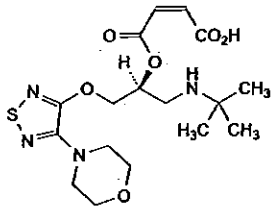
B. (2*RS*)-3-[(1,1-dimethylethyl)amino]-2-[[4-(morpholin-4-yl)-1,2,5-thiadiazol-3-yl]oxy]propan-1-ol,



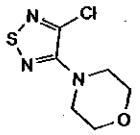
C. (2*RS*)-*N*-(1,1-dimethylethyl)-2,3-bis[[4-(morpholin-4-yl)-1,2,5-thiadiazol-3-yl]oxy]propan-1-amine,



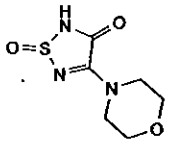
D. 4-(morpholin-4-yl)-1,2,5-thiadiazol-3-ol,



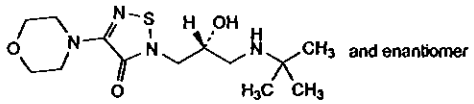
E. (2*Z*)-4-[(1*S*)-1-[[(1,1-dimethylethyl)amino]methyl]-2-[[4-(morpholin-4-yl)-1,2,5-thiadiazol-3-yl]oxy]ethoxy]-4-oxobut-2-enoic acid,



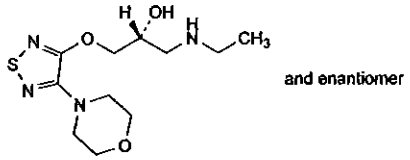
F. 4-(4-chloro-1,2,5-thiadiazol-3-yl)morpholine,



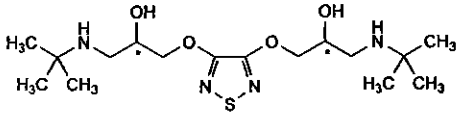
G. 4-(morpholin-4-yl)-1,2,5-thiadiazol-3(2*H*)-one 1-oxide,



H. 2-[(2*RS*)-3-[(1,1-dimethylethyl)amino]-2-hydroxypropyl]-4-(morpholin-4-yl)-1,2,5-thiadiazol-3(2*H*)-one,



I. (2*RS*)-1-(ethylamino)-3-[[4-(morpholin-4-yl)-1,2,5-thiadiazol-3-yl]oxy]propan-2-ol,

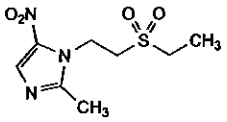


J. 1,1′-[1,2,5-thiadiazol-3,4-diylbis(oxy)]bis[3-[(1,1-dimethylethyl)amino]propan-2-ol].

Ph Eur

Tinidazole

(Ph. Eur. monograph 1051)



$C_8H_{13}N_3O_4S$ 247.3 *19387-91-8*

Action and use
Antiprotozoal; antibacterial.

Ph Eur

DEFINITION

1-[2-(Ethylsulfonyl)ethyl]-2-methyl-5-nitro-1*H*-imidazole.

Content
98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
Almost white or pale yellow, crystalline powder.

Solubility
Practically insoluble in water, soluble in acetone and in methylene chloride, sparingly soluble in methanol.

IDENTIFICATION

First identification: A, C.

Second identification: A, B, D, E.

A. Melting point (*2.2.14*): 125 °C to 128 °C.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 10.0 mg in *methanol R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of this solution to 10.0 mL with *methanol R*.

Spectral range 220-350 nm.

Absorption maximum At 310 nm.

Specific absorbance at the absorption maximum 340 to 360.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *tinidazole CRS*.

D. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 20 mg of *tinidazole CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel GF_{254} plate R.*

Pretreatment Heat at 110 °C for 1 h and allow to cool.

Mobile phase *butanol R, ethyl acetate R* (25:75 *V/V*).

Application 10 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

E. To about 10 mg add about 10 mg of *zinc powder R*, 0.3 mL of *hydrochloric acid R* and 1 mL of *water R*. Heat in a water-bath for 5 min and cool. The solution gives the reaction of primary aromatic amines (*2.3.1*).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5 (*2.2.2, Method II*).

Dissolve 1.0 g in *acetone R* and dilute to 20 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*). *Protect solutions from light.*

Test solution Dissolve 10.0 mg of the substance to be examined in 10.0 mL of *methanol R* and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *tinidazole impurity A CRS* and 5.0 mg of *tinidazole impurity B CRS* in 10.0 mL of *methanol R* and dilute to 100.0 mL with the mobile phase. Dilute 2.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (c) Dilute 1.0 mL of reference solution (b) to 50.0 mL with the mobile phase.

Column:
- *size*: *l* = 0.25 m, Ø = 3.0 mm;
- *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Regular column conditioning by subsequent flushing with 50 mL of *water R*, 100 mL of *methanol R*, 25 mL of *water R* and 100 mL of the mobile phase is recommended.

Mobile phase *acetonitrile R, methanol R, water R* (10:20:70 *V/V/V*).

Flow rate 0.5 mL/min.

Detection Spectrophotometer at 320 nm.

Injection 20 µL.

Run time 1.5 times the retention time of tinidazole.

Relative retention With reference to tinidazole (retention time = about 6 min): impurity A = about 0.6; impurity B = about 0.7.

System suitability Reference solution (b):
- *resolution*: minimum 2.0 between the peaks due to impurities A and B.

Limits:
- *impurities A, B*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.2 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 25 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

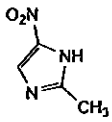
1 mL of *0.1 M perchloric acid* is equivalent to 24.73 mg of $C_8H_{13}N_3O_4S$.

STORAGE

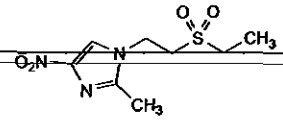
Protected from light.

IMPURITIES

Specified impurities *A, B.*



A. 2-methyl-5-nitro-1*H*-imidazole,

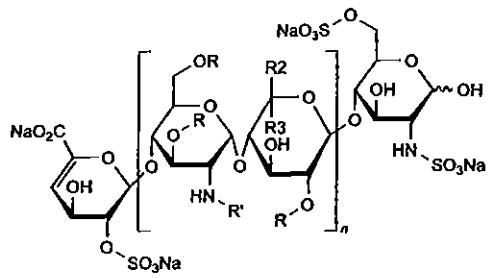


B. 1-[2-(ethylsulfonyl)ethyl]-2-methyl-4-nitro-1*H*-imidazole.

Ph Eur

Tinzaparin Sodium

(Ph. Eur. monograph 1271)



n = 1 to 25 , R = H or SO_3Na , R' = H or SO_3Na or CO-CH_3
R2 = H and R3 = CO_2Na or R2 = CO_2Na and R3 = H

Action and use

Low molecular weight heparin.

Preparation

Tinzaparin Sodium Injection

Ph Eur

DEFINITION

Tinzaparin sodium is the sodium salt of a low-molecular-mass heparin that is obtained by controlled enzymatic depolymerisation of heparin from porcine intestinal mucosa using heparinase from *Flavobacterium heparinum*.

The majority of the components have a 2-*O*-sulfo-4-enepyranosuronic acid structure at the non-reducing end and a 2-*N*,6-*O*-disulfo-D-glucosamine structure at the reducing end of their chain.

Tinzaparin sodium complies with the monograph on Low-molecular-mass heparins (0828) with the modifications and additional requirements below.

The mass-average relative molecular mass ranges between 5500 and 7500 with a characteristic value of about 6500.

The degree of sulfatation is 1.8 to 2.5 per disaccharide unit.

The potency is not less than 70 IU and not more than 120 IU of anti-factor Xa activity per milligram calculated with reference to the dried substance. The ratio of the anti-

factor Xa activity to anti-factor IIa activity is between 1.5 and 2.5.

IDENTIFICATION

Carry out identification test A as described in the monograph *Low-molecular-mass heparins (0828)* using *tinzaparin sodium CRS*.

Carry out identification test C as described in the monograph *Low-molecular-mass heparins (0828)*. The following requirements apply.

The mass-average relative molecular mass ranges between 5500 and 7500. The mass percentage of chains lower than 2000 is not more than 10.0 per cent. The mass percentage of chains between 2000 and 8000 ranges between 60.0 and 72.0 per cent. The mass percentage of chains above 8000 ranges between 22.0 and 36.0 per cent.

TESTS

Appearance of solution

Dissolve 1.0 g in 10 mL of *water R*. The solution is clear (*2.2.1*) and not more intensely coloured than intensity 5 of the range of reference solutions of the most appropriate colour (*2.2.2, Method II*)

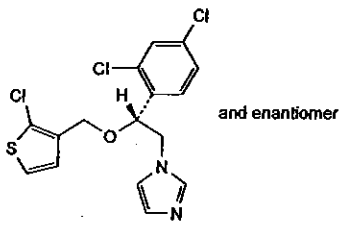
Absorbance (*2.2.25*)

Dissolve 50.0 mg in 100 mL of *0.01 M hydrochloric acid*. The specific absorbance, measured at 231 nm and calculated with reference to the dried substance, is 8.0 to 12.5.

Ph Eur

Tioconazole

(Ph. Eur. monograph 2074)



$C_{16}H_{13}Cl_3N_2OS$ 387.7 *65899-73-2*

Action and use

Antifungal.

Preparation

Tioconazole Nail Solution

Ph Eur

DEFINITION

1-[(2*RS*)-2-[(2-Chlorothiophen-3-yl)methoxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very slightly soluble in water, very soluble in methylene chloride, freely soluble in alcohol.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of tioconazole.*

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 2.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 5 mg of *tioconazole for system suitability CRS* in the mobile phase and dilute to 2.5 mL with the mobile phase.

Column:

— *size*: l = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 170 m^2/g, a pore size of 12 nm and a carbon loading of 10 per cent.

Mobile phase Mix 1 volume of a 1.7 g/L solution of *tetrabutylammonium dihydrogen phosphate R* previously adjusted to pH 7.4 with *dilute ammonia R2* and 3 volumes of *methanol R*.

Flow rate 1 mL/min.

Detection Spectrophotometer at 218 nm.

Injection 20 µL.

Run time 2.5 times the retention time of tioconazole.

System suitability Reference solution (b):

— *resolution*: minimum 1.0 between the peaks due to impurity B and impurity C (locate impurities A, B and C by comparison with the chromatogram provided with *tioconazole for system suitability CRS*).

Limits:

— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 1.7; impurity C = 1.7.

— *impurities A, B, C*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent),

— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent),

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent),

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)

Maximum 0.5 per cent, determined on 1.00 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

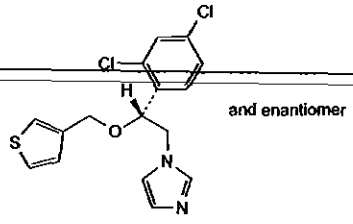
ASSAY

Dissolve 0.300 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

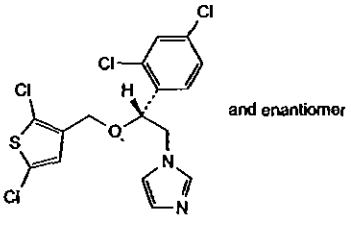
1 mL of *0.1 M perchloric acid* is equivalent to 38.77 mg of $C_{16}H_{13}Cl_3N_2OS$.

STORAGE
Protected from light.

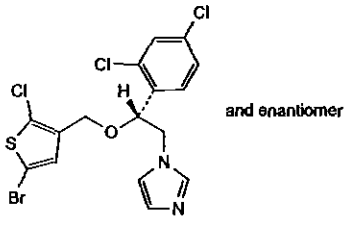
IMPURITIES
Specified impurities *A, B, C.*
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *D.*



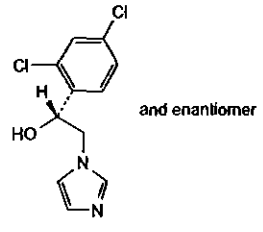
A. 1-[(2*RS*)-2-(2,4-dichlorophenyl)-2-[(thiophen-3-yl) methoxy]ethyl]-1*H*-imidazole,



B. 1-[(2*RS*)-2-(2,4-dichlorophenyl)-2-[(2,5-dichlorothiophen-3-yl)methoxy]ethyl]-1*H*-imidazole,



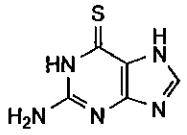
C. 1-[(2*RS*)-2-[(5-bromo-2-chlorothiophen-3-yl)methoxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole,



D. (1*RS*)-1-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-yl) ethanol.

Ph Eur

Tioguanine



$C_5H_5N_5S$ 167.2 *154-42-7*

Action and use
Purine analogue; cytostatic.

Preparation
Tioguanine Tablets

DEFINITION
Tioguanine is 2-aminopurine-6(1*H*)-thione. It contains not less than 96.0% and not more than 102.0% of $C_5H_5N_5S$, calculated with reference to the dried substance.

CHARACTERISTICS
A pale yellow, crystalline powder.
Practically insoluble in *water* and in *ethanol (96%)*.
It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION
Heat a suitable quantity at 105° at a pressure not exceeding 0.7 kPa for 5 hours. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of tioguanine *(RS 340)*.

TESTS

Phosphate
To 0.5 g add 2 mL of 5M *sulfuric acid* and heat on a water bath for 5 minutes. Add, dropwise, 5 mL of *nitric acid* and continue heating until a clear solution is obtained. Cool and add 10 mL of *water* and 0.75 mL of a solution prepared by dissolving 8.3 g of *ammonium molybdate* in 40 mL of *water* and adding 33 mL of 5M *sulfuric acid* and sufficient *water* to produce 100 mL. Add 1.0 mL of *strong aminohydroxynaphthalenesulfonic acid solution*, mix and dilute to 25 mL with *water*. Measure the *absorbance* of the resulting solution at 820 nm, Appendix II B, using in the reference cell a solution prepared in the same manner but omitting the substance being examined. The absorbance is not more than that obtained by treating 1.5 mL of *phosphate standard solution (100 ppm PO_4)* in the same manner, beginning at the words 'add 10 mL of *water* ...'.

Free sulfur
Dissolve 50 mg in 5 mL of 1M *sodium hydroxide*.
The solution is *clear*, Appendix IV A.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve 40 mg of the substance being examined in 100 mL of 0.01M *sodium hydroxide* and dilute 1 volume of the resulting solution to 2 volumes with the mobile phase.

(2) Dilute 1 volume of solution (1) to 100 volumes with a mixture of 1 volume of 0.01M *sodium hydroxide* and 9 volumes of the mobile phase. Further dilute 1 volume of the resulting solution to 5 volumes with the same solvent mixture.

(3) Dissolve 12 mg of *guanine BPCRS* in 100 mL of 0.01M *sodium hydroxide* and dilute 1 volume of the resulting solution to 20 volumes with the mobile phase.

(4) Dissolve 40 mg each of *tioguanine BPCRS* and *guanine BPCRS* in 100 mL of 0.01M *sodium hydroxide* and dilute 1 volume of the resulting solution to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters Atlantis dC18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 248 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

0.05M *anhydrous sodium dihydrogen orthophosphate* adjusted to pH 3.0 with *orthophosphoric acid.*

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks due to tioguanine and guanine is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to guanine is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (3%);

the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of any other *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Loss on drying

When dried at 105° at a pressure not exceeding 0.7 kPa for 5 hours, loses not more than 1.0% of its weight. Use 1 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve 40 mg of the substance being examined in 100 mL of 0.01M *sodium hydroxide* and dilute 1 volume of the resulting solution to 10 volumes with the mobile phase.

(2) Dissolve 40 mg of *tioguanine BPCRS* in 100 mL of 0.01M *sodium hydroxide* and dilute 1 volume of this solution to 10 volumes with the mobile phase.

(3) Dissolve 40 mg each of *tioguanine BPCRS* and *guanine BPCRS* in 100 mL of 0.01M *sodium hydroxide* and dilute 1 volume of the resulting solution to 10 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

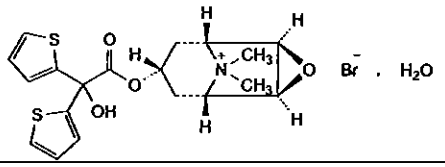
The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to tioguanine and guanine is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of $C_5H_5N_5S$ in the substance being examined from the chromatograms obtained and using the declared content of $C_5H_5N_5S$ in *tioguanine BPCRS.*

Tiotropium Bromide Monohydrate

(Ph. Eur. monograph 2420)



$C_{19}H_{22}BrNO_4S_2,H_2O$ 490.4 *411207-31-3*

Action and use

Anticholinergic (antimuscarinic) bronchodilator.

Ph Eur

DEFINITION

(1*R*,2*R*,4*S*,5*S*,7*s*)-7-[[Hydroxy[di(thiophen-2-yl)]acetyl]oxy]-9,9-dimethyl-3-oxa-9-azatricyclo[3.3.1.$0^{2,4}$]nonan-9-ium bromide monohydrate.

Content

98.5 per cent to 101.5 per cent (anhydrous substance).

CHARACTERS

Appearance

White or yellowish-white powder or crystals.

Solubility

Sparingly soluble in water, soluble in methanol, practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *tiotropium bromide monohydrate CRS.*

B. It gives reaction (a) of bromides (*2.3.1*).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Dissolve 0.2 g in *water R* and dilute to 20 mL with the same solvent.

Impurities G and H

Thin-layer chromatography (*2.2.27*). *Prepare the solutions immediately before use.*

Solvent mixture Dilute 1 volume of a 103 g/L solution of *hydrochloric acid R* to 100 volumes with *methanol R.*

Test solution Dissolve 0.40 g of the substance to be examined in the solvent mixture and dilute to 10 mL with the solvent mixture.

Reference solution (a) Dissolve the contents of a vial of *tiotropium impurity mixture CRS* (containing impurities G and H) in 1.0 mL of the solvent mixture.

Reference solution (b) Mix 0.1 mL of the test solution with 0.1 mL of reference solution (a).

Plate *TLC silica gel F_{254} plate R* (2-10 µm).

Mobile phase *water R, anhydrous formic acid R, acetonitrile R, methylene chloride R* (10:15:35:50 *V/V/V/V*).

Application 10 µL of the test solution and reference solution (a), and 20 µL of reference solution (b).

Development Over 2/3 of the plate.

Drying In air.

Detection Expose to iodine vapour until the spots are clearly visible (about 15 min); remove the plate and examine immediately.

Retardation factors Impurity G = about 0.33; impurity H = about 0.38; tiotropium = about 0.64.

System suitability Reference solution (b):
— the chromatogram shows 3 clearly separated spots.

Limits:
— *impurity G*: any spot due to impurity G is not more intense than the corresponding spot in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *impurity H*: any spot due to impurity H is not more intense than the corresponding spot in the chromatogram obtained with reference solution (a) (0.1 per cent).

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions protected from light.*

Test solution Dissolve 50.0 mg of the substance to be examined in mobile phase B and dilute to 25.0 mL with mobile phase B.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase B. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase B.

Reference solution (b) Dissolve 4 mg of *tiotropium for system suitability 1 CRS* (containing impurity C) in 2.0 mL of mobile phase B.

Column:
— *size*: *l* = 0.15 m, Ø = 3.0 mm;
— *stationary phase*: *propylsilyl silica gel for chromatography R* (3.5 µm);
— *temperature*: 50 °C.

Mobile phase:
— *mobile phase A*: dissolve 1.0 g of *sodium methanesulfonate R* and 5.0 g of *potassium dihydrogen phosphate R* in about 980 mL of *water R*, adjust to pH 3.0 with *dilute phosphoric acid R* and dilute to 1000 mL with *water R*;
— *mobile phase B*: *methanol R1, acetonitrile for chromatography R*, mobile phase A (10:40:50 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	90	10
3 - 17	90 → 80	10 → 20
17 - 28	80 → 25	20 → 75
28 - 30	25	75

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 240 nm.

Injection 5 µL.

Identification of impurities Use the chromatogram supplied with *tiotropium for system suitability 1 CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity C.

Relative retention With reference to tiotropium (retention time = about 15 min): impurity C = about 1.2.

System suitability Reference solution (b):
— *resolution*: minimum 2.4 between the peaks due to tiotropium and impurity C.

Limits:
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)
2.5 per cent to 4.0 per cent, determined on 0.300 g.

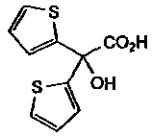
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.35 g in 100 mL of *water R*. Add 10 mL of *dilute nitric acid R2*. Titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (*2.2.20*).

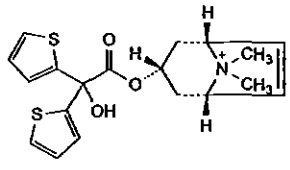
1 mL of *0.1 M silver nitrate* is equivalent to 47.24 mg of $C_{19}H_{22}BrNO_4S_2$.

IMPURITIES
Specified impurities *G, H.*

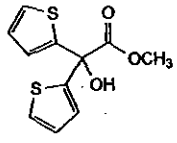
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, C, E, F.*



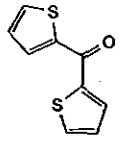
A. hydroxy[di(thiophen-2-yl)]acetic acid,



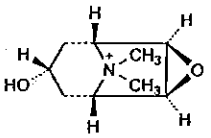
C. (1*R*,3*s*,5*S*)-3-[[hydroxy[di(thiophen-2-yl)]acetyl]oxy]-8,8-dimethyl-8-azabicyclo[3.2.1]oct-6-en-8-ium,



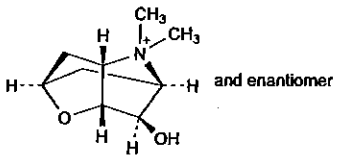
E. methyl hydroxy[di(thiophen-2-yl)]acetate,



F. di(thiophen-2-yl)methanone,



G. (1*R*,2*R*,4*S*,5*S*,7*s*)-7-hydroxy-9,9-dimethyl-3-oxa-9-azatricyclo[3.3.1.0^{2,4}]nonan-9-ium,



H. (1*s*,3*RS*,4*RS*,5*RS*,7*SR*)-4-hydroxy-6,6-dimethyl-2-oxa-6-azatricyclo[3.3.1.0^{3,7}]nonan-6-ium.

Ph Eur

Titanium Dioxide

(Ph. Eur. monograph 0150)

TiO_2 79.9 *13463-67-7*

Action and use
Protective; excipient.

Preparation
Titanium Ointment

Ph Eur

DEFINITION

Content
98.0 per cent to 100.5 per cent.

CHARACTERS

Appearance
White or almost white powder.

Solubility
Practically insoluble in water. It does not dissolve in dilute mineral acids but dissolves slowly in hot concentrated sulfuric acid.

IDENTIFICATION

A. When strongly heated, it becomes pale yellow; the colour disappears on cooling.

B. To 5 mL of solution S (see Tests) add 0.1 mL of *strong hydrogen peroxide solution R*. An orange-red colour appears.

C. To 5 mL of solution S add 0.5 g of *zinc R* in granules. After 45 min, the mixture has a violet-blue colour.

TESTS

Solution S
Mix 0.500 g (*m* g) with 5 g of *anhydrous sodium sulfate R* in a 300 mL long-necked combustion flask. Add 10 mL of *water R* and mix. Add 10 mL of *sulfuric acid R* and boil vigorously, with the usual precautions, until a clear solution is obtained. Cool, add slowly a cooled mixture of 30 mL of *water R* and 10 mL of *sulfuric acid R*, cool again and dilute to 100.0 mL with *water R*.

Appearance of solution
Solution S is not more opalescent than reference suspension II (*2.2.1*) and is colourless (*2.2.2, Method II*).

Acidity or alkalinity
Shake 5.0 g with 50 mL of *carbon dioxide-free water R* for 5 min. Centrifuge or filter until a clear solution is obtained. To 10 mL of the solution add 0.1 mL of *bromothymol blue solution R1*. Not more than 1.0 mL of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Water-soluble substances
Maximum 0.5 per cent.

To 10.0 g add a solution of 0.5 g of *ammonium sulfate R* in 150 mL of *water R* and boil for 5 min. Cool, dilute to 200 mL with *water R* and filter until a clear solution is obtained. Evaporate 100 mL of the solution to dryness in a tared evaporating dish and ignite. The residue weighs a maximum of 25 mg.

Elemental impurities
Any method that fulfils the requirements of general chapter *2.4.20. Determination of elemental impurities* may be used.

Element	Maximum content (ppm)
Antimony	2
Arsenic	1
Barium	20
Lead	5

Iron
Maximum 200 ppm.

To 8 mL of solution S add 4 mL of *water R*. Mix and add 0.05 mL of *bromine water R*. Allow to stand for 5 min and remove the excess of bromine with a current of air. Add 3 mL of *potassium thiocyanate solution R*. Any colour in the solution is not more intense than that in a standard prepared at the same time in the same manner using a mixture of 4 mL of *iron standard solution (2 ppm Fe) R* and 8 mL of a 200 g/L solution of *sulfuric acid R*.

ASSAY

To 300 g of *zinc R* in granules (710) (*2.1.4*) add 300 mL of a 20 g/L solution of *mercuric nitrate R* and 2 mL of *nitric acid R*, shake for 10 min and wash with *water R*. Pack the amalgamated zinc into a glass tube about 400 mm long and about 20 mm in diameter fitted with a tap and a filter plate. Pass through the column 100 mL of *dilute sulfuric acid R* followed by 100 mL of *water R*, making sure that the amalgam is always covered with liquid. Pass slowly at a rate of about 3 mL/min through the column a mixture of 100 mL of *dilute sulfuric acid R* and 100 mL of *water R* followed by 100 mL of *water R*. Collect the eluate in a 500 mL conical flask containing 50.0 mL of a 150 g/L solution of *ferric ammonium sulfate R* in a mixture of 1 volume of *sulfuric acid R* and 3 volumes of *water R*. Add 0.1 mL of *ferroin R* and titrate immediately with *0.1 M ammonium and cerium nitrate* until a greenish colour is obtained (n_1 mL). Pass slowly at a rate of about 3 mL/min through the column a mixture of 50 mL of *dilute sulfuric acid R* and 50 mL of *water R*, followed by 20.0 mL of solution S, a mixture of 50 mL of *dilute sulfuric acid R* and 50 mL of *water R* and finally 100 mL of *water R*. Collect the eluate in a 500 mL conical flask containing 50.0 mL of a 150 g/L solution of *ferric ammonium sulfate R* in a mixture of 1 volume of *sulfuric acid R* and 3 volumes of *water R*. Rinse the lower end of the column with *water R*, add 0.1 mL of *ferroin R* and titrate immediately with *0.1 M ammonium and cerium nitrate* until a greenish colour is obtained (n_2 mL).

Calculate the percentage content of TiO_2 using the following expression:

$$\frac{3.99 \times (n_2 - n_1)}{m}$$

m = mass of the substance to be examined used for the preparation of solution S, in grams.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

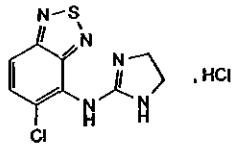
The following characteristic may be relevant for titanium dioxide used as opacifier in solid oral dosage forms and in preparations for cutaneous application.

Particle-size distribution (*2.9.31*)

Ph Eur

Tizanidine Hydrochloride

(Ph. Eur. monograph 2578)



$C_9H_9Cl_2N_5S$ 290.2 *64461-82-1*

Action and use

Alpha$_2$-adrenoceptor agonist; skeletal muscle relaxant.

Ph Eur

DEFINITION

5-Chloro-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-2,1,3-benzothiadiazol-4-amine hydrochloride.

Content

98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish-white, crystalline powder.

Solubility

Soluble in water, very slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *tizanidine hydrochloride CRS.*

B. Solution S (see Tests) gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

pH (*2.2.3*)

3.7 to 5.0 for solution S.

Impurity H

Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.200 g of the substance to be examined in *methanol R* and dilute to 5.0 mL with the same solvent.

Reference solution Dissolve 8.0 mg of *ethylenediamine R* (impurity H) in *methanol R* and dilute to 100.0 mL with the same solvent.

Plate *TLC cellulose plate R.*

Mobile phase *glacial acetic acid R, water R, methanol R* (1:25:75 *V/V/V*).

Application 5 µL.

Development Over 1/2 of the plate.

Drying In a current of cold air.

Detection Spray with a mixture of 15 volumes of a 20 g/L solution of *ninhydrin R* in *glacial acetic acid R* and 85 volumes of *butanol R*, then heat at 100 °C for 5 min.

Relative retention with reference to tizanidine (R_F = about 0.8): impurity H = about 0.3.

Limit:

— *impurity H*: any spot due to impurity H is not more intense than the corresponding spot in the chromatogram obtained with the reference solution (0.2 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 30.0 mg of the substance to be examined in mobile phase A, using sonication if necessary, and dilute to 25.0 mL with mobile phase A. Dilute 5.0 mL of the solution to 50.0 mL with mobile phase A.

Reference solution (a) Dissolve 3 mg of *tizanidine impurity B CRS* in mobile phase A and dilute to 25.0 mL with mobile phase A. Dilute 1.0 mL of the solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with the test solution.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

Reference solution (c) Dissolve 30.0 mg of *tizanidine hydrochloride CRS* in mobile phase A, using sonication if necessary, and dilute to 25.0 mL with mobile phase A. Dilute 5.0 mL of the solution to 50.0 mL with mobile phase A.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3.5 µm);

— *temperature*: 50 °C.

Mobile phase:

— *mobile phase A*: mix 18 volumes of *acetonitrile R1* and 82 volumes of a 3.86 g/L solution of *sodium pentanesulfonate monohydrate R* previously adjusted to pH 3.0 with *phosphoric acid R*;

— *mobile phase B*: mix 20 volumes of a 3.86 g/L solution of *sodium pentanesulfonate monohydrate R* previously adjusted to pH 3.0 with *phosphoric acid R* and 80 volumes of *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	100	0
15 - 25	100 → 0	0 → 100
25 - 27	0	100

Flow rate 0.6 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 10 µL of the test solution and reference solutions (a) and (b).

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity B.

Relative retention With reference to tizanidine (retention time = about 6 min): impurity B = about 1.3.

System suitability Reference solution (a):

— *resolution*: minimum 4.0 between the peaks due to tizanidine and impurity B.

Calculation of percentage contents:

— for each impurity, use the concentration of tizanidine hydrochloride in reference solution (b).

Limits:

— *unspecified impurities*: for each impurity, maximum 0.10 per cent;

— *total*: maximum 0.2 per cent;

— *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

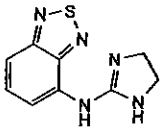
Injection Test solution and reference solution (c).

Calculate the percentage content of $C_9H_9Cl_2N_5S$ taking into account the assigned content of *tizanidine hydrochloride CRS*.

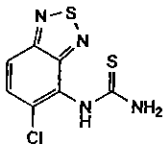
IMPURITIES

Specified impurities *H*.

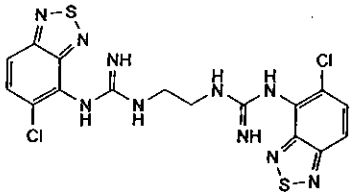
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, E, F, G, I.*



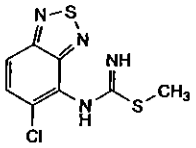
A. *N*-(4,5-dihydro-1*H*-imidazol-2-yl)-2,1,3-benzothiadiazol-4-amine,



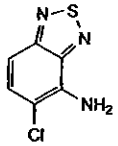
B. *N*-(5-chloro-2,1,3-benzothiadiazol-4-yl)thiourea,



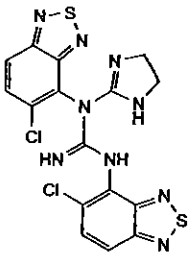
C. 1,1′-ethane-1,2-diylbis[3-(5-chloro-2,1,3-benzothiadiazol-4-yl)guanidine],



D. methyl *N*-(5-chloro-2,1,3-benzothiadiazol-4-yl)carbamimidothioate,

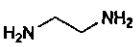


E. 5-chloro-2,1,3-benzothiadiazol-4-amine,

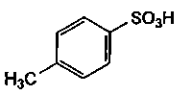


F. 1,3-bis(5-chloro-2,1,3-benzothiadiazol-4-yl)-1-(4,5-dihydro-1*H*-imidazol-2-yl)guanidine,

G. (5-chloro-2,1,3-benzothiadiazol-4-yl)cyanamide,



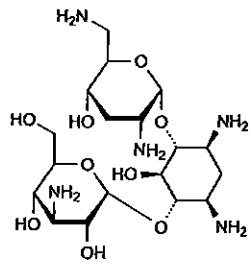
H. ethane-1,2-diamine (ethylenediamine),



I. 4-methylbenzenesulfonic acid (*p*-toluenesulfonic acid).

Tobramycin

(Ph. Eur. monograph 0645)



$C_{18}H_{37}N_5O_9$ 467.5 *32986-56-4*

Action and use
Aminoglycoside antibacterial.

Preparations
Tobramycin and Dexamethasone Eye Drops, Suspension
Tobramycin Eye Drops
Tobramycin Inhalation Powder, Hard Capsule
Tobramycin Injection
Tobramycin Nebuliser Solution

Ph Eur

DEFINITION

4-*O*-(3-Amino-3-deoxy-α-D-glucopyranosyl)-2-deoxy-6-*O*-(2,6-diamino-2,3,6-trideoxy-α-D-*ribo*-hexopyranosyl)-L-streptamine.

Substance produced by *Streptomyces tenebrarius* or obtained by any other means.

Content
97.0 per cent to 102.0 per cent (anhydrous substance).

PRODUCTION

It is produced by methods of manufacture designed to eliminate or minimise substances lowering blood pressure.

CHARACTERS

Appearance
White or almost white powder.

Solubility
Freely soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: A.

Second identification: B, C.

A. Nuclear magnetic resonance spectrometry (*2.2.33*).

Preparation 100 g/L solution in *deuterium oxide R.*

Comparison 100 g/L solution of *tobramycin CRS* in *deuterium oxide R.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 20 mg of the substance to be examined in *water R* and dilute to 5 mL with the same solvent.

Reference solution (a) Dissolve 20 mg of *tobramycin CRS* in *water R* and dilute to 5 mL with the same solvent.

Reference solution (b) Dissolve 4 mg of *neomycin sulfate CRS* and 4 mg of *kanamycin monosulfate CRS* in 1 mL of reference solution (a).

Plate *TLC silica gel plate R.*

Mobile phase *methylene chloride R, concentrated ammonia R, methanol R* (17:33:50 *V/V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying In a current of warm air.

Detection Spray with a mixture of equal volumes of a 2 g/L solution of *1,3-dihydroxynaphthalene R* in *ethanol (96 per cent) R* and a 460 g/L solution of *sulfuric acid R*; heat at 105 °C for 5-10 min.

System suitability The chromatogram obtained with reference solution (b) shows 3 major spots which are clearly separated.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve about 5 mg in 5 mL of *water R.* Add 5 mL of a 1 g/L solution of *ninhydrin R* in *ethanol (96 per cent) R* and heat in a water-bath for 3 min. A violet-blue colour develops.

TESTS

pH (*2.2.3*)
9.0 to 11.0.

Dissolve 1.0 g in 10 mL of *carbon dioxide-free water R.*

Specific optical rotation (*2.2.7*)
+ 138 to + 148 (anhydrous substance).

Dissolve 1.00 g in *water R* and dilute to 25.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 mL with the mobile phase.

Test solution (b) Dilute 10.0 mL of test solution (a) to 100.0 mL with the mobile phase.

Reference solution (a) Dissolve 25.0 mg of *tobramycin CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 100.0 mL with the mobile phase.

Reference solution (c) Dilute 1.0 mL of reference solution (a) to 50.0 mL with the mobile phase.

Reference solution (d) Dissolve 10.0 mg of *kanamycin B sulfate CRS* in 20.0 mL of the mobile phase. To 1.0 mL of this solution, add 2.0 mL of reference solution (a) and dilute to 10.0 mL with the mobile phase.

Reference solution (e) Dilute 10.0 mL of reference solution (a) to 25.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *styrene-divinylbenzene copolymer R* (8 µm) with a pore size of 100 nm;
— *temperature*: 55 °C.

Mobile phase Mixture prepared with *carbon dioxide-free water R* containing 52 g/L of *anhydrous sodium sulfate R*, 1.5 g/L of *sodium octanesulfonate R*, 3 mL/L of *tetrahydrofuran R* stabilised with *butylhydroxytoluene R*, and 50 mL/L of *0.2 M potassium dihydrogen phosphate R* previously adjusted to pH 3.0 with *dilute phosphoric acid R.* Degas.

Flow rate 1.0 mL/min.

Post-column solution *carbonate-free sodium hydroxide solution R* diluted 25-fold with *carbon dioxide-free water R*, which is added pulselessly to the column effluent using a 375 µL polymeric mixing coil.

Flow rate 0.3 mL/min.

Detection Pulsed amperometric detector or equivalent with a gold working electrode, a silver-silver chloride reference electrode and a stainless steel auxiliary electrode which is the cell body, held at respectively + 0.05 V detection, + 0.75 V oxidation and −0.15 V reduction potentials, with pulse durations according to the instrument used. The temperature of the detector is set at 35 °C.

NOTE *to prevent problems due to salt precipitation, the electrochemical cell can be flushed with water R overnight.*

Injection 20 µL using a refrigerated injector (4-8 °C); inject test solution (a) and reference solutions (b), (c) and (d).

Run time 1.5 times the retention time of tobramycin.

Relative retention With reference to tobramycin (retention time = about 18 min): impurity C = about 0.35; impurity B = about 0.40, impurity A = about 0.70.

System suitability:

— *resolution*: minimum 3.0 between the peaks due to impurity A and to tobramycin in the chromatogram obtained with reference solution (d); if necessary, adjust the concentration of sodium octanesulfonate in the mobile phase;

— *signal-to-noise ratio*: minimum 10 for the principal peak in the chromatogram obtained with reference solution (b).

Limits:

— *any impurity*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent) and not more than 1 such peak has an area greater than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (1.5 per cent);

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent).

2-Methyl-1-propanol (*2.4.24, System B*)
Maximum 1.0 per cent *m/m*.

Water (*2.5.12*)
Maximum 8.0 per cent, determined on 0.30 g.

Sulfated ash (*2.4.14*)
Maximum 0.3 per cent, determined on 1.0 g.

Bacterial endotoxins (*2.6.14*)
Less than 2.0 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

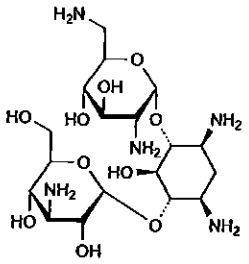
ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution (b) and reference solution (e).

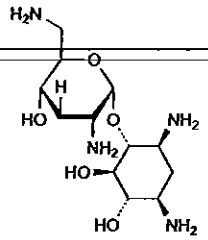
Calculate the percentage content of tobramycin.

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

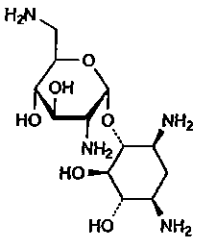
IMPURITIES



A. 4-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-2-deoxy-6-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-L-streptamine (kanamycin B),



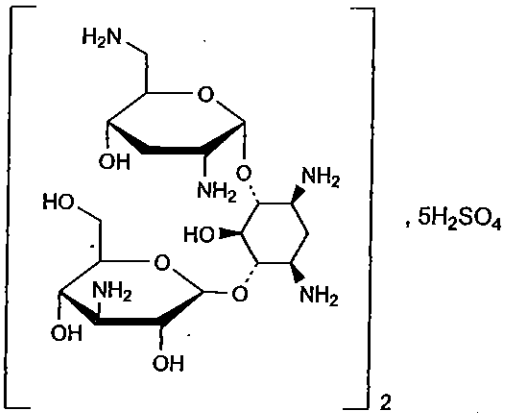
B. 2-deoxy-4-*O*-(2,6-diamino-2,3,6-trideoxy-α-D-*ribo*-hexopyranosyl)-D-streptamine (nebramine),



C. 2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-D-streptamine (neamine).

Ph Eur

Tobramycin Sulfate



$(C_{18}H_{37}N_5O_9)_2,5H_2SO_4$ 1425.42 *79645-27-5*

Action and use
Aminoglycoside antibacterial.

Preparation
Tobramycin Oral Solution

DEFINITION

4-*O*-(3-Amino-3-deoxy-α-D-glucopyranosyl)-2-deoxy-6-*O*-(2,6-diamino-2,3,6-trideoxy-α-D-*ribo*-hexopyranosyl)-L-streptamine sulfate.

Substance produced by *Streptomyces tenebrarius* or obtained by any other means.

It contains not less than 97.0% and not more than 102.0% of $(C_{18}H_{37}N_5O_9)_2,5H_2SO_4$, calculated with reference to the anhydrous substance.

CHARACTERISTICS

A white to off-white powder.

Soluble in *water*.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve 90 mg of the substance being examined in sufficient *water* to produce 10 mL.

(2) 0.6% w/v of *tobramycin BPCRS in water*.

(3) 0.4% w/v of each of *kanamycin monosulfate BPCRS*, *neomycin sulfate EPCRS* and *tobramycin BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* (Merck silica gel 60 plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of warm air, spray with a mixture of equal volumes of a 0.2% w/v solution of *naphthalene-1,3-diol* in *ethanol (96%)* and a 46% w/v solution of *sulfuric acid* and heat at 105° for 5 to 10 minutes.

MOBILE PHASE

17 volumes of *dichloromethane*, 33 volumes of 13.5M *ammonia* and 50 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated principal spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position, colour and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as that of the principal peak in the chromatogram obtained with solution (2).

C. Yields the reactions characteristic of *sulfates*, Appendix VI.

TESTS

Acidity or alkalinity

pH of a 0.4% w/v solution, 6.0 to 8.0, Appendix V L.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve 50 mg of the substance being examined in 7 mL of *water*, adjust the pH to 5.5 with 0.5M *sulfuric acid* and add sufficient water to produce 10 mL.

(2) Dilute 1 volume of solution (1) to 100 volumes with *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel for chromatography*.

(b) Use the mobile phase as described below.

(c) Apply 1 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, allow it to dry in air, heat at 110° for 10 minutes and spray with a 20% v/v solution of *sodium hypochlorite solution* in *water*. Dry the plate in air and then spray with a solution prepared in the following manner. Dissolve 1.1 g of *potassium iodide* in 60 mL of *water*, boil for 15 minutes and slowly add a suspension containing 1.5 g of *soluble starch* in 10 mL of *water*. Add 25 mL of *water*, boil for 10 minutes, allow to cool and dilute to 100 mL with *water*.

MOBILE PHASE

20 volumes of *water*, 30 volumes of *ethanol* and 50 volumes of a 29.2% w/v solution of *sodium chloride* in *water*.

LIMITS

In the chromatogram obtained with solution (1):

any *secondary spot* is not more intense than the principal spot in the chromatogram obtained with solution (2) (1%).

Sulfated ash

Not more than 1% w/w, Appendix IX A, when determined by the following method. Moisten the charred residue with 2 mL of *nitric acid* and 5 drops of *sulfuric acid*.

Water

Not more than 2.0% w/w, Appendix IX C.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Derivatise solutions (1), (2) and (4) prior to analysis.

(1) Dissolve 30 mg of the substance being examined in sufficient *water* to produce 100 mL.

(2) Add 1 mL of 0.5M *sulfuric acid* to 50 mg of *tobramycin BPCRS*, dissolve in *water*, add sufficient *water* to produce 50 mL and mix. Dilute 1 volume of this solution to 5 volumes with *water*.

(3) Dilute 1 volume of a 0.024% w/v solution of *1-naphtholbenzein* in *acetonitrile* to 5 volumes with derivatised solution (2).

(4) *water* (blank solution).

Derivatise solutions (1), (2) and (4) using the following method. The solutions should be heated at the same temperature and for the same time as indicated below.

Transfer 4 mL of each solution separately into 50-mL volumetric flasks. To each solution add 10 mL of a 1% w/v solution of *1-fluoro-2,4-dinitrobenzene* in *ethanol (96%)* and 10 mL of a solution prepared by diluting 40 mL of a 1.5% w/v solution of *tris(hydroxymethyl)methylamine* in *water* to 200 mL with *dimethyl sulfoxide* and mixing. Heat in a water bath at 60° for 50 minutes. Remove the flasks, allow to stand for 10 minutes and add *acetonitrile* to about 2 mL below the meniscus. Allow to cool to room temperature and add sufficient *acetonitrile* to produce 50 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (30 cm × 3.9 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil 5 C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 365 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Dissolve 2.0 g of *tris(hydroxymethyl)aminomethane* in 800 mL of *water*, add 20 mL of 0.5M *sulfuric acid* and sufficient *acetonitrile* to produce 2000 mL. Allow to cool and filter (using a 0.2-µm, or finer, filter).

When the chromatograms are recorded under the prescribed conditions, the retention of 1-*naphtholbenzein* relative to tobramycin (retention time, about 20 minutes) is about 0.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to 1-naphtholbenzein and tobramycin is at least 4.0.

DETERMINATION OF CONTENT

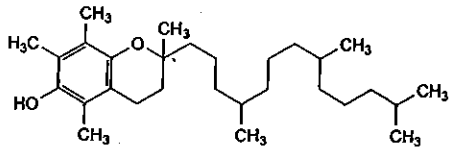
Calculate the content of $(C_{18}H_{37}N_5O_9)_2,5H_2SO_4$ in the substance being examined using the declared content of ~~$C_{18}H_{37}N_5O_9$ in *tobramycin BPCRS*. Each mg of~~ $C_{18}H_{37}N_5O_9$ is equivalent to 1.5245 mg of $(C_{18}H_{37}N_5O_9)_2,5H_2SO_4$.

STORAGE

Tobramycin Sulfate should be stored in an airtight container.

all-rac-Alpha-Tocopherol

(all-rac-α-Tocopherol, Ph. Eur. monograph 0692)



$C_{29}H_{50}O_2$ 430.7 *10191-41-0*

Action and use

Used in prevention and treatment of vitamin E deficiencies.

Ph Eur

DEFINITION

All-*rac*-2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)-3,4-dihydro-2*H*-1-benzopyran-6-ol.

Content

96.0 per cent to 102.0 per cent.

CHARACTERS

Appearance

Clear, colourless or yellowish-brown, viscous, oily liquid.

Solubility

Practically insoluble in water, freely soluble in acetone, in anhydrous ethanol, in methylene chloride and in fatty oils.

IDENTIFICATION

First identification: A, B.

Second identification: A, C.

A. Optical rotation (*2.2.7*): −0.01° to + 0.01°.

Dissolve 2.50 g in *anhydrous ethanol R* and dilute to 25.0 mL with the same solvent.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *α-tocopherol CRS*.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in 2 mL of *cyclohexane R*.

Reference solution Dissolve 10 mg of *α-tocopherol CRS* in 2 mL of *cyclohexane R*.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *ether R, cyclohexane R* (20:80 *V/V*).

Application 10 µL.

Development Over 2/3 of the plate.

Drying In a current of air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Related substances

Gas chromatography (*2.2.28*): use the normalisation procedure.

Internal standard solution Dissolve 1.0 g of *squalane R* in *cyclohexane R* and dilute to 100.0 mL with the same solvent.

Test solution (a) Dissolve 0.100 g of the substance to be examined in 10.0 mL of the internal standard solution.

Test solution (b) Dissolve 0.100 g of the substance to be examined in 10 mL of *cyclohexane R*.

Reference solution (a) Dissolve 0.100 g of *α-tocopherol CRS* in 10.0 mL of the internal standard solution.

Reference solution (b) Dissolve 10 mg of the substance to be examined and 10 mg of *α-tocopheryl acetate R* in *cyclohexane R* and dilute to 100.0 mL with the same solvent.

Reference solution (c) Dissolve 10 mg of *all-rac-α-tocopherol for peak identification CRS* (containing impurities A and B) in *cyclohexane R* and dilute to 1 mL with the same solvent.

Reference solution (d) Dilute 1.0 mL of test solution (b) to 100.0 mL with *cyclohexane R*. Dilute 1.0 mL of this solution to 10.0 mL with *cyclohexane R*.

Column:
- *material*: fused silica;
- *size*: *l* = 30 m, Ø = 0.25 mm;
- *stationary phase*: *methylpolysiloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1 mL/min.

Split ratio 1:100.

Temperature:
- *column*: 280 °C;
- *injection port and detector*: 290 °C.

Detection Flame ionisation.

Injection 1 µL of test solution (b) and reference solutions (b), (c) and (d).

Run time Twice the retention time of all-*rac*-α-tocopherol.

Identification of impurities Use the chromatogram supplied with *all-rac-α-tocopherol for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A and B.

Relative retention With reference to all-*rac*-α-tocopherol (retention time = about 9 min): squalane = about 0.5; impurity A = about 0.7; impurity B = about 0.8; impurities C and D = about 1.05 (eluting immediately after the all-*rac*-α-tocopherol peak).

System suitability Reference solution (b):
- *resolution*: minimum 3.5 between the peaks due to all-*rac*-α-tocopherol and α-tocopheryl acetate.

Limits:
— *impurity A*: maximum 0.5 per cent;
— *impurity B*: maximum 1.5 per cent;
— *sum of impurities C and D*: maximum 1.0 per cent;
— *any other impurity*: for each impurity, maximum 0.25 per cent;
— *total*: maximum 2.5 per cent;
— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (d) (0.1 per cent).

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

ASSAY

Gas chromatography (*2.2.28*) as described in the test for related substances with the following modifications.

Injection Test solution (a) and reference solution (a).

System suitability Reference solution (a):
— *symmetry factor*: minimum 0.6 for the principal peak.

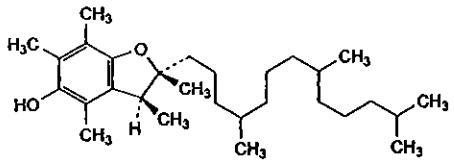
Calculate the percentage content of $C_{29}H_{50}O_2$ from the declared content of *α-tocopherol CRS*.

STORAGE

Under an inert gas, protected from light.

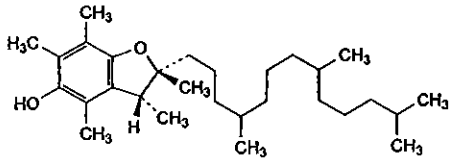
IMPURITIES

Specified impurities *A, B, C, D.*



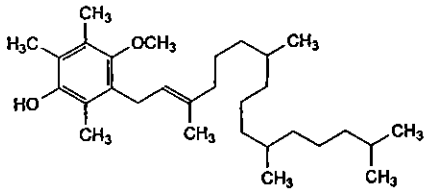
and diastereoisomers

A. all-*rac-trans*-2,3,4,6,7-pentamethyl-2-(4,8,12-trimethyltridecyl)-2,3-dihydrobenzofuran-5-ol,

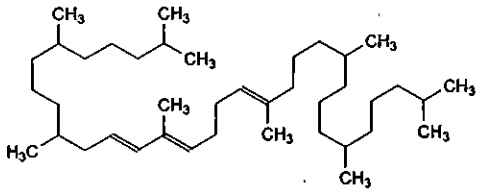


and diastereoisomers

B. all-*rac-cis*-2,3,4,6,7-pentamethyl-2-(4,8,12-trimethyltridecyl)-2,3-dihydrobenzofuran-5-ol,



C. 4-methoxy-2,3,6-trimethyl-5-[(all-*RS,E*)-3,7,11,15-tetramethylhexadec-2-enyl]phenol,

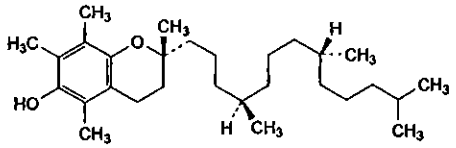


D. (all-*RS*,all-*E*)-2,6,10,14,19,23,27,31-octamethyldotriaconta-12,14,18-triene.

Ph Eur

RRR-Alpha-Tocopherol

(RRR-α-Tocopherol, Ph. Eur. monograph 1256)



$C_{29}H_{50}O_2$ 430.7 *59-02-9*

Action and use

Used in prevention and treatment of vitamin E deficiencies.

Ph Eur

DEFINITION

(2*R*)-2,5,7,8-Tetramethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-3,4-dihydro-2*H*-1-benzopyran-6-ol.

Content

94.5 per cent to 102.0 per cent.

CHARACTERS

Appearance

Clear, colourless or yellowish-brown, viscous, oily liquid.

Solubility

Practically insoluble in water, freely soluble in acetone, in anhydrous ethanol, in methylene chloride and in fatty oils.

IDENTIFICATION

First identification: A, B.

Second identification: A, C.

A. Optical rotation (*2.2.7*): + 0.05° to + 0.10°.

Dissolve 2.50 g in *anhydrous ethanol R* and dilute to 25.0 mL with the same solvent.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *α-tocopherol CRS*.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in 2 mL of *cyclohexane R*.

Reference solution Dissolve 10 mg of *α-tocopherol CRS* in 2 mL of *cyclohexane R*.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *ether R, cyclohexane R* (20:80 *V/V*).

Application 10 µL.

Development Over 2/3 of the plate.

Drying In a current of air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Related substances

Gas chromatography (*2.2.28*): use the normalisation procedure.

Internal standard solution Dissolve 1.0 g of *squalane R* in *cyclohexane R* and dilute to 100.0 mL with the same solvent.

Test solution (a) Dissolve 0.100 g of the substance to be examined in 10.0 mL of the internal standard solution.

Test solution (b) Dissolve 0.100 g of the substance to be examined in 10.0 mL of *cyclohexane R*.

Reference solution (a) Dissolve 0.100 g of *α-tocopherol CRS* in 10.0 mL of the internal standard solution.

Reference solution (b) Dissolve 10 mg of *α-tocopherol R* and 10 mg of *α-tocopheryl acetate R* in *cyclohexane R* and dilute to 100.0 mL with the same solvent.

Column:
- *material*: fused silica;
- *size*: *l* = 30 m, Ø = 0.25 mm;
- *stationary phase*: *methylpolysiloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R.*

Flow rate 1 mL/min.

Split ratio 1:100.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 15	280
Injection port		290
Detector		290

Detection Flame ionisation.

Injection 1 µL of test solution (b) and reference solution (b).

System suitability Reference solution (b):
- *resolution*: minimum 3.5 between the peaks due to α-tocopherol and α-tocopheryl acetate.

Limits:
- *total*: maximum 4.0 per cent;
- *disregard limit*: 0.1 per cent.

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

ASSAY

Gas chromatography (*2.2.28*) as described in the test for related substances with the following modifications.

Injection Test solution (a) and reference solution (a).

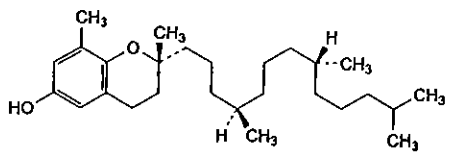
System suitability Reference solution (a):
- *symmetry factor*: minimum 0.6 for the principal peak.

Calculate the percentage content of $C_{29}H_{50}O_2$ taking into account the assigned content of *α-tocopherol CRS*.

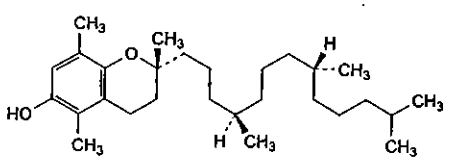
STORAGE

Under an inert gas, protected from light.

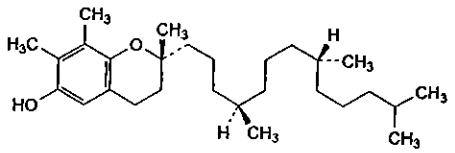
IMPURITIES



A. (2*R*)-2,8-dimethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-3,4-dihydro-2*H*-1-benzopyran-6-ol (*RRR*-δ-tocopherol),



B. (2*R*)-2,5,8-trimethyl-2-[(4*R*,8*R*)4,8,12-trimethyltridecyl]-3,4-dihydro-2*H*-1-benzopyran-6-ol (*RRR*-β-tocopherol),

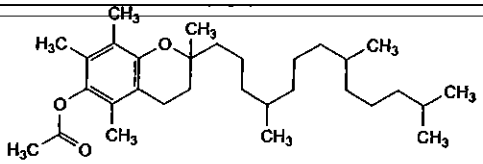


C. (2*R*)-2,7,8-trimethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-3,4-dihydro-2*H*-1-benzopyran-6-ol (*RRR*-γ-tocopherol).

Ph Eur

all-rac-Alpha-Tocopheryl Acetate

(all-rac-α-Tocopheryl Acetate, Ph. Eur. monograph 0439)



$C_{31}H_{52}O_3$ 472.7 *7695-91-2*

Action and use
Used in prevention and treatment of vitamin E deficiencies.

Ph Eur

DEFINITION

all-*rac*-2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)-3,4-dihydro-2*H*-1-benzopyran-6-yl acetate.

Content
96.5 per cent to 102.0 per cent.

CHARACTERS

Appearance
Clear, colourless or slightly greenish-yellow, viscous, oily liquid.

Solubility
Practically insoluble in water, freely soluble in acetone, in anhydrous ethanol and in fatty oils.

IDENTIFICATION

First identification: A, B.

Second identification: A, C.

A. Optical rotation (*2.2.7*): −0.01° to + 0.01°.

Dissolve 2.50 g in *anhydrous ethanol R* and dilute to 25.0 mL with the same solvent.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *α-tocopheryl acetate CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve about 10 mg of the substance to be examined in 2 mL of *cyclohexane R.*

Reference solution Dissolve about 10 mg of *α-tocopheryl acetate CRS* in 2 mL of *cyclohexane R.*

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *ether R, cyclohexane R* (20:80 *V/V*).

Application 10 µL.

Development Over 2/3 of the plate.

Drying In a current of air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the

principal spot in the chromatogram obtained with the reference solution.

TESTS

Related substances

Gas chromatography (*2.2.28*): use the normalisation procedure.

Internal standard solution Dissolve 1.0 g of *squalane R* in *cyclohexane R* and dilute to 100.0 mL with the same solvent.

Test solution (a) Dissolve 0.100 g of the substance to be examined in 10.0 mL of the internal standard solution.

Test solution (b) Dissolve 0.100 g of the substance to be examined in 10 mL of *cyclohexane R*.

Reference solution (a) Dissolve 0.100 g of *α-tocopheryl acetate CRS* in 10.0 mL of the internal standard solution.

Reference solution (b) Dissolve 10 mg of the substance to be examined and 10 mg of *α-tocopherol R* in *cyclohexane R* and dilute to 100.0 mL with the same solvent.

Reference solution (c) Dissolve 10 mg of *all-rac-α-tocopheryl acetate for peak identification CRS* (containing impurities A and B) in *cyclohexane R* and dilute to 1 mL with the same solvent.

Reference solution (d) Dilute 1.0 mL of test solution (b) to 100.0 mL with *cyclohexane R*. Dilute 1.0 mL of this solution to 10.0 mL with *cyclohexane R*.

Column:
- *material*: fused silica;
- *size*: *l* = 30 m, Ø = 0.25 mm;
- *stationary phase*: *methylpolysiloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1 mL/min.

Split ratio 1:100.

Temperature:
- *column*: 280 °C;
- *injection port and detector*: 290 °C.

Detection Flame ionisation.

Injection 1 µL of test solution (b) and reference solutions (a), (b), (c) and (d); inject directly onto the column or via a sufficiently inert, glass-lined injection port using an automatic injection device or other reproducible injection method.

Run time Twice the retention time of all-*rac*-α-tocopheryl acetate.

Identification of impurities Use the chromatogram supplied with *all-rac-α-tocopheryl acetate for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A and B.

Relative retention With reference to all-*rac*-α-tocopheryl acetate (retention time = about 15 min):
squalane = about 0.4; impurity A = about 0.7; impurity B = about 0.8; impurity C = about 0.9; impurities D and E = about 1.05 (eluting immediately after the all-*rac*-α-tocopheryl acetate peak).

System suitability:
- *resolution*: minimum 3.5 between the peaks due to impurity C and all-*rac*-α-tocopheryl acetate in the chromatogram obtained with reference solution (b);
- in the chromatogram obtained with reference solution (a), the area of the peak due to impurity C is not greater than 0.2 per cent of the area of the peak due to all-*rac*-α-tocopheryl acetate.

Limits:
- *impurities A, C*: for each impurity, maximum 0.5 per cent;
- *impurity B*: maximum 1.5 per cent;
- *sum of impurities D and E*: maximum 1.0 per cent;
- *any other impurity*: for each impurity, maximum 0.25 per cent;
- *total*: maximum 2.5 per cent;
- *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (d) (0.1 per cent).

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

ASSAY

Gas chromatography (*2.2.28*) as described in the test for related substances with the following modifications.

Injection Test solution (a) and reference solution (a).

System suitability Reference solution (a):
- *symmetry factor*: minimum 0.6 for the principal peak.

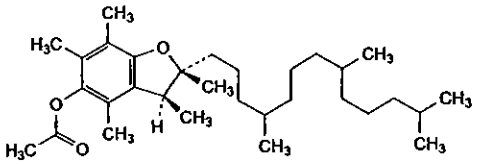
Calculate the percentage content of $C_{31}H_{52}O_3$ from the declared content of *α-tocopheryl acetate CRS*.

STORAGE

Protected from light.

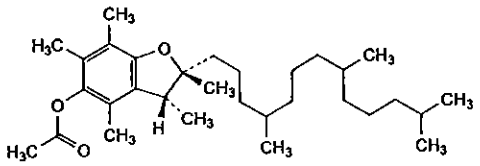
IMPURITIES

Specified impurities *A, B, C, D, E.*



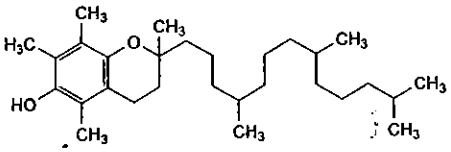
and diastereoisomers

A. all-*rac-trans*-2,3,4,6,7-pentamethyl-2-(4,8,12-trimethyltridecyl)-2,3-dihydrobenzofuran-5-yl acetate,

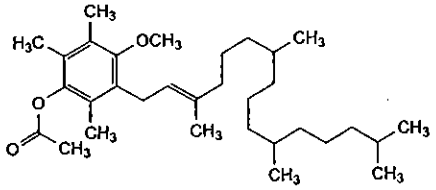


and diastereoisomers

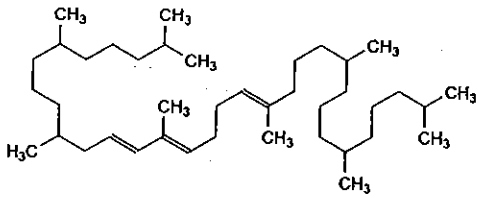
B. all-*rac-cis*-2,3,4,6,7-pentamethyl-2-(4,8,12-trimethyltridecyl)-2,3-dihydrobenzofuran-5-yl acetate,



C. all-*rac*-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-3,4-dihydro-2*H*-1-benzopyran-6-ol (all-*rac*-α-tocopherol),



D. 4-methoxy-2,3,6-trimethyl-5-[(all-*RS,E*)-3,7,11,15-tetramethylhexadec-2-enyl]phenyl acetate,

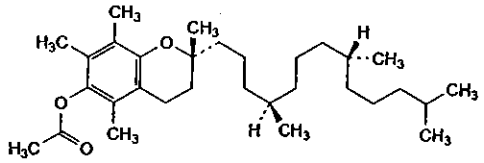


E. (all-*RS*,all-*E*)-2,6,10,14,19,23,27,31-octamethyldotriaconta-12,14,18-triene.

Ph Eur

RRR-Alpha-Tocopheryl Acetate

(RRR-α-Tocopheryl Acetate, Ph. Eur. monograph 1257)



$C_{31}H_{52}O_3$ 472.7

Action and use
Used in prevention and treatment of vitamin E deficiencies.

Ph Eur

DEFINITION
(2*R*)-2,5,7,8-Tetramethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-3,4-dihydro-2*H*-1-benzopyran-6-yl acetate.

Content
95.0 per cent to 101.0 per cent.

CHARACTERS
Appearance
Clear, colourless or slightly greenish-yellow, viscous, oily liquid.

Solubility
Practically insoluble in water, freely soluble in acetone, in anhydrous ethanol and in fatty oils, soluble in ethanol (96 per cent).

IDENTIFICATION
First identification: A, B.

Second identification: A, C.

A. Optical rotation (*2.2.7*): + 0.25° to + 0.35°.

Dissolve 2.50 g in *anhydrous ethanol R* and dilute to 25.0 mL with the same solvent.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *α-tocopheryl acetate CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution (a) Dissolve 10 mg of the substance to be examined in 2 mL of *cyclohexane R.*

Test solution (b) In a ground-glass stoppered tube, dissolve about 10 mg of the substance to be examined in 2 mL of *2.5 M alcoholic sulfuric acid R.* Heat on a water-bath for 5 min. Cool and add 2 mL of *water R* and 2 mL of *cyclohexane R.* Shake for 1 min. Use the upper layer.

Reference solution (a) Dissolve 10 mg of *α-tocopheryl acetate CRS* in 2 mL of *cyclohexane R.*

Reference solution (b) Prepare as described for test solution (b), using *α-tocopheryl acetate CRS* instead of the substance to be examined.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *ether R, cyclohexane R* (20:80 *V/V*).

Application 10 µL.

Development Over 2/3 of the plate.

Drying In a current of air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with test solution (a) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a). In the chromatograms obtained with test solution (b) and reference solution (b), there may be 2 spots depending on the degree of hydrolysis: the spot with the higher R_F value is due to α-tocopheryl acetate and corresponds to the spot in the chromatogram obtained with reference solution (a); the spot with the lower R_F value is due to α-tocopherol.

TESTS
Related substances
Gas chromatography (*2.2.28*): use the normalisation procedure.

Internal standard solution Dissolve 1.0 g of *squalane R* in *cyclohexane R* and dilute to 100.0 mL with the same solvent.

Test solution (a) Dissolve 0.100 g of the substance to be examined in 10.0 mL of the internal standard solution.

Test solution (b) Dissolve 0.100 g of the substance to be examined in 10.0 mL of *cyclohexane R.*

Reference solution (a) Dissolve 0.100 g of *α-tocopheryl acetate CRS* in 10.0 mL of the internal standard solution.

Reference solution (b) Dissolve 10 mg of *α-tocopherol R* and 10 mg of *α-tocopheryl acetate R* in *cyclohexane R* and dilute to 100.0 mL with the same solvent.

Column:
- *material:* fused silica;
- *size:* *l* = 30 m, Ø = 0.25 mm;
- *stationary phase: methylpolysiloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R.*

Flow rate 1 mL/min.

Split ratio 1:100.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 15	280
Injection port		290
Detector		290

Detection Flame ionisation.

Injection 1 µL of test solution (b) and reference solutions (a) and (b); inject directly onto the column or via a sufficiently inert, glass-lined injection port using an automatic injection device or other reproducible injection method.

System suitability:
- *resolution:* minimum 3.5 between the peaks due to α-tocopherol and α-tocopheryl acetate in the chromatogram obtained with reference solution (b);
- in the chromatogram obtained with reference solution (a), the area of the peak due to α-tocopherol is not greater

than 0.2 per cent of the area of the peak due to α-tocopheryl acetate.

Limits:
— *total*: maximum 4.0 per cent;
— *disregard limit*: 0.1 per cent.

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

ASSAY

Gas chromatography (*2.2.28*) as described in the test for related substances with the following modifications.

Injection Test solution (a) and reference solution (a).

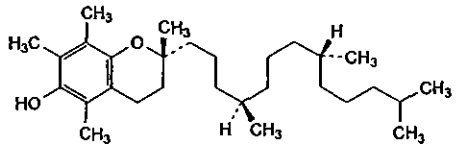
System suitability Reference solution (a):
— *symmetry factor*: minimum 0.6 for the principal peak.

Calculate the percentage content of $C_{31}H_{52}O_3$ taking into account the assigned content of *α-tocopheryl acetate CRS*.

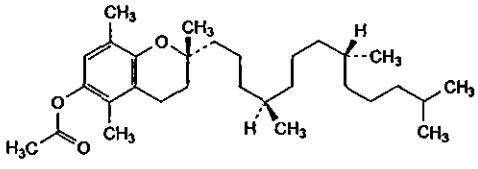
STORAGE

Protected from light.

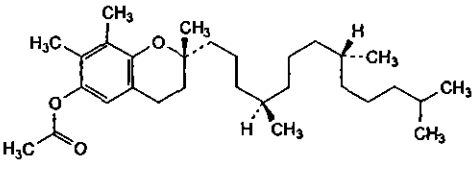
IMPURITIES



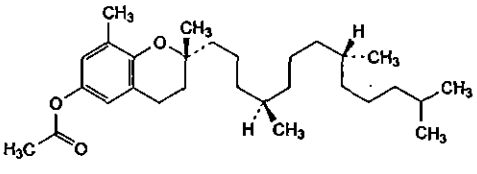
A. (2*R*)-2,5,7,8-tetramethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-3,4-dihydro-2*H*-1-benzopyran-6-ol (*RRR*-α-tocopherol),



B. (2*R*)-2,5,8-trimethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-3,4-dihydro-2*H*-1-benzopyran-6-yl acetate (*RRR*-β-tocopheryl acetate),



C. (2*R*)-2,7,8-trimethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-3,4-dihydro-2*H*-1-benzopyran-6-yl acetate (*RRR*-γ-tocopheryl acetate),



D. (2*R*)-2,8-dimethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-3,4-dihydro-2*H*-1-benzopyran-6-yl acetate (*RRR*-δ-tocopheryl acetate).

Ph Eur

Alpha Tocopheryl Acetate Concentrate (Powder Form)

(α-Tocopheryl Acetate Concentrate (Powder Form) Ph. Eur. monograph 0691)

Action and use

Used in prevention and treatment of vitamin E deficiencies.

Ph Eur

DEFINITION

Preparation obtained either by finely dispersing *all-rac-α-Tocopheryl acetate (0439)* in a suitable carrier of suitable quality (for example gelatin, acacia, carbohydrates, lactoproteins or a mixture thereof) or by adsorbing *all-rac-α-Tocopheryl acetate (0439)* on silicic acid of suitable quality.

Content

90.0 per cent to 115.0 per cent of the α-tocopheryl acetate content stated on the label, which is not less than 25 g per 100 g of concentrate.

CHARACTERS

Appearance

Almost white, yellowish or light brown, small particles.

Solubility

Practically insoluble or swells or forms a dispersion in water, depending on the formulation.

IDENTIFICATION

First identification: B.

Second identification: A.

A. Thin-layer chromatography (*2.2.27*).

Test solution To a quantity of the preparation to be examined corresponding to 50 mg of α-tocopheryl acetate add 5 mL of *0.01 M hydrochloric acid* and treat with ultrasound at 60 °C. Add 5 mL of *anhydrous ethanol R* and 10 mL of *cyclohexane R*, shake for 1 min and centrifuge for 5 min. Use the upper layer.

Reference solution Dissolve 50 mg of *α-tocopheryl acetate CRS* in *cyclohexane R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *ether R, cyclohexane R* (20:80 *V/V*).

Application 10 µL.

Development 3/4 of the plate.

Drying In a current of air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

B. Examine the chromatograms obtained in the assay.

Results:
— the principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a);
— in the chromatogram obtained with reference solution (c) no additional principal peak is observed when compared with the chromatogram obtained with the test solution.

ASSAY

Gas chromatography (*2.2.28*).

Internal standard solution Dissolve 1.0 g of *squalane R* in *cyclohexane R* and dilute to 500.0 mL with the same solvent.

Test solution Weigh accurately a quantity of the preparation to be examined corresponding to about 0.100 g of α-tocopheryl acetate into a 250 mL conical flask. Add 20 mL of *1 M hydrochloric acid* and sonicate at 70 °C for 20 min. Add 50 mL of *anhydrous ethanol R* and 50.0 mL of the internal standard solution. Mix thoroughly for 30 min using a magnetic stirrer. Allow the 2 layers to separate and use the upper layer.

Reference solution (a) Dissolve 0.100 g of *α-tocopheryl acetate CRS* in 50.0 mL of the internal standard solution.

Reference solution (b) Dissolve 10 mg of *α-tocopherol R* and 10 mg of *α-tocopheryl acetate CRS* in 5.0 mL of *cyclohexane R*.

Reference solution (c) Mix 1.0 mL of the test solution and 1.0 mL of reference solution (a).

Column:
— *material*: fused silica;
— *size*: *l* = 30 m, Ø = 0.25 mm;
— *stationary phase*: *methylpolysiloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1 mL/min.

Split ratio 1:100.

Temperature:
— *column*: 280 °C;
— *injection port and detector*: 290 °C.

Detection Flame ionisation.

Injection 1 µL; inject directly onto the column or via a sufficiently inert, glass-lined injection port.

Run time 1.1 times the retention time of α-tocopheryl acetate.

Relative retention With reference to α-tocopheryl acetate (retention time = about 12 min): squalane = about 0.5; α-tocopherol = about 0.9.

System suitability:
— *resolution*: minimum 3.5 between the peaks due to α-tocopherol and α-tocopheryl acetate in the chromatogram obtained with reference solution (b);
— in the chromatogram obtained with reference solution (a), the area of the peak due to α-tocopherol is not greater than 0.002 times the area of the peak due to α-tocopheryl acetate (0.2 per cent).

Calculate the percentage content of $C_{31}H_{52}O_3$ from the declared content of *α-tocopheryl acetate CRS*.

STORAGE

In an airtight, well-filled container, protected from light.

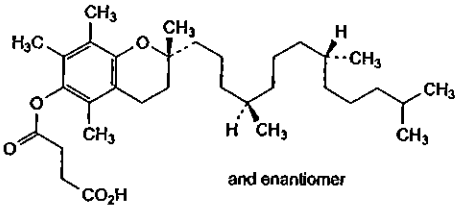
LABELLING

The label states the content of α-tocopheryl acetate, expressed in grams per 100 g of concentrate.

Ph Eur

Alpha Tocopheryl Hydrogen Succinate

(DL-α-Tocopheryl Hydrogen Succinate, Ph. Eur. monograph 1258)



$C_{33}H_{54}O_5$ 530.8

Action and use

Used in prevention and treatment of vitamin E deficiencies.

Ph Eur

DEFINITION

(2*RS*)-2,5,7,8-Tetramethyl-2-[(4*RS*,8*RS*)-4,8,12-trimethyltridecyl]-3,4-dihydro-2*H*-1-benzopyran-6-yl hydrogen succinate.

Content

96.0 per cent to 102.0 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, very soluble in methylene chloride, soluble in acetone and in anhydrous ethanol.

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Absorbance (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *RRR-α-tocopheryl hydrogen succinate CRS*.

C. Thin-layer chromatography (*2.2.27*).

Test solution (a) Dissolve 10 mg of the substance to be examined in 2 mL of *cyclohexane R*.

Test solution (b) In a ground-glass-stoppered tube, dissolve 10 mg of the substance to be examined in 2 mL of *2.5 M alcoholic sulfuric acid R*. Heat on a water-bath for 5 min. Cool and add 2 mL of *water R* and 2 mL of *cyclohexane R*. Shake for 1 min. Use the upper layer.

Reference solution (a) Dissolve 10 mg of *RRR-α-tocopheryl hydrogen succinate CRS* in 2 mL of *cyclohexane R*.

Reference solution (b) Prepare as described for test solution (b), using *RRR-α-tocopheryl hydrogen succinate CRS* instead of the substance to be examined.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *glacial acetic acid R, ether R, cyclohexane R* (0.2:20:80 *V/V/V*).

Application 10 µL.

Development Over a path of 15 cm.

Drying In a current of air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spot in the chromatogram obtained with test solution (a) is similar in position and size to the principal spot in the chromatogram obtained with reference

solution (a). In the chromatograms obtained with test solution (b) and reference solution (b), there are 2 spots: the spot with the higher R_F value is due to α-tocopherol, the spot with the lower R_F value is due to DL-α-tocopheryl hydrogen succinate and corresponds to the spot obtained with reference solution (a). Depending on the degree of hydrolysis, the lower spot may be weak or even absent.

Detection B Spray with a mixture of 10 volumes of *hydrochloric acid R*, 40 volumes of a 2.5 g/L solution of *ferric chloride R* in *ethanol (96 per cent) R* and 40 volumes of a 10 g/L solution of *phenanthroline hydrochloride R* in *ethanol (96 per cent) R*.

Results B In the chromatograms obtained with test solution (b) and reference solution (b), the spot due to α-tocopherol is orange.

~~D. Optical rotation (see Tests).~~

TESTS

Optical rotation (*2.2.7*)

−0.01° to + 0.01°.

Dissolve 2.50 g in *anhydrous ethanol R* and dilute to 25.0 mL with the same solvent.

Absorbance (*2.2.25*)

Solution A Dissolve 0.150 g in *anhydrous ethanol R* and dilute to 100 mL with the same solvent.

Test solution (a) Dilute 10.0 mL of solution A to 100.0 mL with *anhydrous ethanol R*.

Test solution (b) Dilute 20.0 mL of solution A to 50.0 mL with *anhydrous ethanol R*.

Absorption maximum At 284 nm for test solution (a).

Absorption minimum At 254 nm for test solution (b).

Specific absorbance at the absorption maximum 35 to 38 for test solution (a).

Specific absorbance at the absorption minimum 6.0 to 8.0 for test solution (b).

Acid value (*2.5.1*)

101 to 108, determined on 1.00 g.

Free tocopherol

Maximum 1.0 per cent.

Dissolve 0.500 g in 100 mL of *0.25 M alcoholic sulfuric acid R*. Add 20 mL of *water R* and 0.1 mL of a 2.5 g/L solution of *diphenylamine R* in *sulfuric acid R*. Titrate with *0.01 M ammonium and cerium sulfate* until a blue colour is obtained that persists for at least 5 s. Carry out a blank titration.

1 mL of *0.01 M ammonium and cerium sulfate* is equivalent to 2.154 mg of free tocopherol.

Related substances

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Gas chromatography (*2.2.28*).

Internal standard solution Dissolve 0.300 g of *dotriacontane R* in *hexane R* and dilute to 100.0 mL with the same solvent.

Test solution Weigh 30.0 mg of the substance to be examined into a 20 mL vial. Add 2.0 mL of *methanol R*, 1.0 mL of *dimethoxypropane R* and 0.1 mL of *hydrochloric acid R*. Cap tightly and sonicate. Allow to stand in the dark for 1 h ± 5 min. Remove from the dark, uncap and evaporate just to dryness on a steam bath with the aid of a stream of nitrogen. Add 10.0 mL of the internal standard solution. Vortex into solution.

Reference solution Weigh 30.0 mg of *RRR-α-tocopheryl hydrogen succinate CRS* into a 20 mL vial. Add 2.0 mL of *methanol R*, 1.0 mL of *dimethoxypropane R* and 0.1 mL of *hydrochloric acid R*. Cap tightly and sonicate. Allow to stand in the dark for 1 h ± 5 min. Remove from the dark, uncap and evaporate just to dryness on a steam bath with the aid of a stream of nitrogen. Add 10.0 mL of the internal standard solution. Vortex into solution.

Column:
- *material*: fused silica;
- *size*: l = 15 m, Ø = 0.32 mm;
- *stationary phase*: *methylpolysiloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R*.

Flow rate 3-6 mL/min.

Split ratio 1:10 to 1:20.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 10	200 → 250
	10 - 20	250
Injection port		300
Detector		330

Detection Flame ionisation.

Injection 1 µL; inject directly onto the column or via a glass-lined injection port using an automatic injection device or some other reproducible injection method.

System suitability Reference solution:
- *resolution*: minimum 12.0 between the peaks due to dotriacontane and DL-α-tocopheryl hydrogen succinate.

Interference test Dissolve 0.100 g of the substance to be examined in *hexane R* and dilute to 50.0 mL with the same solvent. Inject 1 µL of the solution and record the chromatogram. If a peak is detected with the same retention time as that of the peak due to dotriacontane, calculate the area of this peak relative to the peak area of the substance to be examined. If the relative peak area is greater than 0.5 per cent, use the corrected peak area $S'_{D(corr)}$ for the final calculation.

$$S'_{D(corr)} = S'_D - \frac{S_I \times S'_T}{S_{TI}}$$

S'_D = area of the peak due to dotriacontane in the chromatogram obtained with the test solution;

S_I = area of the peak with the same retention time as that of the peak due to dotriacontane in the chromatogram obtained in the interference test;

S'_T = area of the peak due to DL-α-tocopheryl hydrogen succinate in the chromatogram obtained with the test solution;

S_{TI} = area of the peak due to DL-α-tocopheryl hydrogen succinate in the chromatogram obtained in the interference test.

Measure the areas of the peaks due to *RRR-α-tocopheryl hydrogen succinate CRS* (S_T) and dotriacontane (S_D) in the chromatogram obtained with the reference solution and the areas of the peaks due to DL-α-tocopheryl hydrogen succinate (S'_T) and dotriacontane (S'_D) in the chromatogram obtained with the test solution.

Determine the response factor (RF) for DL-α-tocopheryl hydrogen succinate from the areas of the peaks due to *RRR-α-tocopheryl hydrogen succinate CRS* and dotriacontane in the

chromatogram obtained with the reference solution, using the following expression:

$$\frac{S_D \times m_T}{S_T \times m_D}$$

Calculate the percentage content of DL-α-tocopheryl hydrogen succinate using the following expression:

$$\frac{100 \times S'_T \times m_D \times RF}{S'_{D(corr)} \times m}$$

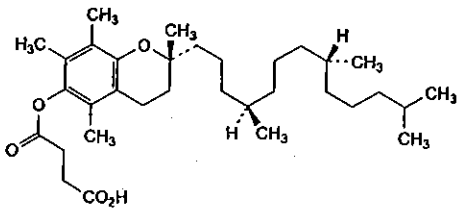
S_D = area of the peak due to dotriacontane in the chromatogram obtained with the reference solution;
$S'_{D(corr)}$ = corrected area of the peak due to dotriacontane in the chromatogram obtained with the test solution;
S_T = area of the peak due to *RRR-α-tocopheryl hydrogen succinate CRS* in the chromatogram obtained with the reference solution;
S'_T = area of the peak due to DL-α-tocopheryl hydrogen succinate in the chromatogram obtained with the test solution;
m_D = mass of dotriacontane in the test solution and in the reference solution, in milligrams;
m_T = mass of *RRR-α-tocopheryl hydrogen succinate CRS* in the reference solution, in milligrams;
m = mass of the substance to be examined in the test solution, in milligrams.

STORAGE
Protected from light.

Ph Eur

RRR-Alpha-Tocopheryl Hydrogen Succinate

(RRR-α-Tocopheryl Hydrogen Succinate, Ph. Eur. monograph 1259)



$C_{33}H_{54}O_5$ 530.8 *4345-03-3*

Action and use
Used in prevention and treatment of vitamin E deficiencies.

Ph Eur

DEFINITION
(2*R*)-2,5,7,8-Tetramethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-3,4-dihydro-2*H*-1-benzopyran-6-yl hydrogen succinate.

Content
96.0 per cent to 102.0 per cent.

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, very soluble in methylene chloride, soluble in acetone and in anhydrous ethanol.

IDENTIFICATION
First identification: B, D.
Second identification: A, C, D.

A. Absorbance (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *RRR-α-tocopheryl hydrogen succinate CRS.*

C. Thin-layer chromatography (*2.2.27*).
Test solution (a) Dissolve 10 mg of the substance to be examined in 2 mL of *cyclohexane R.*
Test solution (b) In a ground-glass-stoppered tube, dissolve 10 mg of the substance to be examined in 2 mL of *2.5 M alcoholic sulfuric acid R.* Heat on a water-bath for 5 min. Cool and add 2 mL of *water R* and 2 mL of *cyclohexane R.* Shake for 1 min. Use the upper layer.
Reference solution (a) Dissolve 10 mg of *RRR-α-tocopheryl hydrogen succinate CRS* in 2 mL of *cyclohexane R.*
Reference solution (b) Prepare as described for test solution (b), using *RRR-α-tocopheryl hydrogen succinate CRS* instead of the substance to be examined.
Plate *TLC silica gel F_{254} plate R.*
Mobile phase *glacial acetic acid R, ether R, cyclohexane R* (0.2:20:80 *V/V/V*).
Application 10 µL.
Development Over a path of 15 cm.
Drying In a current of air.
Detection A Examine in ultraviolet light at 254 nm.
Results A The principal spot in the chromatogram obtained with test solution (a) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a). In the chromatograms obtained with test solution (b) and reference solution (b), there are 2 spots: the spot with the higher R_F value is due to α-tocopherol, the spot with the lower R_F value is due to *RRR*-α-tocopheryl hydrogen succinate and corresponds to the spot obtained with reference solution (a). Depending on the degree of hydrolysis, the lower spot may be weak or even absent.
Detection B Spray with a mixture of 10 volumes of *hydrochloric acid R*, 40 volumes of a 2.5 g/L solution of *ferric chloride R* in *ethanol (96 per cent) R* and 40 volumes of a 10 g/L solution of *phenanthroline hydrochloride R* in *ethanol (96 per cent) R.*
Results B In the chromatograms obtained with test solution (b) and reference solution (b), the spot due to α-tocopherol is orange.

D. After saponification, the resulting *RRR*-α-tocopherol is dextrorotatory (*2.2.7*). The specific optical rotation after oxidation to the quinone form is not less than + 24.
Carry out the test avoiding exposure to actinic light Transfer 1.0 g to a round bottomed, ground-glass-stoppered, 250 mL flask, dissolve in 30 mL of *anhydrous ethanol R* and heat under reflux for 3 min. While the solution is boiling, add, through the condenser, 20 mL of *2 M alcoholic potassium hydroxide R.* Continue heating under reflux for 20 min and, without cooling, add 4.0 mL of *hydrochloric acid R* dropwise through the condenser. Cool, rinse the condenser with 10 mL of *anhydrous ethanol R*, transfer the contents of the flask to a 500 mL separating funnel, and rinse the flask with 4 quantities, each of 25 mL, of *water R* and 4 quantities, each of 25 mL, of *ether R.* Add the rinsings to the separating funnel. Shake vigorously for 2 min, allow the layers to separate and collect each of the 2 layers in individual separating funnels. Shake the aqueous layer with 2 quantities, each of 50 mL, of *ether R* and add these extracts to the main ether extract. Wash the combined ether extracts with

4 quantities, each of 100 mL, of *water R* and discard the washings.

To the ether solution add 40 mL of a 100 g/L solution of *potassium ferricyanide R* in an 8 g/L solution of *sodium hydroxide R* and shake for 3 min. Wash the ether solution with 4 quantities, each of 50 mL, of *water R*, discard the washings and dry the ether layer over *anhydrous sodium sulfate R*. Evaporate the ether on a water-bath under reduced pressure or in an atmosphere of nitrogen until a few millilitres remain, then complete the evaporation removing the last traces of ether without the application of heat. Immediately dissolve the residue in 25.0 mL of *trimethylpentane R* and determine the optical rotation.

Calculate the specific optical rotation of the substance in the test solution using as c the number of grams equivalent to α-tocopherol (factor 0.811) in 1000 mL.

TESTS

Absorbance (*2.2.25*)

Solution A Dissolve 0.150 g in *anhydrous ethanol R* and dilute to 100 mL with the same solvent.

Test solution (a) Dilute 10.0 mL of solution A to 100.0 mL with *anhydrous ethanol R*.

Test solution (b) Dilute 20.0 mL of solution A to 50.0 mL with *anhydrous ethanol R*.

Absorption maximum At 284 nm for test solution (a).

Absorption minimum At 254 nm for test solution (b).

Specific absorbance at the absorption maximum 35 to 38 for test solution (a).

Specific absorbance at the absorption minimum 6.0 to 8.0 for test solution (b).

Acid value (*2.5.1*)

101 to 108, determined on 1.00 g.

Free tocopherol

Maximum 1.0 per cent.

Dissolve 0.500 g in 100 mL of *0.25 M alcoholic sulfuric acid R*. Add 20 mL of *water R* and 0.1 mL of a 2.5 g/L solution of *diphenylamine R* in *sulfuric acid R*. Titrate with *0.01 M ammonium and cerium sulfate* until a blue colour is obtained that persists for at least 5 s. Carry out a blank titration.

1 mL of *0.01 M ammonium and cerium sulfate* is equivalent to 2.154 mg of free tocopherol.

Related substances

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Gas chromatography (*2.2.28*).

Internal standard solution Dissolve 0.300 g of *dotriacontane R* in *hexane R* and dilute to 100.0 mL with the same solvent.

Test solution Weigh 30.0 mg of the substance to be examined into a 20 mL vial. Add 2.0 mL of *methanol R*, 1.0 mL of *dimethoxypropane R* and 0.1 mL of *hydrochloric acid R*. Cap tightly and sonicate. Allow to stand in the dark for 1 h ± 5 min. Remove from the dark, uncap and evaporate just to dryness on a steam bath with the aid of a stream of nitrogen. Add 10.0 mL of the internal standard solution. Vortex into solution.

Reference solution Weigh 30.0 mg of *RRR-α-tocopheryl hydrogen succinate CRS* into a 20 mL vial. Add 2.0 mL of *methanol R*, 1.0 mL of *dimethoxypropane R* and 0.1 mL of *hydrochloric acid R*. Cap tightly and sonicate. Allow to stand in the dark for 1 h ± 5 min. Remove from the dark, uncap and evaporate just to dryness on a steam bath with the aid of a stream of nitrogen. Add 10.0 mL of the internal standard solution. Vortex into solution.

Column:
- *material*: fused silica;
- *size*: l = 15 m, Ø = 0.32 mm;
- *stationary phase*: *methylpolysiloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R*.

Flow rate 3-6 mL/min.

Split ratio 1:10 to 1:20.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 10	200 → 250
	10 - 20	250
Injection port		300
Detector		330

Detection Flame ionisation.

Injection 1 µL; inject directly onto the column or via a glass-lined injection port using an automatic injection device or some other reproducible injection method.

System suitability Reference solution:
- *resolution*: minimum 12.0 between the peaks due to dotriacontane and *RRR*-α-tocopheryl hydrogen succinate.

Interference test Dissolve 0.100 g of the substance to be examined in *hexane R* and dilute to 50.0 mL with the same solvent. Inject 1 µL of the solution and record the chromatogram. If a peak is detected with the same retention time as that of the peak due to dotriacontane, calculate the area of this peak relative to the peak area of the substance to be examined. If the relative peak area is greater than 0.5 per cent, use the corrected peak area $S'_{D(corr)}$ for the final calculation.

$$S'_{D(corr)} = S'_D - \frac{S_I \times S'_T}{S_{TI}}$$

S'_D = area of the peak due to dotriacontane in the chromatogram obtained with the test solution;

S_I = area of the peak with the same retention time as that of the peak due to dotriacontane in the chromatogram obtained in the interference test;

S'_T = area of the peak due to *RRR*-α-tocopheryl hydrogen succinate in the chromatogram obtained with the test solution;

S_{TI} = area of the peak due to *RRR*-α-tocopheryl hydrogen succinate in the chromatogram obtained in the interference test.

Measure the areas of the peaks due to *RRR-α-tocopheryl hydrogen succinate CRS* (S_T) and dotriacontane (S_D) in the chromatogram obtained with the reference solution and the areas of the peaks due to *RRR*-α-tocopheryl hydrogen succinate (S'_T) and dotriacontane (S'_D) in the chromatogram obtained with the test solution.

Determine the response factor (RF) for *RRR*-α-tocopheryl hydrogen succinate from the areas of the peaks due to *RRR-α-tocopheryl hydrogen succinate CRS* and dotriacontane in the chromatogram obtained with the reference solution, using the following expression:

$$\frac{S_D \times m_T}{S_T \times m_D}$$

Calculate the percentage content of *RRR*-α-tocopheryl hydrogen succinate using the following expression:

$$\frac{100 \times S'_T \times m_D \times RF}{S'_{D(corr)} \times m}$$

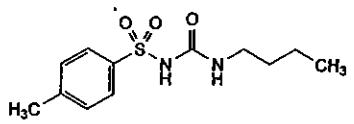
S_D = area of the peak due to dotriacontane in the chromatogram obtained with the reference solution;
$S'_{D(corr)}$ = corrected area of the peak due to dotriacontane in the chromatogram obtained with the test solution;
S_T = area of the peak due to *RRR-α-tocopheryl hydrogen succinate CRS* in the chromatogram obtained with the reference solution;
S'_T = area of the peak due to *RRR*-α-tocopheryl hydrogen succinate in the chromatogram obtained with the test solution;
m_D = mass of dotriacontane in the test solution and in the reference solution, in milligrams;
m_T = mass of *RRR-α-tocopheryl hydrogen succinate CRS* in the reference solution, in milligrams;
m = mass of the substance to be examined in the test solution, in milligrams.

STORAGE
Protected from light.

Ph Eur

Tolbutamide

(Ph. Eur. monograph 0304)



$C_{12}H_{18}N_2O_3S$ 270.3 *64-77-7*

Action and use
Inhibition of ATP-dependent potassium channels (sulfonylurea); treatment of diabetes mellitus.

Preparation
Tolbutamide Tablets

Ph Eur

DEFINITION
1-Butyl-3-[(4-methylphenyl)sulfonyl]urea.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, soluble in acetone and in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION
First identification: A, C.
Second identification: A, B, D.

A. Melting point (*2.2.14*): 126 °C to 130 °C.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution (a) Dissolve 25.0 mg in *methanol R* and dilute to 100.0 mL with the same solvent.

Test solution (b) Dilute 10.0 mL of test solution (a) to 250.0 mL with *methanol R*.

Spectral range 245-300 nm for test solution (a); 220-235 nm for test solution (b).

Absorption maxima At 258 nm, 263 nm and 275 nm for test solution (a); at 228 nm for test solution (b).

Shoulder At 268 nm for test solution (a).

Specific absorbance at the absorption maximum 480 to 520 for test solution (b).

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison tolbutamide CRS.

D. To 0.2 g add 8 mL of a 500 g/L solution of *sulfuric acid R* and heat under a reflux condenser for 30 min. Allow to cool. Crystals are formed which, after recrystallisation from hot *water R* and drying at 105 °C, melt (*2.2.14*) at 135 °C to 140 °C.

TESTS
Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 0.2 g in 5 mL of *dilute sodium hydroxide solution R* and add 5 mL of *water R*.

pH (*2.2.3*)
4.5 to 5.5.

To 2.0 g add 50 mL of *carbon dioxide-free water R* and heat at 70 °C for 5 min. Cool rapidly and filter.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 50 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 10 mg of *toluenesulfonamide R* (impurity A) and 10 mg of *toluenesulfonylurea R* (impurity B) in the mobile phase and dilute to 10.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 100.0 mL with the mobile phase.

Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 35 volumes of *acetonitrile R1* and 65 volumes of a 1.36 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 3.5 with *phosphoric acid R*.

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 20 µL.

Run time 1.5 times the retention time of tolbutamide.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurities A and B.

Relative retention With reference to tolbutamide (retention time = about 18 min): impurity B = about 0.2; impurity A = about 0.3.

System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the peaks due to impuritites A and B.

Limits:
- — *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- — *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
- — *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

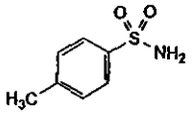
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.250 g in a mixture of 20 mL of *water R* and 40 mL of *ethanol (96 per cent) R*. Titrate with *0.1 M sodium hydroxide*, using 1 mL of *phenolphthalein solution R* as indicator.

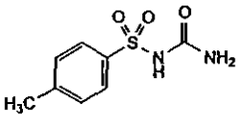
1 mL of *0.1 M sodium hydroxide* is equivalent to 27.03 mg of $C_{12}H_{18}N_2O_3S$.

STORAGE
In an airtight container.

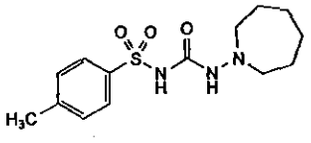
IMPURITIES
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C.*



A. (4-methylphenyl)sulfonamide (toluenesulfonamide),



B. 1-[(4-methylphenyl)sulfonyl]urea (toluenesulfonylurea),

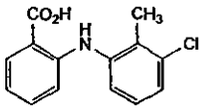


C. 1-azepan-1-yl-3-[(4-methylphenyl)sulfonyl]urea (tolazamide).

Ph Eur

Tolfenamic Acid

(Ph. Eur. monograph 2039)



$C_{14}H_{12}ClNO_2$ 261.7 *13710-19-5*

Action and use
Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Ph Eur

DEFINITION
2-(3-Chloro-2-methylanilino)benzoic acid.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or slightly yellow, crystalline powder.

Solubility
Practically insoluble in water, soluble in dimethylformamide, sparingly soluble in anhydrous ethanol and in methylene chloride. It dissolves in dilute solutions of alkali hydroxides.

mp
About 213 °C.

IDENTIFICATION
First identification: B.
Second identification: A, C.

A. Dissolve 20 mg in a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol R* and dilute to 100 mL with the same mixture of solvents. Dilute 5.0 mL of the solution to 50 mL with a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol R*. Examined between 250 nm and 380 nm (*2.2.25*), the solution shows 2 absorption maxima, at 286 nm and 345 nm. The ratio of the absorbance measured at the maximum at 286 nm to that measured at the maximum at 345 nm is 1.2 to 1.4.

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *tolfenamic acid CRS.*

C. Thin-layer chromatography (*2.2.27*).
Test solution Dissolve 25 mg of the substance to be examined in a mixture of 1 volume of *methanol R* and 3 volumes of *methylene chloride R* and dilute to 10 mL with the same mixture of solvents.
Reference solution Dissolve 25 mg of *tolfenamic acid CRS* in a mixture of 1 volume of *methanol R* and 3 volumes of *methylene chloride R* and dilute to 10 mL with the same mixture of solvents.
Plate *TLC silica gel GF_{254} plate R.*
Mobile phase *glacial acetic acid R, dioxan R, toluene R* (1:25:90 *V/V/V*).
Application 10 µL.
Development Over 2/3 of the plate.
Drying In a current of warm air.
Detection Ultraviolet light at 254 nm.
Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in 5 mL of *anhydrous ethanol R* and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 25.0 mg of *2-chlorobenzoic acid R* (impurity A) and 25.0 mg of *3-chloro-2-methylaniline R* (impurity B) in 5 mL of *anhydrous ethanol R* and dilute to 50.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 50.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 10.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 100.0 mL with the mobile phase.

Column:

— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *glacial acetic acid R, water for chromatography R, anhydrous ethanol R* (0.2:35:65 *V/V/V*).

Flow rate 0.8 mL/min.

Detection Spectrophotometer at 232 nm.

Injection 20 µL.

Run time 3 times the retention time of tolfenamic acid.

Relative retention With reference to tolfenamic acid (retention time = about 15 min): impurity A = about 0.25; impurity B = about 0.34.

System suitability Reference solution (a):

— *resolution*: minimum 2.5 between the peaks due to impurity A and to impurity B.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *impurity B*: not more than half the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.05 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.01 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g with the aid of ultrasound in 100 mL of *anhydrous ethanol R*. Add 0.1 mL of *phenol red solution R* and titrate with *0.1 M sodium hydroxide*.

1 mL of *0.1 M sodium hydroxide* is equivalent to 26.17 mg of $C_{14}H_{12}ClNO_2$.

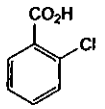
STORAGE

Protected from light.

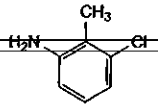
IMPURITIES

Specified impurities *A, B.*

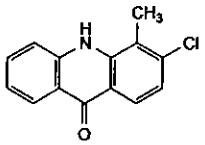
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C.*



A. 2-chlorobenzoic acid,



B. 3-chloro-2-methylaniline,

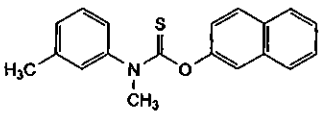


C. 3-chloro-4-methylacridin-9(10*H*)-one.

Ph Eur

Tolnaftate

(Ph. Eur. monograph 1158)



$C_{19}H_{17}NOS$ 307.4 *2398-96-1*

Action and use

Antifungal.

Ph Eur

DEFINITION

O-Naphthalen-2-yl methyl(3-methylphenyl)carbamothioate.

Content

97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish-white powder.

Solubility

Practically insoluble in water, freely soluble in acetone and in methylene chloride, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *tolnaftate CRS.*

TESTS

Impurity D

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.400 g of the substance to be examined in 2 mL of *methylene chloride R*. Extract with 3 quantities, each of 3 mL, of *0.01 M hydrochloric acid*. Combine the aqueous phases and dilute to 10.0 mL with *0.01 M hydrochloric acid*.

Reference solution (a) Dissolve 20.0 mg of *N-methyl-m-toluidine R* (impurity D) in 50.0 mL of *methylene chloride R*.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 100.0 mL with *methylene chloride R*. Take 2.0 mL of this solution and extract with 3 quantities, each of 3 mL, of *0.01 M hydrochloric acid*. Combine the aqueous phases and dilute to 10.0 mL with *0.01 M hydrochloric acid*.

Reference solution (c) Dissolve 10 mg of the substance to be examined in 25 mL of *methanol R*. Add 2 mL of this solution to 2 mL of reference solution (a) and dilute to 25 mL with *methanol R*.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A*: *trifluoroacetic acid R, methanol R, water R* (0.1:10:90 *V/V/V*);
- *mobile phase B*: *trifluoroacetic acid R, water R, methanol R* (0.1:10:90 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	70	30
3 - 8	70 → 0	30 → 100
8 - 20	0	100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 100 µL of the test solution and reference solution (b); 10 µL of reference solution (c).

Relative retention With reference to tolnaftate (retention time = about 15 min): impurity D = about 0.25.

System suitability:
- *resolution*: minimum 5.0 between the peaks due to impurity D and tolnaftate in the chromatogram obtained with reference solution (c);
- *symmetry factor*: maximum 1.9 for the peak due to impurity D in the chromatogram obtained with reference solution (b).

Limit:
- *impurity D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (20 ppm).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 25 mg of the substance to be examined in 5 mL of *methanol R* and dilute to 25.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *methanol R*. Dilute 1.0 mL of this solution to 10.0 mL with *methanol R*.

Reference solution (b) Dissolve 5 mg of *tolnaftate for system suitability CRS* (containing resolution component A) in 5.0 mL of *methanol R*.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A*: *trifluoroacetic acid R, water R, methanol R* (0.1:30:70 *V/V/V*);
- *mobile phase B*: *trifluoroacetic acid R, water R, methanol R* (0.1:10:90 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 12	100	0
12 - 30	100 → 0	0 → 100
30 - 33	0	100

Flow rate 1.0 mL/ min.

Detection Spectrophotometer at 254 nm.

Injection 10 µL.

Relative retention With reference to tolnaftate (retention time = about 18 min): resolution component A = about 0.7.

System suitability Reference solution (b):
- *resolution*: minimum 5.0 between the peaks due to resolution component A and tolnaftate.

Limits:
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying at 60 °C at a pressure not exceeding 0.7 kPa for 3 h.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 50.0 mg in *methanol R* and dilute to 250.0 mL with the same solvent. Dilute 2.0 mL of this solution to 50.0 mL with *methanol R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 257 nm.

Calculate the content of $C_{19}H_{17}NOS$ taking the specific absorbance to be 720.

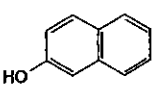
STORAGE

Protected from light.

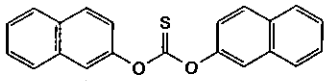
IMPURITIES

Specified impurities *D*.

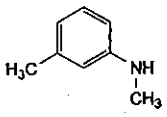
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B*.



A. naphthalen-2-ol (β-naphthol),



B. *O,O*-dinaphthalen-2-yl carbonothioate,

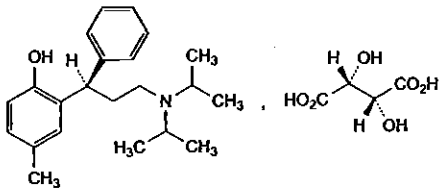


D. *N*,3-dimethylaniline (*N*-methyl-*m*-toluidine).

Ph Eur

Tolterodine Tartrate

(Ph. Eur. monograph 2781)



$C_{26}H_{37}NO_7$ 475.6 *124937-52-6*

Action and use
Anticholinergic.

Preparations
Tolterodine Prolonged-release Capsules
Tolterodine Tablets

Ph Eur

DEFINITION
2-[(1*R*)-3-[Bis(1-methylethyl)amino]-1-phenylpropyl]-4-methylphenol (2*R*,3*R*)-2,3-dihydroxybutanedioate.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Sparingly soluble in water, slightly soluble in anhydrous ethanol, practically insoluble in heptane.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *tolterodine tartrate CRS.*
B. Enantiomeric purity (see Tests).
C. It gives reaction (b) of tartrates (*2.3.1*).

TESTS

Enantiomeric purity
Liquid chromatography (*2.2.29*).
Solution A Dissolve 0.29 g of *sodium dihydrogen phosphate monohydrate R* and 0.47 g of *disodium hydrogen phosphate dihydrate R* in 1 L of *water R*. Dissolve 1.04 g of *tetrabutylammonium bromide R* in this solution.
Test solution Dissolve 4.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.
Reference solution (a) Dissolve 2 mg of *tolterodine for system suitability CRS* (containing impurity F) in the mobile phase and dilute to 50.0 mL with the mobile phase.
Reference solution (b) Dilute 0.5 mL of the test solution to 100.0 mL with the mobile phase.
Column:
— *size*: *l* = 0.10 m, Ø = 2 mm;
— *stationary phase*: *α1-acid-glycoprotein silica gel for chiral separation R* (5 µm).
Mobile phase *2-methylpropanol R*, solution A (7:93 *V/V*).
Flow rate 0.2 mL/min.
Detection Spectrophotometer at 220 nm.
Injection 20 µL.
Run time 1.5 times the retention time of tolterodine.
Relative retention With reference to tolterodine (retention time = about 23 min): impurity F = about 0.9.
System suitability Reference solution (a):
— *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to impurity F and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to tolterodine.
Calculation of percentage content:
— for impurity F, use the concentration of tolterodine tartrate in reference solution (b).
Limit:
— *impurity F*: maximum 0.6 per cent.

Related substances
Liquid chromatography (*2.2.29*).
Test solution Dissolve 25 mg of the substance to be examined in 10 mL of *methanol R1*, sonicate until dissolution is complete and dilute to 25.0 mL with *methanol R1*.
Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *methanol R1*. Dilute 1.0 mL of this solution to 10.0 mL with *methanol R1*.
Reference solution (b) Dissolve 2 mg of *tolterodine impurity E CRS* in *methanol R1* and dilute to 10.0 mL with *methanol R1*. Dilute 1.0 mL of the solution to 20.0 mL with *methanol R1*.
Reference solution (c) Dissolve 25 mg of the substance to be examined in 10 mL of *methanol R1*. Sonicate until dissolution is complete. Add 5 mL of reference solution (b) and dilute to 25.0 mL with *methanol R1*.
Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).
Mobile phase:
— *mobile phase A*: mix 5 volumes of *triethylamine R2* and 450 volumes of a 2.88 g/L solution of *ammonium dihydrogen phosphate R*; adjust to pH 5.9 with a 50 per cent *V/V* solution of *phosphoric acid R*; add 550 volumes of *methanol R1*;
— *mobile phase B*: *methanol R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 25	100	0
25 - 45	100 → 80	0 → 20

Flow rate 1.5 mL/min.
Detection Spectrophotometer at 220 nm.

Injection 20 µL of the test solution and reference solutions (a) and (c).

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peak due to impurity E.

Relative retention With reference to tolterodine (retention time = about 7 min): tartaric acid = about 0.2; impurity B = about 0.9.

System suitability Reference solution (c):
— *resolution*: minimum 1.5 between the peaks due to impurity E and tolterodine.

Calculation of percentage contents:
— for each impurity, use the concentration of tolterodine tartrate in reference solution (a).

Limits:
— ~~*unspecified impurities*: for each impurity, maximum~~ 0.10 per cent;
— *total*: maximum 0.3 per cent;
— *reporting threshold*: 0.05 per cent; disregard the peak due to tartaric acid.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

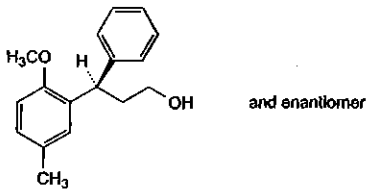
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.400 g in 30.0 mL of *anhydrous acetic acid R* and sonicate until dissolution is complete. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

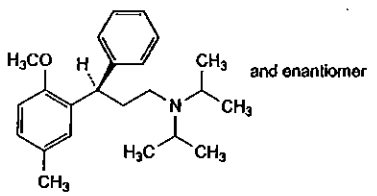
1 mL of *0.1 M perchloric acid* is equivalent to 47.56 mg of $C_{26}H_{37}NO_7$.

IMPURITIES
Specified impurities *F.*

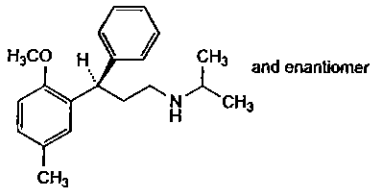
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, C, D, E, G.*



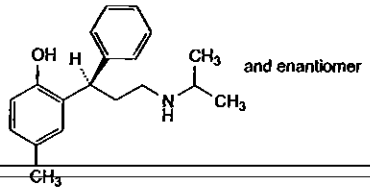
A. (3*RS*)-3-(2-methoxy-5-methylphenyl)-3-phenylpropan-1-ol,



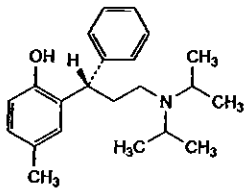
C. (3*RS*)-3-(2-methoxy-5-methylphenyl)-*N*,*N*-bis(1-methylethyl)-3-phenylpropan-1-amine,



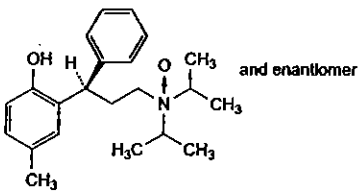
D. (3*RS*)-3-(2-methoxy-5-methylphenyl)-*N*-(1-methylethyl)-3-phenylpropan-1-amine,



E. 4-methyl-2-[(1*RS*)-3-[(1-methylethyl)amino]-1-phenylpropyl]phenol,



F. 2-[(1*S*)-3-[bis(1-methylethyl)amino]-1-phenylpropyl]-4-methylphenol,

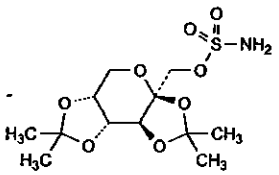


G. 2-[(1*R*)-3-[bis(1-methylethyl)amino]-1-phenylpropyl]-4-methylphenol *N*-oxide.

Ph Eur

Topiramate

(Ph. Eur. monograph 2616)



$C_{12}H_{21}NO_8S$ 339.4 *97240-79-4*

Action and use
Antiepileptic.

Ph Eur

DEFINITION
2,3:4,5-Bis-*O*-(propan-2,2-diyl)-β-D-fructopyranose 1-sulfamate.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Slightly soluble in water, freely soluble in methanol and in methylene chloride.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *topiramate CRS*.

TESTS

Specific optical rotation (*2.2.7*)
−32 to −29 (anhydrous substance).

Dissolve 0.200 g in *methanol R* and dilute to 50.0 mL with the same solvent.

Impurity A
Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 40.0 mg of the substance to be examined in 1.0 mL of *methanol R*.

Reference solution (a) Dissolve 10.0 mg of *topiramate impurity A CRS* in *methanol R* and dilute to 50.0 mL with the same solvent. Dilute 2.0 mL of the solution to 5.0 mL with *methanol R*.

Reference solution (b) Dissolve 40 mg of the substance to be examined in 1 mL of reference solution (a).

Plate *TLC silanised silica gel plate R*.

Pretreatment Wash the plate with *methanol R* and allow to dry in air.

Mobile phase *methanol R*, *acetonitrile R*, 29 g/L solution of *sodium chloride R* (15:35:50 *V/V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with a solution prepared by dissolving 3 g of *phenol R* in 95 mL of *ethanol (96 per cent) R* and carefully adding 5 mL of *sulfuric acid R*; allow to dry in air, then heat at 125 °C for 10 min. Use transmittance mode.

Retardation factors Topiramate = about 0.43; impurity A = about 0.55.

System suitability:
— the chromatogram obtained with reference solution (b) shows 2 clearly separated spots due to topiramate and impurity A.

Limit:
— *impurity A*: any spot due to impurity A is not more intense than the corresponding spot in the chromatogram obtained with reference solution (a) (0.2 per cent).

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R1*, mobile phase A (20:80 *V/V*).

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Test solution (b) Dilute 1.0 mL of test solution (a) to 20.0 mL with the solvent mixture. Dilute 5.0 mL of this solution to 50.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 15.0 mg of *topiramate impurity E CRS* in the solvent mixture and dilute to 50.0 mL with the solvent mixture. Dilute 5.0 mL of the solution to 200.0 mL with the solvent mixture.

Reference solution (c) Dissolve 5 mg of the substance to be examined in 1 mL of a mixture of 2 volumes of *acetonitrile R1*, 4 volumes of *water for chromatography R* and 4 volumes of *anhydrous formic acid R*. Incubate at 60 °C for 30 min (*in situ* preparation of impurity C). Dilute 20 µL of the solution to 1 mL with a 25 mg/L solution of *fructose R* (impurity E).

Reference solution (d) Dissolve 25.0 mg of *topiramate CRS* in 10.0 mL of the solvent mixture and dilute to 100.0 mL with the solvent mixture. Dilute 5.0 mL of the solution to 50.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.10 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped solid core pentafluorophenylpropylsilyl silica gel for chromatography R* (2.6 µm);
— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: 1.93 g/L solution of *ammonium acetate R* adjusted to pH 3.5 with *glacial acetic acid R*;
— *mobile phase B*: *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	80	20
5 - 15	80 → 50	20 → 50
15 - 15.1	50 → 80	50 → 20

Flow rate 1.0 mL/min.

Detection Charged aerosol detector:
— *range*: 100 pA;
— *filter*: none.

Injection 20 µL of test solution (a) and reference solutions (a), (b) and (c).

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity E.

Relative retention With reference to topiramate (retention time = about 5.6 min): impurity E = about 0.18; impurity C = about 0.25.

System suitability Reference solution (c):
— *resolution*: minimum 4.5 between the peaks due to impurities E and C.

Calculation of percentage contents:
— for impurity E, use the concentration of impurity E in reference solution (b) and the height of the peak due to impurity E;
— for impurities other than E, use the concentration of topiramate in reference solution (a).

Limits:
— *impurity E*: maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.2 per cent;
— *reporting threshold*: 0.05 per cent.

Water (*2.5.12*)
Maximum 0.5 per cent, determined on 1.000 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

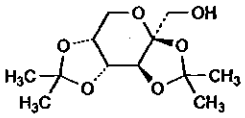
Injection Test solution (b) and reference solution (d).

Calculate the percentage content of $C_{12}H_{21}NO_8S$ taking into account the assigned content of *topiramate CRS*.

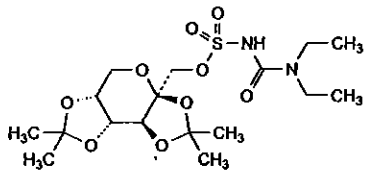
IMPURITIES

Specified impurities *A, E.*

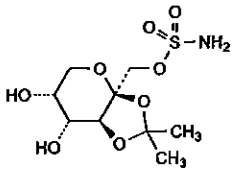
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D.*



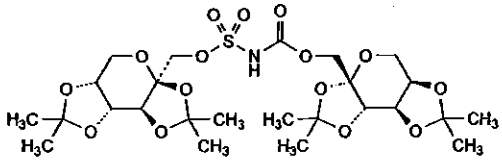
A. 2,3:4,5-bis-*O*-(propan-2,2-diyl)-β-D-fructopyranose,



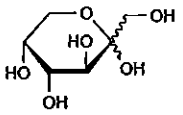
B. 2,3:4,5-bis-*O*-(propan-2,2-diyl)-β-D-fructopyranose 1-[*N*-(diethylcarbamoyl)sulfamate],



C. 2,3-*O*-(propan-2,2-diyl)-β-D-fructopyranose 1-sulfamate,



D. 2,3:4,5-bis-*O*-(propan-2,2-diyl)-β-D-fructopyranose 1-[*N*-[[[2,3:4,5-bis-*O*-(propan-2,2-diyl)-β-D-fructopyranosyl]oxy]carbamoyl]sulfamate],



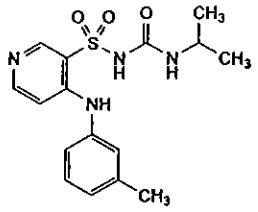
E. D-fructopyranose (fructose).

Ph Eur

Torasemide

Anhydrous Torasemide

(Ph. Eur. monograph 2132)



$C_{16}H_{20}N_4O_3S$ 348.4 *56211-40-6*

Action and use

Thiazide-like diuretic.

Ph Eur

DEFINITION

1-(1-Methylethyl)-3-[[4-[(3-methylphenyl)amino]pyridin-3-yl]sulfonyl]urea.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, slightly soluble in ethanol (96 per cent). It is sparingly soluble in dilute solutions of alkali hydroxides and slightly soluble in dilute acids.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *anhydrous torasemide CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Solution A Dissolve 2.7 g of *potassium dihydrogen phosphate R* in 950 mL of *water R*, adjust to pH 3.5 with *phosphoric acid R* and dilute to 1000 mL with *water R*.

Test solution Dissolve 20.0 mg of the substance to be examined in 15 mL of *methanol R* and sonicate for 15 min. Add 22.5 mL of solution A, cool to room temperature and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 2.0 mg of *torasemide for system suitability CRS* (containing impurities A, B, C and D) in 2.5 mL of *methanol R* and dilute to 5.0 mL with solution A.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (c) Dissolve the contents of a vial of *torasemide impurity E CRS* in 0.5 mL of *methanol R*. Add 0.5 mL of solution A.

Column:

— *size: l* = 0.125 m, Ø = 4.0 mm;

— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 30 °C.

Mobile phase *methanol R*, solution A (40:60 *V/V*).

Flow rate 0.8 mL/min.

Detection Spectrophotometer at 288 nm.

Injection 20 µL.

Run time 2.5 times the retention time of torasemide.

Identification of impurities Use the chromatogram supplied with *torasemide for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C and D; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity E.

Relative retention With reference to torasemide (retention time = about 10 min): impurity A = about 0.3; impurity B = about 0.4; impurity C = about 0.5; impurity E = about 0.7; impurity D = about 2.3.

System suitability:

— *resolution*: minimum 3.0 between the peaks due to impurities B and C in the chromatogram obtained with reference solution (a);

— *signal-to-noise ratio*: minimum 100 for the principal peak in the chromatogram obtained with reference solution (b).

Limits:

— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 5.1; impurity B = 0.76;

— *impurity B*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *impurities A, C, D, E*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.6 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

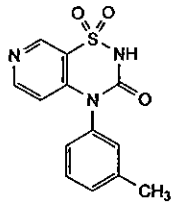
1 mL of *0.1 M perchloric acid* is equivalent to 34.84 mg of $C_{16}H_{20}N_4O_3S$.

STORAGE

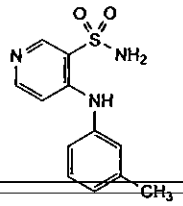
Protected from light.

IMPURITIES

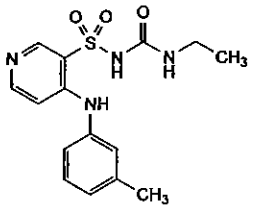
Specified impurities *A, B, C, D, E.*



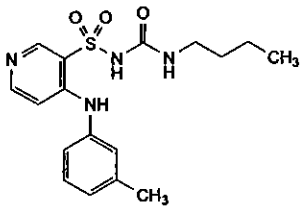
A. 4-(3-methylphenyl)-2*H*-pyrido[4,3-*e*]-1,2,4-thiadiazin-3(4*H*)-one 1,1-dioxide,



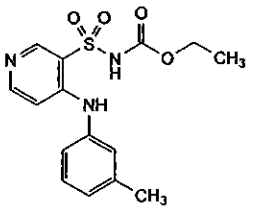
B. 4-[(3-methylphenyl)amino]pyridine-3-sulfonamide,



C. 1-ethyl-3-[[4-[(3-methylphenyl)amino]pyridin-3-yl]sulfonyl]urea,



D. 1-butyl-3-[[4-[(3-methylphenyl)amino]pyridin-3-yl]sulfonyl]urea,

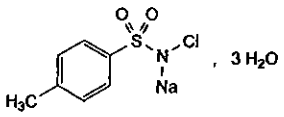


E. ethyl [[4-[(3-methylphenyl)amino]pyridin-3-yl]sulfonyl]carbamate.

Ph Eur

Tosylchloramide Sodium

(Ph. Eur. monograph 0381)



$C_7H_7ClNNaO_2S,3H_2O$ 281.7

Action and use
Antiseptic.

Ph Eur

DEFINITION
Sodium *N*-chloro-4-methylbenzenesulfonimidate trihydrate.

Content
98.0 per cent to 103.0 per cent of $C_7H_7ClNNaO_2S,3H_2O$.

CHARACTERS

Appearance
White or slightly yellow, crystalline powder.

Solubility
Freely soluble in water, soluble in ethanol (96 per cent).

IDENTIFICATION
A. Solution S (see Tests) turns *red litmus paper R* blue and then bleaches it.

B. To 10 mL of solution S add 10 mL of *dilute hydrogen peroxide solution R*. A white precipitate is formed which dissolves on heating. Filter the hot solution and allow to cool. White crystals are formed which, when washed and dried at 100-105 °C, melt (*2.2.14*) at 137 °C to 140 °C.

C. Ignite cautiously 1 g, because of the risk of deflagration. Dissolve the residue in 10 mL of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

D. The solution prepared for identification test C gives reaction (a) of sulfates (*2.3.1*).

E. The solution prepared for identification test C gives reaction (b) of sodium (*2.3.1*).

TESTS

Solution S
Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 mL with the same solvent.

Appearance of solution
Solution S is not more opalescent than reference suspension II (*2.2.1*) and is colourless (*2.2.2, Method II*).

pH (*2.2.3*)
8.0 to 10.0 for solution S.

Ortho compound
To 2 g add 10 mL of *water R*, mix, add 1 g of *sodium metabisulfite R* and heat to boiling. Cool to 0 °C, filter rapidly and wash with 3 quantities, each of 5 mL, of iced *water R*. The precipitate, dried *in vacuo* (*2.2.32*) at a pressure not exceeding 0.6 kPa, melts (*2.2.14*) at a minimum of 134 °C.

Residue insoluble in anhydrous ethanol
Maximum 2 per cent.

Shake 1.00 g with 20 mL of *anhydrous ethanol R* for 30 min, filter on a tared filter, wash any residue with 5 mL of *anhydrous ethanol R* and dry at 100-105 °C. The residue weighs a maximum of 20 mg.

ASSAY
Dissolve 0.125 g in 100 mL of *water R* in a ground-glass-stoppered flask. Add 1 g of *potassium iodide R* and 5 mL of *dilute sulfuric acid R*. Allow to stand for 3 min. Titrate with *0.1 M sodium thiosulfate*, using 1 mL of *starch solution R* as indicator.

1 mL of *0.1 M sodium thiosulfate* is equivalent to 14.08 mg of $C_7H_7ClNNaO_2S,3H_2O$.

STORAGE
In an airtight container, protected from light.

Ph Eur

Tragacanth

(Ph. Eur. monograph 0532)

When Powdered Tragacanth is prescribed or demanded, material complying with the requirements below with the exception of Identification test A shall be dispensed or supplied.

9000-65-1

Ph Eur

DEFINITION
Air-hardened, gummy exudate, flowing naturally or obtained by incision from the trunk and branches of *Astragalus gummifer* Labill. and certain other species of *Astragalus* from western Asia.

IDENTIFICATION
A. Tragacanth occurs in thin, flattened, ribbon-like, white or pale yellow, translucent strips, about 30 mm long and 10 mm wide and up to 1 mm thick, more or less curved, horny, with a short fracture; the surface is marked by fine longitudinal striae and concentric transverse ridges. It may also contain pieces similar in shape but somewhat thicker, more opaque and more difficult to fracture.

B. Microscopic examination (*2.8.23*). The powder is white or almost white and forms a mucilaginous gel with about 10 times its mass of *water R*. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R*. The powder shows in the gummy mass numerous stratified cellular membranes that turn slowly violet when treated with *iodinated zinc chloride solution R*. The gummy mass includes starch grains, isolated or in small groups, usually rounded in shape and sometimes deformed, with diameters varying between 4 µm and 10 µm, occasionally up to 20 µm, and a central hilum visible between crossed nicol prisms.

C. Examine the chromatograms obtained in the test for acacia.

Results The chromatogram obtained with the test solution shows 3 zones due to galactose, arabinose and xylose. A faint yellowish zone at the solvent front and a greyish-green zone between the zones due to galactose and arabinose may be present.

D. Moisten 0.5 g of the powdered herbal drug (355) (*2.9.12*) with 1 mL of *ethanol (96 per cent) R* and add gradually, while shaking, 50 mL of *water R* until a homogeneous mucilage is obtained. To 5 mL of the mucilage add 5 mL of *water R* and 2 mL of *barium hydroxide solution R*. A slight flocculent precipitate is formed. Heat on a water-bath for 10 min. An intense yellow colour develops.

TESTS

Acacia

Thin-layer chromatography (*2.2.27*).

Test solution To 100 mg of the powdered herbal drug (355) (*2.9.12*) in a thick-walled centrifuge test-tube, add 2 mL of a 100 g/L solution of *trifluoroacetic acid R*, shake vigorously to dissolve the forming gel, stopper the test-tube and heat the mixture at 120 °C for 1 h. Centrifuge the resulting hydrolysate, transfer the clear supernatant carefully into a 50 mL flask, add 10 mL of *water R* and evaporate the solution to dryness under reduced pressure. To the resulting clear film add 0.1 mL of *water R* and 0.9 mL of *methanol R*. Centrifuge to separate the amorphous precipitate, collect the supernatant and, if necessary, dilute to 1 mL with *methanol R*.

Reference solution Dissolve 10 mg of *arabinose R*, 10 mg of *galactose R*, 10 mg of *rhamnose R* and 10 mg of *xylose R* in 1 mL of *water R* and dilute to 10 mL with *methanol R*.

Plate *TLC silica gel plate R*.

Mobile phase 16 g/L solution of *sodium dihydrogen phosphate R*, *butanol R*, *acetone R* (10:40:50 *V/V/V*).

Application 10 µL as bands.

Development A Over a path of 10 cm.

Drying A In a current of warm air for a few minutes.

Development B Over a path of 15 cm using the same mobile phase.

Drying B At 110 °C for 10 min.

Detection Spray with *anisaldehyde solution R* and dry at 110 °C for 10 min.

Results The chromatogram obtained with the reference solution shows 4 clearly separated coloured zones due to galactose (greyish-green or green), arabinose (yellowish-green), xylose (greenish-grey or yellowish-grey) and rhamnose (yellowish-green), in order of increasing R_F value; the chromatogram obtained with the test solution does not show a yellowish-green zone corresponding to the zone of rhamnose in the chromatogram obtained with the reference solution.

Methylcellulose

Examine the chromatograms obtained in the test for acacia.

Results The chromatogram obtained with the test solution does not show a red zone near the solvent front.

Sterculia gum

A. Place 0.2 g of the powdered herbal drug (355) (*2.9.12*) in a 10 mL ground-glass-stoppered cylinder graduated in 0.1 mL. Add 10 mL of *ethanol (60 per cent V/V) R* and shake. Any gel formed occupies not more than 1.5 mL.

B. To 1.0 g of the powdered herbal drug (355) (*2.9.12*) add 100 mL of *water R* and shake. Add 0.1 mL of *methyl red solution R*. Not more than 5.0 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Foreign matter

Maximum 1.0 per cent.

Place 2.0 g of the powdered herbal drug (355) (*2.9.12*) in a 250 mL round-bottomed flask and add 95 mL of *methanol R*. Swirl to moisten the powder and add 60 mL of *hydrochloric acid R1*. Add a few glass beads about 4 mm in diameter and heat on a water-bath under a reflux condenser for 3 h, shaking occasionally. Remove the glass beads and filter the hot suspension *in vacuo* through a sintered-glass filter (160) (*2.1.2*). Rinse the flask with a small quantity of *water R* and pass the rinsings through the filter. Wash the residue on the filter with about 40 mL of *methanol R* and dry to constant mass at 110 °C (about 1 h). Allow to cool in a desiccator and weigh. The residue weighs a maximum of 20 mg.

Flow time

Minimum 10 s, or minimum 50 s if the substance to be examined is to be used for the preparation of emulsions.

Place 1.0 g of the powdered herbal drug (125-250) (*2.9.12*) in a 1000 mL round-bottomed flask with a ground-glass stopper, add 8.0 mL of *ethanol (96 per cent) R* and close the flask. Disperse the suspension over the inner surface of the flask by shaking, taking care not to wet the stopper. Open the flask and add as a single portion 72.0 mL of *water R*. Stopper the flask and shake vigorously for 3 min. Allow to stand for 24 h and shake vigorously again for 3 min. Eliminate air bubbles by applying vacuum above the mucilage for 5 min. Transfer the mucilage to a 50 mL cylinder. Dip in the mucilage a piece of glass tubing 200 mm long and 6.0 mm in internal diameter and graduated at 20 mm and 120 mm from the lower end; the tubing must not be rinsed with surface-active substances. When the mucilage has reached the upper mark, close the tube with a finger. Withdraw the closed tube, remove the finger and measure with a stop-watch the time needed for the meniscus to reach the lower graduation. Carry out this operation 4 times and determine the average value of the last 3 determinations.

Total ash (*2.4.16*)

Maximum 4.0 per cent.

Microbial contamination

TAMC: acceptance criterion 10^4 CFU/g (*2.6.12*).

TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

Absence of *Escherichia coli* (*2.6.13*).

Absence of *Salmonella* (*2.6.13*).

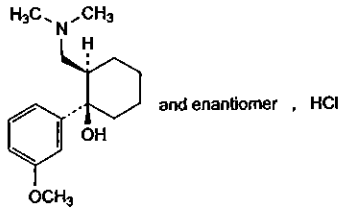
LABELLING

The label states whether or not the contents are suitable for preparing emulsions.

Ph Eur

Tramadol Hydrochloride

(Ph. Eur. monograph 1681)



$C_{16}H_{26}ClNO_2$ 299.8 *36282-47-0*

Action and use

µ-Opioid receptor (OP3, MOR) agonist and noradrenaline reuptake inhibitor; analgesic.

Preparations

Tramadol Capsules

Tramadol Prolonged-release Capsules

Tramadol Prolonged-release Tablets

Ph Eur

DEFINITION

(1*RS*,2*RS*)-2-[(Dimethylamino)methyl]-1-(3-methoxyphenyl) cyclohexan-1-ol hydrochloride.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water and in methanol, very slightly soluble in acetone.

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 180 °C to 184 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *tramadol hydrochloride CRS.*

C. Examine the chromatograms obtained in the test for impurity E.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S
Dissolve 1.0 g in *water R* and dilute to 20.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity
To 10 mL of solution S, add 0.2 mL of *methyl red solution R* and 0.2 mL of *0.01 M hydrochloric acid*. The solution is red. Not more than 0.4 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to yellow.

Optical rotation (*2.2.7*)
−0.10° to + 0.10°, determined on solution S.

Impurity E
Thin-layer chromatography (*2.2.27*).

Test solution (a) Dissolve 0.10 g of the substance to be examined in *methanol R* and dilute to 2.0 mL with the same solvent.

Test solution (b) Dilute 1 mL of test solution (a) to 10 mL with *methanol R*.

Reference solution (a) Dissolve 25 mg of *tramadol hydrochloride CRS* in *methanol R* and dilute to 5 mL with the same solvent.

Reference solution (b) Dissolve 5.0 mg of *tramadol impurity E CRS* in *methanol R* and dilute to 5.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with *methanol R*.

Reference solution (c) Dissolve 5 mg of *tramadol impurity A CRS* in 1 mL of reference solution (a).

Plate *TLC silica gel F_{254} plate R.*

Pretreatment Wash the plate with *methanol R*.

Mobile phase *concentrated ammonia R, 2-propanol R, toluene R* (1:19:80 *V/V/V*).

Application 10 µL.

Development Over 2/3 of the plate. Add *concentrated ammonia R* to one trough of a twin trough tank then saturate the plate for 20 min. Just before developing, add the mobile phase to the other trough. Place the plate in the trough, ensuring that the layer of silica gel faces the middle of the tank.

Drying In air.

Detection Expose to iodine vapour for 1 h, then examine in ultraviolet light at 254 nm.

System suitability The chromatogram obtained with reference solution (c) shows 2 clearly separated spots.

Limit Test solution (a):
— *impurity E*: any spot due to impurity E is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent).

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.15 g of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dilute 2.0 mL of the test solution to 10.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve 5 mg of *tramadol impurity A CRS* in 4 mL of the test solution and dilute to 100 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.0 mm;
— *stationary phase*: *base-deactivated end-capped octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase 295 volumes of *acetonitrile R* and 705 volumes of a mixture of 0.2 mL of *trifluoroacetic acid R* and 100 mL of *water for chromatography R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 270 nm.

Injection 20 µL.

Run time 4 times the retention time of tramadol.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity A.

Relative retention With reference to tramadol (retention time = about 6 min): impurity A = about 0.85.

System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the peaks due to impurity A and tramadol.

Calculation of percentage contents:
— for each impurity, use the concentration of tramadol hydrochloride in reference solution (a).

Limits:
— *impurity A*: maximum 0.2 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.4 per cent;
— *reporting threshold*: 0.02 per cent.

Water (*2.5.12*)
Maximum 0.5 per cent, determined on 1.000 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

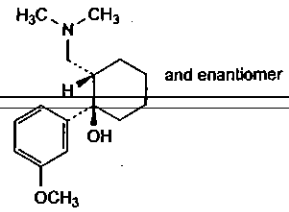
ASSAY

Dissolve 0.180 g in 50 mL of *ethanol (96 per cent) R*. Titrate with *0.1 M ethanolic sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

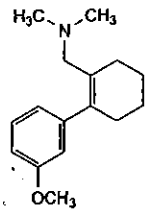
1 mL of *0.1 M ethanolic sodium hydroxide* is equivalent to 29.98 mg of $C_{16}H_{26}ClNO_2$.

STORAGE
Protected from light.

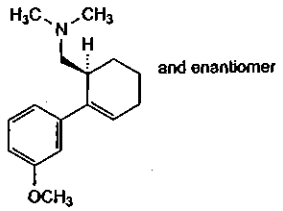
IMPURITIES
Specified impurities *A, E.*
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D.*



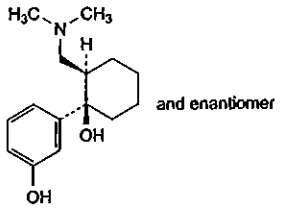
A. (1*RS*,2*SR*)-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexan-1-ol,



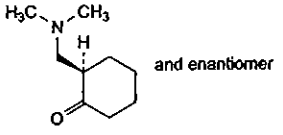
B. [2-(3-methoxyphenyl)cyclohex-1-en-1-yl]-*N*,*N*-dimethylmethanamine,



C. [(1*RS*)-2-(3-methoxyphenyl)cyclohex-2-en-1-yl]-*N*,*N*-dimethylmethanamine,



D. (1*RS*,2*RS*)-2-[(dimethylamino)methyl]-1-(3-hydroxyphenyl)cyclohexan-1-ol,

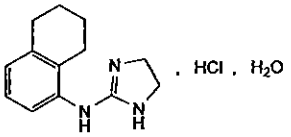


E. (2*RS*)-2-[(dimethylamino)methyl]cyclohexan-1-one.

Ph Eur

Tramazoline Hydrochloride Monohydrate

(Ph. Eur. monograph 1597)



$C_{13}H_{18}ClN_3,H_2O$ 269.8 *74195-73-6*

Action and use
Alpha-adrenoceptor agonist.

Ph Eur

DEFINITION
N-(5,6,7,8-Tetrahydronaphthalen-1-yl)-4,5-dihydro-1*H*-imidazol-2-amine hydrochloride monohydrate.

Content
98.5 per cent to 101.5 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Soluble in water and in ethanol (96 per cent).

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *tramazoline hydrochloride monohydrate CRS.*
B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S
Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

pH (*2.2.3*)
4.9 to 6.3 for solution S.

Related substances
Liquid chromatography (*2.2.29*).
Solvent mixture *acetonitrile R, water R* (50:50 *V/V*).
Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.
Reference solution (a) Dissolve 5.0 mg of *tramazoline impurity A CRS* and 5.0 mg of *tramazoline impurity B CRS* in 5 mL of the solvent mixture and add 5 mL of the test solution.
Reference solution (b) Dilute 0.2 mL of reference solution (a) to 100 mL with the solvent mixture.
Column:
— *size*: *l* = 0.125 m, Ø = 4 mm;
— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).
Mobile phase 2.0 g/L solution of *sodium dodecyl sulfate R* in a mixture of 6 volumes of *2-propanol R*, 42 volumes of *acetonitrile R1* and 52 volumes of *water for chromatography R*.
Flow rate 1.2 mL/min.
Detection Spectrophotometer at 215 nm.

Injection 5 µL.

Run time 3 times the retention time of tramazoline.

Relative retention With reference to tramazoline (retention time = about 6.5 min): impurity A = about 0.71; impurity B = about 0.86.

System suitability Reference solution (a):
- the chromatogram obtained shows 3 clearly separated peaks;
- *resolution*: minimum 1.5 between the peak due to impurity B and tramazoline.

Limits:
- *impurities A, B*: for each impurity, not more than 3 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) (0.10 per cent);
- *sum of impurities other than A and B*: not more than twice the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *disregard limit*: 0.2 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) (0.02 per cent).

Water (*2.5.12*)
6.2 per cent to 7.2 per cent, determined on 0.500 g.

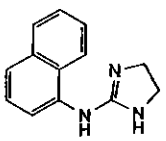
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 2.000 g in a mixture of 5 mL of *0.1 M hydrochloric acid* and 75 mL of *ethanol (96 per cent) R*. Carry out a potentiometric titration (*2.2.20*) using *1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

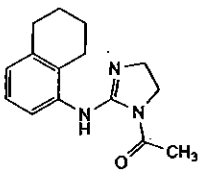
1 mL of *1 M sodium hydroxide* is equivalent to 251.8 mg of $C_{13}H_{18}ClN_3$.

IMPURITIES
Specified impurities *A, B.*

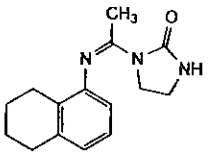
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) C.



A. *N*-(naphthalen-1-yl)-4,5-dihydro-1*H*-imidazol-2-amine,



B. 1-[2-[(5,6,7,8-tetrahydronaphthalen-1-yl)amino]-4,5-dihydro-1*H*-imidazol-1-yl]ethan-1-one,

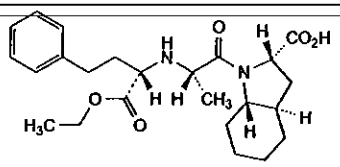


C. 1-[(1*Z*)-*N*-(5,6,7,8-tetrahydronaphthalen-1-yl) ethanimidoyl]imidazolidin-2-one.

Ph Eur

Trandolapril

(Ph. Eur. monograph 2245)



$C_{24}H_{34}N_2O_5$ 430.5 *87679-37-6*

Action and use
Angiotensin converting enzyme inhibitor.

Preparation
Trandolapril Capsules

Ph Eur

DEFINITION
(2*S*,3a*R*,7a*S*)-1-[(2*S*)-2-[[(2*S*)-1-Ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white powder.

Solubility
Practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in anhydrous ethanol.

IDENTIFICATION
A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *trandolapril CRS.*

TESTS
Appearance of solution
The solution is not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

Dissolve 1.0 g in *methanol R* and dilute to 10 mL with the same solvent.

Specific optical rotation (*2.2.7*)
−18.5 to −16.5 (anhydrous substance).

Dissolve 1.0 g in *anhydrous ethanol R* and dilute to 50.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 25 mg of the substance to be examined in mobile phase A and dilute to 10.0 mL with mobile phase A.

Reference solution (a) Dilute 1.0 mL of the test solution to 10.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 100.0 mL with mobile phase A.

Reference solution (b) Dissolve 5 mg of *trandolapril impurity C CRS* and 5 mg of *trandolapril impurity D CRS* in mobile phase A and dilute to 5 mL with mobile phase A. Dilute 1 mL of the solution to 20 mL with mobile phase A.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer R* (3.5 µm);
- *temperature*: 40 °C.

Mobile phase:
- *mobile phase A*: mix 25 volumes of *acetonitrile R1* and 75 volumes of a 6.8 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 2.5 ± 0.1 with *phosphoric acid R*;
- *mobile phase B*: mix equal volumes of *acetonitrile R1* and a 6.8 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 2.2 ± 0.1 with *phosphoric acid R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	95	5
20 - 35	95 → 5	5 → 95
35 - 45	5	95

Flow rate 1.3 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities C and D.

Relative retention With reference to trandolapril (retention time = about 14.5 min): impurity C = about 2.1; impurity D = about 2.5.

System suitability Reference solution (b):
- *resolution*: minimum 4 between the peaks due to impurities C and D.

Limits:
- *correction factor*: for the calculation of content, multiply the peak area of impurity C by 2.2;
- *impurity D*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *impurity C*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *sum of impurities other than D*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.32*)
Maximum 0.2 per cent, determined on 1.000 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 2.0 g in a porcelain or quartz crucible.

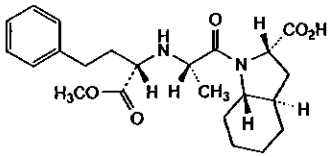
ASSAY
Dissolve 0.300 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 43.05 mg of $C_{24}H_{34}N_2O_5$.

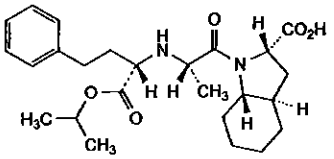
STORAGE
Protected from light.

IMPURITIES
Specified impurities *C, D.*

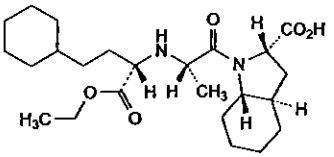
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, E, F.*



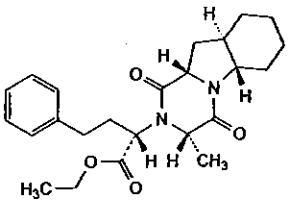
A. (2*S*,3a*R*,7a*S*)-1-[(2*S*)-2-[[(2*S*)-1-methoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid (methyl ester derivative),



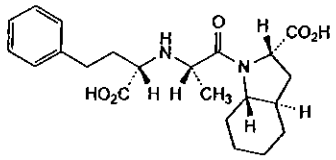
B. (2*S*,3a*R*,7a*S*)-1-[(2*S*)-2-[[(2*S*)-1-oxo-4-phenyl-1-[(propan-2-yl)oxy]butan-2-yl]amino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid (isopropyl ester derivative),



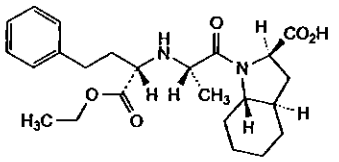
C. (2*S*,3a*R*,7a*S*)-1-[(2*S*)-2-[[(2*S*)-4-cyclohexyl-1-ethoxy-1-oxobutan-2-yl]amino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid (hexahydrotrandolapril),



D. ethyl (2*S*)-2-[(3*S*,5a*S*,9a*R*,10a*S*)-3-methyl-1,4-dioxodecahydropyrazino[1,2-*a*]indol-2(1*H*)-yl]-4-phenylbutanoate (trandolapril diketopiperazine),



E. (2*S*,3a*R*,7a*S*)-1-[(2*S*)-2-[[(1*S*)-1-carboxy-3-phenylpropyl]amino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid (trandolaprilate),

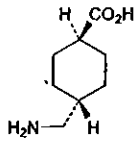


F. (2*R*,3a*R*,7a*S*)-1-[(2*S*)-2-[[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]octahydro-1*H*-indole-2-carboxylic acid.

Ph Eur

Tranexamic Acid

(Ph. Eur. monograph 0875)



$C_8H_{15}NO_2$ 157.2 *1197-18-8*

Action and use
Antifibrinolytic.

Preparations
Tranexamic Acid Injection
Tranexamic Acid Mouthwash
Tranexamic Acid Oral Solution
Tranexamic Acid Tablets

Ph Eur

DEFINITION

(1*r*,4*r*)-4-(Aminomethyl)cyclohexane-1-carboxylic acid.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water and in glacial acetic acid, practically insoluble in acetone and in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *tranexamic acid CRS.*

TESTS

pH (*2.2.3*)
7.0 to 8.0.

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.200 g of the substance to be examined in *water R* and dilute to 20.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *water R*. Dilute 1.0 mL of this solution to 20.0 mL with *water R*.

Reference solution (b) Dissolve 5.0 mg of *tranexamic acid impurity D CRS* in *water R* and dilute to 50.0 mL with the same solvent.

Reference solution (c) Dilute 5.0 mL of reference solution (b) to 100.0 mL with *water R*.

Reference solution (d) Dissolve 2.5 mg of *tranexamic acid impurity E CRS* in *water R* and dilute to 50.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with *water R*.

Reference solution (e) Dissolve 2.5 mg of *tranexamic acid impurity C CRS*, 2.5 mg of *tranexamic acid impurity F CRS* and 7.5 mg of *tranexamic acid impurity B CRS* in 25 mL of *water R*. Mix 1 mL of the solution, 1 mL of reference solution (b) and 18 mL of the test solution.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Dissolve 11.0 g of *anhydrous sodium dihydrogen phosphate R* in 500 mL of *water for chromatography R* and add 5 mL of *triethylamine R* and 1.4 g of *sodium laurilsulfate R1*; adjust to pH 2.0 with *phosphoric acid R* and dilute to 600 mL with *water for chromatography R*; add 400 mL of *methanol R2*.

Flow rate 0.9 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 40 µL of the test solution and reference solutions (a), (c), (d) and (e).

Run time 2.5 times the retention time of tranexamic acid.

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peak due to impurity D; use the chromatogram obtained with reference solution (d) to identify the peak due to impurity E; use the chromatogram obtained with reference solution (e) to identify the peaks due to impurities B, C and F.

Relative retention With reference to tranexamic acid (retention time = about 10 min): impurity F = about 0.3; impurity C = about 1.1; impurity D = about 1.2; impurity E = about 1.3; impurity B = about 1.5.

System suitability Reference solution (e):
— *resolution*: minimum 2.0 between the peaks due to tranexamic acid and impurity C; minimum 1.5 between the peaks due to impurities C and D.

Calculation of percentage contents:
— *correction factors*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 1.3; impurity C = 0.4; impurity F = 0.6;
— for impurities C and D, use the concentration of impurity D in reference solution (c);
— for impurities E and F, use the concentration of impurity E in reference solution (d);
— for impurities other than C, D, E and F, use the concentration of tranexamic acid in reference solution (a).

Limits:
— *impurity B*: maximum 0.15 per cent;

— *impurities C, D, E, F*: for each impurity, maximum 0.05 per cent;
— *unspecified impurities*: for each impurity, maximum 0.05 per cent;
— *total*: maximum 0.2 per cent;
— *reporting threshold*: 0.03 per cent.

Halides expressed as chlorides (*2.4.4*)
Maximum 140 ppm.

Dissolve 1.2 g in *water R* and dilute to 50 mL with the same solvent.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

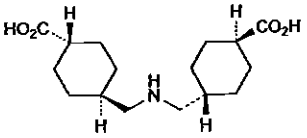
Dissolve 0.140 g in 20 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 15.72 mg of $C_8H_{15}NO_2$.

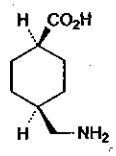
IMPURITIES

Specified impurities *B, C, D, E, F.*

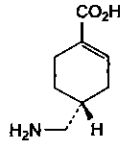
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A.*



A. (1*r*,4*r*,1′*r*,4′*r*)-4,4′-[azanediylbis(methylene)]di(cyclohexane-1-carboxylic acid),

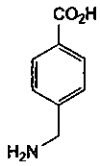


B. (1*s*,4*s*)-4-(aminomethyl)cyclohexane-1-carboxylic acid,

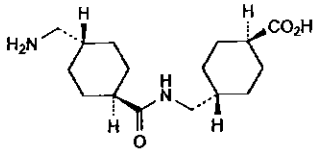


and enantiomer

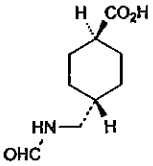
C. (4*RS*)-4-(aminomethyl)cyclohex-1-ene-1-carboxylic acid,



D. 4-(aminomethyl)benzoic acid,



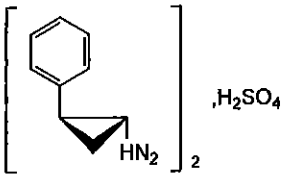
E. (1*r*,4*r*)-4-[[(1*r*,4*r*)-4-(aminomethyl)cyclohexane-1-carboxamido]methyl]cyclohexane-1-carboxylic acid,



F. (1*r*,4*r*)-4-(formamidomethyl)cyclohexane-1-carboxylic acid.

Ph Eur

Tranylcypromine Sulfate



and enantiomer

$(C_9H_{11}N)_2,H_2SO_4$ 364.5 *13492-01-8*

Action and use
Monoamine oxidase inhibitor; antidepressant.

Preparation
Tranylcypromine Tablets

DEFINITION
Tranylcypromine Sulfate is (1*RS*,2*SR*)-2-phenylcyclopropylamine sulfate. It contains not less than 98.0% and not more than 101.0% of $(C_9H_{11}N)_2,H_2SO_4$, calculated with reference to the dried substance.

CHARACTERISTICS
A white or almost white, crystalline powder.

Soluble in *water*; very slightly soluble in *ethanol (96%)* and in *ether*.

IDENTIFICATION
A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of tranylcypromine sulfate *(RS 345)*.

B. Yields the reactions characteristic of *sulfates*, Appendix VI.

TESTS

Related substances
Carry out the method for *gas chromatography*, Appendix III B, using the following solutions in solution A.

Dissolve 10 mg of *4-chloroaniline* (internal standard) in sufficient 0.1M *hydrochloric acid* to produce 20 mL (solution A).

(1) Add 5 mL of 1M *sodium hydroxide* to 1 mL of solution A, extract with 10 mL of *dichloromethane*, add 1 mL of *trifluoroacetic anhydride* to the dichloromethane extract and allow to stand for 10 minutes. Evaporate the solution at a

pressure of 2 kPa using a rotary evaporator and a water bath at 20° and dissolve the residue in 2 mL of *dichloromethane*.

(2) Dissolve 0.1 g of the substance being examined in 5 mL of *water*, add 1 mL of 5M *sodium hydroxide*, extract with 10 mL of *dichloromethane*, add 1 mL of *trifluoroacetic anhydride* to the dichloromethane extract and allow to stand for 10 minutes. Evaporate the solution at a pressure of 2 kPa using a rotary evaporator and a water bath at 20° and dissolve the residue in 2 mL of *dichloromethane*.

(3) Dissolve 0.1 g of the substance being examined in 5 mL of *water*, add 1 mL of solution A and 1 mL of 5M *sodium hydroxide*, extract with 10 mL of *dichloromethane*, add 1 mL of *trifluoroacetic anhydride* to the dichloromethane extract and allow to stand for 10 minutes. Evaporate the solution at a pressure of 2 kPa using a rotary evaporator and a water bath at 20° and dissolve the residue in 2 mL of *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a glass column (1.5 m × 4 mm) packed with *acid-washed, silanised diatomaceous support* (100 to 120 mesh) coated with 3% w/w of cyanopropylmethylphenyl methyl silicone fluid (OV-225 is suitable).

(b) Use *helium* as the carrier gas at 1.7 mL per minute.

(c) Use isothermal conditions maintained at 170°.

LIMITS

In the chromatogram obtained with solution (3), the area of any *secondary peak* is not greater than that of the peak due to the trifluoracetyl derivative of 4-chloroaniline (0.5%).

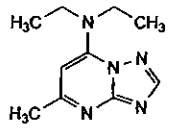
Loss on drying
When dried to constant weight at 105°, loses not more than 0.5% of its weight. Use 1 g.

Sulfated ash
Not more than 0.1%, Appendix IX A.

ASSAY
Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.3 g and determining the end point potentiometrically. Each mL of *0.1M perchloric acid VS* is equivalent to 36.45 mg of $(C_9H_{11}N)_2,H_2SO_4$.

Trapidil

(Ph. Eur. monograph 1576)



$C_{10}H_{15}N_5$ 205.3 *15421-84-8*

Action and use
Antiplatelet agent; vasodilator.

Ph Eur

DEFINITION
N,N-Diethyl-5-methyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water, soluble in ethanol and in methylene chloride.

mp
About 102 °C.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *trapidil CRS*.

TESTS
Solution S
Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity
To 10 mL of solution S add 0.2 mL of *methyl red solution R* and 0.2 mL of *0.01 M hydrochloric acid*. The solution is red. Add 0.4 mL of *0.01 M sodium hydroxide*. The solution is yellow.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dissolve 5.0 mg of *trapidil impurity A CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 50.0 mL with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *trapidil impurity B CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 50.0 mL with the mobile phase.

Reference solution (c) Mix equal volumes of reference solution (a) and reference solution (b).

Column:
— *size*: *l* = 0.125 m, Ø = 4.0 mm,
— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (5 µm),

Mobile phase 50 mL of *methanol R*, 75 mL of *acetonitrile R* and 800 mL of a 1.7 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 2.45 with *phosphoric acid R*; dilute to 1000 mL with *water R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 205 nm.

Injection 10 µL.

Run time 3 times the retention time of trapidil.

System suitability:
— *resolution*: minimum of 4.0 between the peaks due to impurity A and impurity B in the chromatogram obtained with reference solution (c).

Limits:
— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
— *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),
— *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent).
— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.01 per cent).

Chlorides (*2.4.4*)
Maximum 100 ppm.

Dissolve 0.25 g in 10 mL of *water R* and dilute to 15 mL with *water R*. Prepare the standard using 5 mL of *chloride standard solution (5 ppm Cl) R*.

Ammonium (*2.4.1*)
Maximum 20 ppm.

0.50 g complies with limit test A. Prepare the standard using 0.1 mL of *ammonium standard solution (100 ppm NH_4) R*.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 3 h.

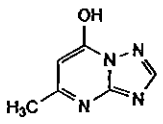
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.180 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

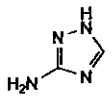
1 mL of *0.1 M perchloric acid* is equivalent to 20.53 mg of $C_{10}H_{15}N_5$.

STORAGE
Protected from light.

IMPURITIES



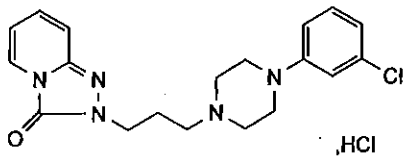
A. 5-methyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-ol,



B. 1,2,4-triazol-3-amine.

Ph Eur

Trazodone Hydrochloride



$C_{19}H_{22}ClN_5O,HCl$ 408.3 *25332-39-2*

Action and use
Monoamine reuptake inhibitor; antidepressant.

Preparations
Trazodone Capsules
Trazodone Tablets

DEFINITION
Trazodone Hydrochloride is 2,3-[4-(3-chloro) phenylpiperazin-1-yl]propyl-1,2,4-triazolo[4,3-*a*]pyridin-3 (2*H*)-one hydrochloride. It contains not less than 99.0% and not more than 101.0% of $C_{19}H_{22}ClN_5O,HCl$, calculated with reference to the dried substance.

PRODUCTION
The method of manufacture is such that the level of impurity F, 1-(3-chloropropyl)-3-chlorophenylpiperazine, is not more than 2.5 ppm when determined by a suitable method.

CHARACTERISTICS
A white or almost white, crystalline powder.

Soluble in *water*; sparingly soluble in *ethanol (96%)*; practically insoluble in *ether*.

IDENTIFICATION
A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of trazodone hydrochloride *(RS 346)*. In the preparation of the disc, avoid excessive grinding when triturating the substance being examined with *potassium chloride*.

B. Yields the reactions characteristic of *chlorides*, Appendix VI.

TESTS
Acidity
pH of a 1% w/v solution, 3.9 to 4.5, Appendix V L.

3-Chloroaniline
To 10 mL of a 1% w/v solution of the substance being examined in a mixture of equal volumes of *water* and *ethanol* add 2 mL of a freshly prepared 5% w/v solution of *4-dimethylaminobenzaldehyde* in *ethanol* and 0.1 mL of *hydrochloric acid*. Shake well and allow to stand for 5 minutes. Any yellow colour produced at 5 minutes from the preparation of the solution is not more intense than that produced by treating at the same time and in the same manner 10 mL of a 1 µg per mL solution of *3-chloroaniline* in a mixture of equal volumes of *water* and *ethanol* beginning at the words 'add 2 mL...' (100 ppm).

Related substances
Carry out the procedures protected from light. The combined nominal total content of impurities determined in tests A and B below is not more than 1.0% and no single unknown impurity is more than 0.1%.

A. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) 0.1% w/v of the substance being examined.

(2) 0.0001% w/v of the substance being examined.

(3) 0.00005% w/v of the substance being examined.

(4) 0.1% w/v of *trazodone hydrochloride impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters XTerra RP18 or Phenomenex Prodigy are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2.0 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for 3 times the retention time of the principal peak.

MOBILE PHASE

0.4 volumes of *diethylamine*, 350 volumes of *acetonitrile* and 650 volumes of *water*. If necessary, adjust the proportions of *acetonitrile* and *water* in the mobile phase to obtain a retention time of about 10 minutes for the principal peak.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to impurity C and trazodone is at least 2.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity D, identified from reference chromatogram A supplied with *trazodone hydrochloride impurity standard BPCRS*, is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* with a retention time of less than or equal to impurity E, identified from reference chromatogram A supplied with *trazodone hydrochloride impurity standard BPCRS*, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (3) (0.05%).

B. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) 0.1% w/v of the substance being examined.

(2) 0.0001% w/v of the substance being examined.

(3) 0.00005% w/v of the substance being examined.

(4) 0.1% w/v of *trazodone hydrochloride impurity standard BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Waters XTerra RP18 or Phenomenex Prodigy are suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.7 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

(g) For solution (1), allow the chromatography to proceed for at least 5 times the retention time of the principal peak.

MOBILE PHASE

0.4 volumes of *diethylamine*, 320 volumes of *water* and 680 volumes of *acetonitrile*. If necessary, adjust the proportions of *acetonitrile* and *water* in the mobile phase to obtain a retention time of about 2.5 minutes for the principal peak.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks due to trazodone and impurity E is at least 3.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* with a retention time longer than that of impurity E identified from reference chromatogram B, supplied with *trazodone hydrochloride impurity standard BPCRS*, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (3) (0.05%).

Loss on drying

When dried to constant weight at 105° at a pressure of 3.5 to 6.5 kPa, loses not more than 0.5% of its weight. Use 1 g.

Sulfated ash

Not more than 0.2%, Appendix IX A.

ASSAY

~~*Carry out the procedure protected from light.*~~

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in the mobile phase.

(1) 0.01% w/v of the substance being examined.

(2) 0.01% w/v of *trazodone hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Spherisorb C8 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 40°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

40 volumes of a 0.115% w/v solution of *diammonium hydrogen orthophosphate*, previously adjusted to pH 6.0 with 10% v/v *orthophosphoric acid* or 1M *sodium hydroxide*, and 60 volumes of *methanol*.

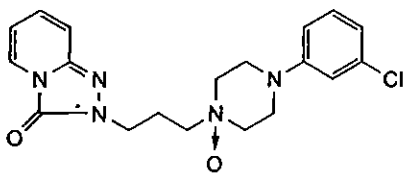
DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{22}ClN_5O$,HCl in the substance being examined using the declared content of $C_{19}H_{22}ClN_5O$, HCl in *trazodone hydrochloride BPCRS*.

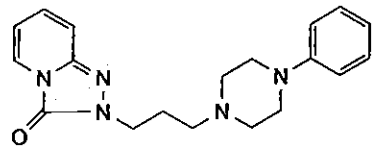
STORAGE

Trazodone Hydrochloride should be kept in an airtight container and protected from light.

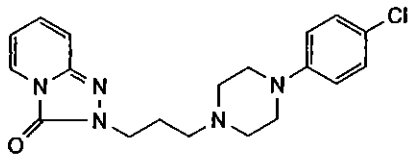
IMPURITIES



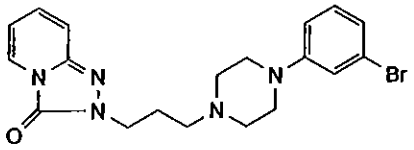
A. 4-(3-chlorophenyl)-1-[3-(3-oxo-2,3-dihydro-1,2,4-triazolo[4,3-*a*]pyridin-2-yl)propyl]piperazine N^1-oxide



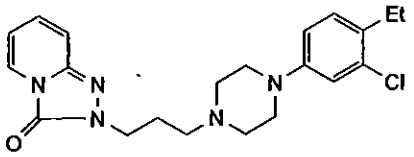
B. 2-[3-(4-phenylpiperazin-1-yl)propyl]-1,2,4-triazolo[4,3-*a*]pyridin-3(2*H*)-one



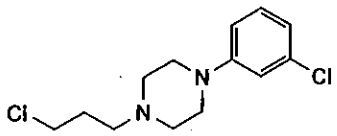
C. 2-3-[4-(4-chlorophenyl)piperazin-1-yl]propyl-1,2,4-triazolo[4,3-*a*]pyridin-3(2*H*)-one



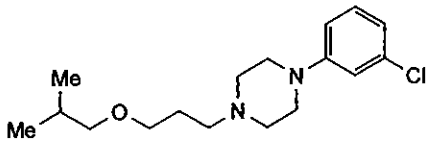
D. 2-3-[4-(3-bromophenyl)piperazin-1-yl]propyl-1,2,4-triazolo[4,3-*a*]pyridin-3(2*H*)-one



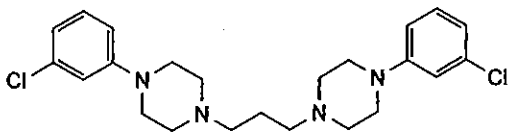
E. 2-3-[4-(3-chloro-4-ethylphenyl)piperazin-1-yl]propyl-1,2,4-triazolo[4,3-*a*]pyridin-3(2*H*)-one



F. 1-(3-chloropropyl)-3chlorophenylpiperazine



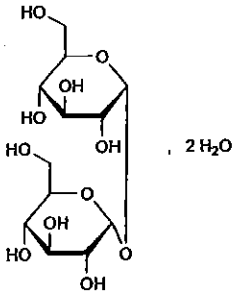
G. 3-[4-(3-chlorophenyl)piperazin-1-yl]propyl isobutyl ether



H. 1,3-bis-[4-(3-chlorophenyl)piperazin-1-yl]propane

Trehalose Dihydrate

(Ph. Eur. monograph 2297)



$C_{12}H_{22}O_{11},2H_2O$ 378.3 *6138-23-4*

Ph Eur

DEFINITION

α-D-Glucopyranosyl α-D-glucopyranoside dihydrate (α,α-trehalose dihydrate). It is obtained by enzymatic modification of starch.

Content
97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water, slightly soluble in methanol, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *trehalose dihydrate CRS.*

B. Dissolve 2 g in 5 mL of *water R*. To 1 mL of this solution add 0.4 mL of a 50 g/L solution of *α-naphthol R* in *ethanol (96 per cent) R* and mix thoroughly. Carefully add 2 mL of *sulfuric acid R*. A violet colour develops at the interface.

C. Dissolve 1 g in 25 mL of *water R*. To 2 mL of this solution add 1 mL of *dilute hydrochloric acid R* and mix. Keep the solution for 20 min at room temperature. Add 4 mL of a 40 g/L solution of *sodium hydroxide R* and 2 mL of a 40 g/L solution of *glycine R* and mix. Heat the solution in a water-bath for 10 min. No brown colour develops.

TESTS

Solution S
Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)
4.5 to 6.5 for solution S.

Specific optical rotation (*2.2.7*)
+ 197 to + 201 (anhydrous substance), determined on solution S.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in *water R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dissolve 0.100 g of *trehalose dihydrate CRS* in *water R* and dilute to 10.0 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *water R*.

Reference solution (c) Dilute 5.0 mL of reference solution (b) to 25.0 mL with *water R*.

Reference solution (d) Dissolve 25 mg of *glucose R* (impurity A) and 25 mg of *maltotriose R* in *water R*, add 2.5 mL of reference solution (a) and dilute to 10.0 mL with *water R*.

Column:
— *size*: l = 0.3 m, Ø = 8 mm;
— *stationary phase*: *strong cation-exchange resin (sodium form) R* (6 µm);
— *temperature*: 80 °C.

Mobile phase *water R*.

Flow rate 0.4 mL/min.

Detection Refractometer maintained at 40 °C.

Injection 20 µL of the test solution and reference solutions (b), (c) and (d).

Run time Twice the retention time of trehalose.

Identification of impurities Use the chromatogram obtained with reference solution (d) to identify the peaks due to impurities A and B; impurity B has the same retention time as maltotriose.

Relative retention With reference to trehalose (retention time = about 15 min): impurity B = about 0.9; impurity A = about 1.2.

System suitability Reference solution (d):
— *resolution*: minimum 1.5 between the peaks due to maltotriose and trehalose.

Limits:
— *impurities A, B*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);
— *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Chlorides (*2.4.4*)
Maximum 125 ppm.

Dilute 4 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 200 ppm.

Dilute 7.5 mL of solution S to 15 mL with *distilled water R*.

Soluble starch
Dissolve 1 g in 10 mL of *water R*. Add 0.1 mL of *iodine solution R1*. No blue colour develops.

Water (*2.5.12*)
9.0 per cent to 11.0 per cent, determined on 0.10 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

Microbial contamination
If intended for use in the manufacture of parenteral preparations:
— TAMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

If not intended for use in the manufacture of parenteral preparations:
— TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*);
— TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*);
— absence of *Escherichia coli* (*2.6.13*);
— absence of *Salmonella* (*2.6.13*).

Bacterial endotoxins (*2.6.14*)
If intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins:
— less than 4 IU/g for parenteral preparations having a concentration of 100 g/L or less of trehalose dihydrate;
— less than 2.5 IU/g for parenteral preparations having a concentration of more than 100 g/L of trehalose dihydrate.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

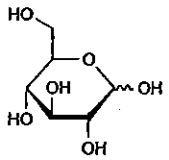
Calculate the percentage content of trehalose from the declared content of *trehalose dihydrate CRS*.

LABELLING

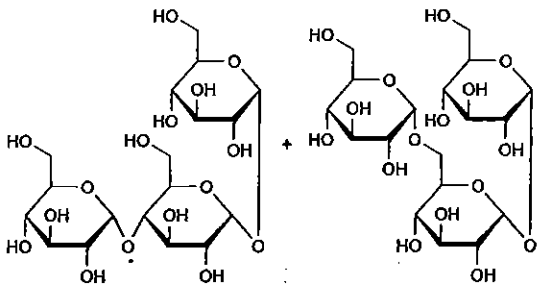
The label states:
— where applicable, the maximum concentration of bacterial endotoxins;
— where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

IMPURITIES

Specified impurities *A, B.*



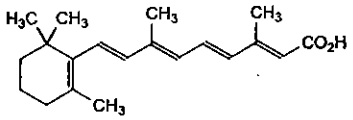
A. D-glucopyranose (glucose),



B. oligosaccharides, mainly glucosyltrehalose: mixture of α-D-glucopyranosyl α-D-glucopyranosyl-(1→4)-α-D-glucopyranoside (4-*O*-glucosyltrehalose or α-D-maltosyl α-D-glucoside) and α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl α-D-glucopyranoside (6-*O*-glucosyltrehalose or α-D-isomaltosyl α-D-glucoside).

Tretinoin

(Ph. Eur. monograph 0693)



$C_{20}H_{28}O_2$ 300.4 *302-79-4*

Action and use
Vitamin A analogue (retinoid); treatment of acne.

Preparations
Tretinoin Gel
Tretinoin Solution
Tretinoin, Hydrocortisone and Hydroquinone Cream

Ph Eur

DEFINITION

(2*E*,4*E*,6*E*,8*E*)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenoic acid.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
Yellow or light orange, crystalline powder.

Solubility
Practically insoluble in water, sparingly soluble in methylene chloride, slightly soluble in ethanol (96 per cent).

mp
About 182 °C, with decomposition.

It is sensitive to air, heat and light, especially in solution.

Carry out all operations as rapidly as possible and avoid exposure to actinic light; use freshly prepared solutions.

IDENTIFICATION

First identification: A.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *tretinoin CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *methylene chloride R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 10 mg of *tretinoin CRS* in *methylene chloride R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel GF_{254} plate R.*

Mobile phase *glacial acetic acid R, acetone R, peroxide-free ether R, cyclohexane R* (2:4:40:54 *V/V/V/V*).

Application 5 µL.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. Dissolve about 5 mg in 2 mL of *antimony trichloride solution R*. An intense red colour develops and later becomes violet.

TESTS

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 50.0 mL with the same solvent.

Reference solution (a) Dissolve 10.0 mg of *isotretinoin CRS* (impurity A) in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (b) Mix 1.0 mL of reference solution (a) and 0.5 mL of the test solution and dilute to 25.0 mL with *methanol R*.

Reference solution (c) Dilute 0.5 mL of the test solution to 100.0 mL with *methanol R*.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase *glacial acetic acid R, water R, methanol R* (5:225:770 *V/V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 355 nm.

Injection 10 µL.

Run time 1.2 times the retention time of tretinoin.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity A.

Relative retention With reference to tretinoin (retention time = about 29 min): impurity A = about 0.75.

System suitability Reference solution (b):
— *resolution*: minimum 5.0 between the peaks due to impurity A and tretinoin.

Limits:
— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);
— *unspecified impurities*: for each impurity, not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent);
— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for Pharmaceutical use (2034)* do not apply.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* for 16 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 70 mL of *acetone R*. Titrate with *0.1 M tetrabutylammonium hydroxide in 2-propanol*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M tetrabutylammonium hydroxide in 2-propanol* is equivalent to 30.04 mg of $C_{20}H_{28}O_2$.

STORAGE

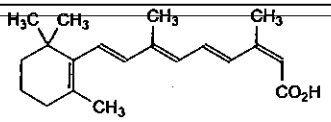
Under an inert gas, in an airtight container, protected from light.

It is recommended that the contents of an opened container be used as soon as possible and any unused part be protected by an atmosphere of inert gas.

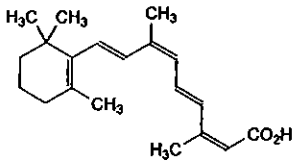
IMPURITIES

Specified impurities *A.*

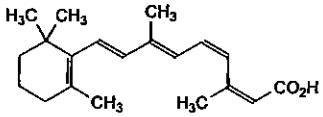
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D, F, G.*



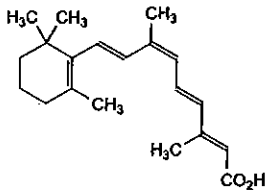
A. (2*Z*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenoic acid (isotretinoin),



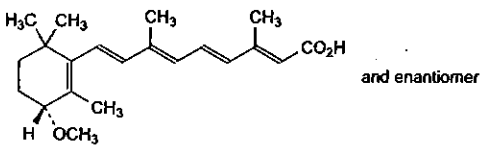
B. (2*Z*,4*E*,6*Z*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenoic acid (9,13-di-*cis*-retinoic acid),



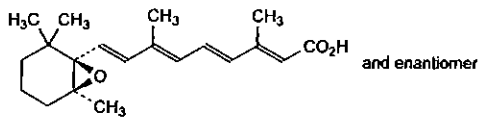
C. (2*Z*,4*Z*,6*E*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenoic acid (11,13-di-*cis*-retinoic acid),



D. (2*E*,4*E*,6*Z*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenoic acid (9-*cis*-retinoic acid),



F. (2*E*,4*E*,6*E*,8*E*)-9-[(3*RS*)-3-methoxy-2,6,6-trimethylcyclohex-1-enyl]-3,7-dimethylnona-2,4,6,8-tetraenoic acid (*rac*-4-methoxytretinoin),

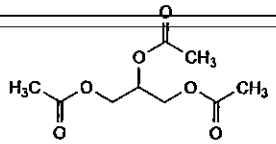


G. (2*E*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)nona-2,4,6,8-tetraenoic acid (*rac*-5,6-epoxytretinoin).

Ph Eur

Triacetin

(Ph. Eur. monograph 1106)



$C_9H_{14}O_6$ 218.2 *102-76-1*

Action and use

Antifungal.

Ph Eur

DEFINITION

Propane-1,2,3-triyl triacetate.

Content

97.0 per cent to 100.5 per cent (anhydrous substance).

CHARACTERS

Appearance

Clear, colourless, slightly viscous oily liquid.

Solubility

Soluble in water, miscible with ethanol (96 per cent) and toluene.

bp

About 260 °C.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of triacetin.*

TESTS

Appearance

It is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Acidity

Dissolve 5.00 g in 25 mL of *anhydrous ethanol R*, previously neutralised to 0.2 mL of *phenolphthalein solution R* and add 0.20 mL of *0.1 M sodium hydroxide*. The pink colour of the mixture persists for 15 s.

Relative density (*2.2.5*)

1.159 to 1.164.

Refractive index (*2.2.6*)

1.429 to 1.432.

Water (*2.5.12*)

Maximum 0.2 per cent, determined on 5.00 g.

ASSAY

Introduce 0.300 g into a 250 mL borosilicate glass flask fitted with a reflux condenser. Add 25.0 mL of 0.5 M alcoholic potassium hydroxide and a few glass beads. Attach

the condenser and heat under reflux for 30 min. Add 1 mL of *phenolphthalein solution R1* and titrate immediately with *0.5 M hydrochloric acid*. Carry out a blank test under the same conditions. Calculate the content from the difference in consumption of alkali in the main and the blank procedure.

1 mL of 0.5 M alcoholic potassium hydroxide is equivalent to 36.37 mg of $C_9H_{14}O_6$.

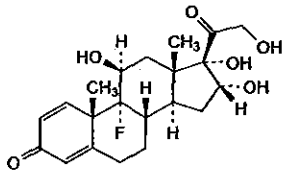
STORAGE

In a well-filled container.

Ph Eur

Triamcinolone

(Ph. Eur. monograph 1376)



$C_{21}H_{27}FO_6$ 394.4 *124-94-7*

Action and use

Glucocorticoid.

Preparation

Triamcinolone Tablets .

Ph Eur

DEFINITION

9-Fluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione.

Content

97.0 per cent to 103.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Practically insoluble in water, slightly soluble in methanol, practically insoluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *triamcinolone CRS*.

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness, dry the residues at 60 °C at a pressure not exceeding 0.7 kPa and record new spectra using the residues.

B. Thin-layer chromatography (*2.2.27*). *Prepare the solutions immediately before use and protect from light. Examine the plate under ultraviolet light immediately after development.*

Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dissolve 20 mg of *triamcinolone CRS* in *methanol R* and dilute to 20 mL with the same solvent.

Reference solution (b) Dissolve 10 mg of *dexamethasone CRS* in reference solution (a) and dilute to 10 mL with the same solution.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*.

Application 5 µL.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability The chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Specific optical rotation (*2.2.7*)

+ 65 to + 72 (anhydrous substance).

Dissolve 0.100 g in *dimethylformamide R* and dilute to 10.0 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use and protect from light.*

Test solution Dissolve 25.0 mg of the substance to be examined in a mixture of equal volumes of *methanol R* and *water R* and dilute to 10.0 mL with the same mixture of solvents.

Reference solution (a) Dissolve 2 mg of *triamcinolone CRS* and 2 mg of *triamcinolone impurity C CRS* in a mixture of equal volumes of *methanol R* and *water R* and dilute to 100.0 mL with the same mixture of solvents.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with a mixture of equal volumes of *methanol R* and *water R*.

Blank *methanol R*.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase A mixture prepared as follows: in a 1000 mL volumetric flask mix 525 mL of *methanol R* with 400 mL of *water R* and allow to equilibrate; adjust the volume to 1000 mL with *water R* and mix again.

Flow rate 1 mL/min.

Detection Spectrophotometer set at 238 nm.

Injection 20 µL.

Run time 4.5 times the retention time of triamcinolone.

Retention time Triamcinolone = about 11 min.

System suitability Reference solution (a):

— *resolution*: minimum of 1.8 between the peaks due to triamcinolone and to impurity C.

Limits:

— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent) and not more than 2 such peaks have an area greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),

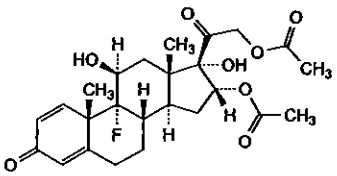
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent),
— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.12*)
Maximum 1.0 per cent, determined on 0.500 g.

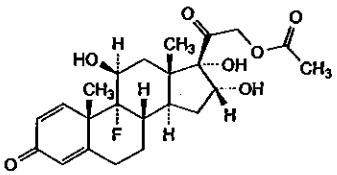
ASSAY
Prepare the solutions immediately before use and protect from light.
Dissolve 50.0 mg in *alcohol R* and dilute to 50.0 mL with the same solvent. Dilute 2.0 mL of the solution to 100.0 mL with *alcohol R*. Measure the absorbance (*2.2.25*) at the maximum at 238 nm.
Calculate the content of $C_{21}H_{27}FO_6$ taking the specific absorbance to be 389.

STORAGE
Protected from light.

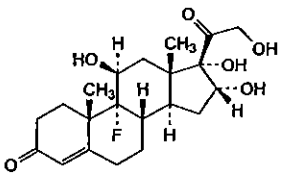
IMPURITIES



A. 9-fluoro-11β,17-dihydroxy-3,20-dioxopregna-1,4-diene-16α,21-diyl diacetate (triamcinolone 16,21-diacetate),



B. 9-fluoro-11β,16α,17-trihydroxy-3,20-dioxopregna-1,4-dien-21-yl acetate (triamcinolone 21-acetate),

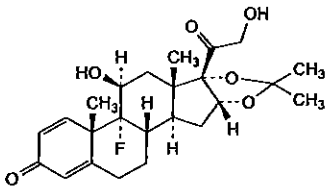


C. 9-fluoro-11β,16α,17,21-tetrahydroxypregn-4-ene-3,20-dione (pretriamcinolone).

Ph Eur

Triamcinolone Acetonide

(Ph. Eur. monograph 0533)



$C_{24}H_{31}FO_6$ 434.5 *76-25-5*

Action and use
Glucocorticoid.

Preparations
Triamcinolone Cream
Triamcinolone Acetonide Injection
Triamcinolone Acetonide Nasal Spray
Triamcinolone Ointment
Triamcinolone Dental Paste

Ph Eur

DEFINITION
9-Fluoro-11β,21-dihydroxy-16α,17-(1-methylethylidenedioxy)pregna-1,4-diene-3,20-dione.

Content
97.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, sparingly soluble in ethanol (96 per cent).
It shows polymorphism (*5.9*).

IDENTIFICATION
First identification: A, C.
Second identification: B, D.
A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *triamcinolone acetonide CRS.*
If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *methanol R* and evaporate to dryness. Using the residues, prepare halogen salt discs or mulls in *liquid paraffin R* and record new spectra.
B. Thin-layer chromatography (*2.2.27*). *Prepare the solutions immediately before use and protect from light.*
Test solution Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.
Reference solution (a) Dissolve 20 mg of *triamcinolone acetonide CRS* in *methanol R* and dilute to 20 mL with the same solvent.
Reference solution (b) Dissolve 10 mg of *triamcinolone hexacetonide CRS* in reference solution (a) and dilute to 10 mL with reference solution (a).
Plate *TLC silica gel F_{254} plate R.*
Mobile phase Add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R.*

Application 5 µL.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm, immediately after development.

System suitability Reference solution (b):

— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (c).

D. Mix about 5 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, add 1 mL of *water R*, 0.05 mL of *phenolphthalein solution R1* and about 1 mL of *dilute hydrochloric acid R* to render the solution colourless. Filter. To a freshly prepared mixture of 0.1 mL of *alizarin S solution R* and 0.1 mL of *zirconyl nitrate solution R*, add 1.0 mL of the filtrate. Mix, allow to stand for 5 min and compare the colour of the solution to that of a blank prepared in the same manner. The test solution is yellow and the blank is red.

TESTS

Specific optical rotation (*2.2.7*)

+ 110 to + 117 (anhydrous substance).

Dissolve 0.100 g in *ethanol (96 per cent) R* and dilute to 20.0 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Test solution Dissolve 25.0 mg of the substance to be examined in mobile phase B and dilute to 25.0 mL with mobile phase B.

Reference solution (a) Dissolve 5 mg of *triamcinolone acetonide for system suitability CRS* (containing impurities B and C) in mobile phase B and dilute to 5.0 mL with mobile phase B.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase B. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase B.

Reference solution (c) Dissolve 25.0 mg of *triamcinolone acetonide CRS* in mobile phase B and dilute to 25.0 mL with mobile phase B.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A*: *acetonitrile R, water for chromatography R* (32:68 *V/V*);

— *mobile phase B*: *water for chromatography R, acetonitrile R* (35:65 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	100	0
20 - 40	100 → 0	0 → 100

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL of the test solution and reference solutions (a) and (b).

Identification of impurities Use the chromatogram supplied with *triamcinolone acetonide for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities B and C.

Relative retention With reference to triamcinolone acetonide (retention time = about 16 min): impurity C = about 0.7; impurity B = about 0.8.

System suitability Reference solution (a):

— *resolution*: minimum 2.5 between the peaks due to impurities C and B.

Limits:

— *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *impurity C*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.12*)

Maximum 2.0 per cent, determined on 0.500 g.

ASSAY

Carry out the assay protected from light.

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Mobile phase Mobile phase A.

Injection Test solution and reference solution (c).

Run time 1.5 times the retention time of triamcinolone acetonide.

Retention time Triamcinolone acetonide = about 16 min.

Calculate the percentage content of $C_{24}H_{31}FO_6$ taking into account the assigned content of *triamcinolone acetonide CRS*.

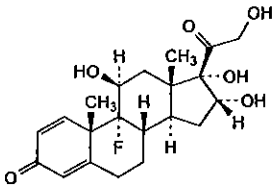
STORAGE

Protected from light.

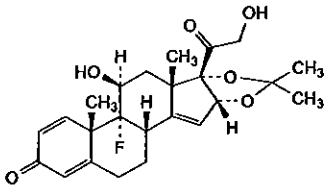
IMPURITIES

Specified impurities *B, C.*

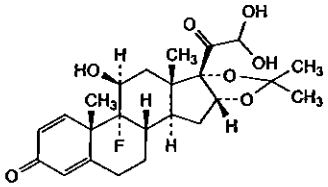
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, D, E, F.*



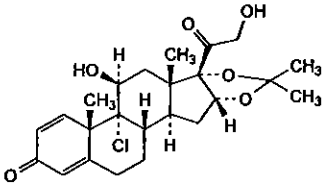
A. 9-fluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione (triamcinolone),



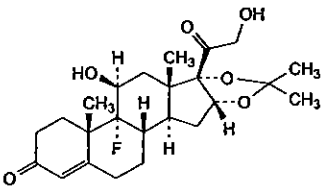
B. 9-fluoro-11β,21-dihydroxy-16α,17-(1-methylethylidenedioxy)pregna-1,4,14-triene-3,20-dione (Δ14-triamcinolone acetonide),



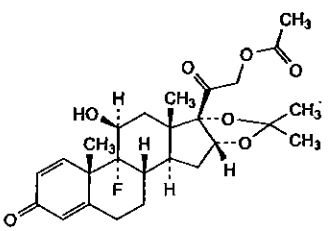
C. 9-fluoro-11β,21,21-trihydroxy-16α,17-(1-methylethylidenedioxy)pregna-1,4-diene-3,20-dione (triamcinolone acetonide 21-aldehyde hydrate),



D. 9-chloro-11β,21-dihydroxy-16α,17-(1-methylethylidenedioxy)pregna-1,4-diene-3,20-dione (9α-chloro triamcinolone acetonide),



E. 9-fluoro-11β,21-dihydroxy-16α,17-(1-methylethylidenedioxy)pregna-4-ene-3,20-dione (1,2-dihydrotriamcinolone acetonide),

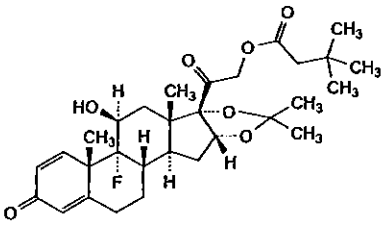


F. 9-fluoro-11β-hydroxy-16α,17-(1-methylethylidenedioxy)-3,20-dioxopregna-1,4-dien-21-yl acetate (21-acetate triamcinolone acetonide).

Ph Eur

Triamcinolone Hexacetonide

(Ph. Eur. monograph 0867)



$C_{30}H_{41}FO_7$ 532.6 *5611-51-8*

Action and use
Glucocorticoid.

Preparation
Triamcinolone Hexacetonide Injection

Ph Eur

DEFINITION

9-Fluoro-11β-hydroxy-2′,2′-dimethyl-3,20-dioxo-(16β*H*)-[1,3]dioxolo[4′,5′:16,17]pregna-1,4-dien-21-yl 3,3-dimethylbutanoate.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, sparingly soluble in anhydrous ethanol and in methanol, practically insoluble in heptane.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *triamcinolone hexacetonide CRS.*

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (c).

TESTS

Specific optical rotation (*2.2.7*)
+ 95 to + 101 (anhydrous substance).

Dissolve 0.100 g in *methylene chloride R* and dilute to 10.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Test solution (a) Dissolve 25.0 mg of the substance to be examined in *methanol R* and dilute to 10.0 mL with the same solvent.

Test solution (b) Dilute 1.0 mL of test solution (a) to 5.0 mL with *methanol R.*

Reference solution (a) Dissolve 5 mg of *triamcinolone hexacetonide for system suitability CRS* (containing impurities B and C) in *methanol R* and dilute to 2.0 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of test solution (a) to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (c) Dissolve 25.0 mg of *triamcinolone hexacetonide CRS* in *methanol R* and dilute to 10.0 mL with the same solvent. Dilute 1.0 mL of the solution to 5.0 mL with *methanol R*.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *water for chromatography R, methanol R* (25:75 *V/V*).

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL of test solution (a) and reference solutions (a) and (b).

Run time Twice the retention time of triamcinolone hexacetonide.

Identification of impurities Use the chromatogram supplied with *triamcinolone hexacetonide for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities B and C.

Relative retention With reference to triamcinolone hexacetonide (retention time = about 8 min): impurity B (epimer 1) = about 0.79; impurity B (epimer 2) = about 0.81; impurity C = about 1.3.

System suitability Reference solution (a):
— *resolution*: minimum 4.0 between the peaks due to triamcinolone hexacetonide and impurity C.

Calculation of percentage contents:
— for each impurity, use the concentration of triamcinolone hexacetonide in reference solution (b).

Limits:
— *impurity B*: for the sum of the areas of the 2 epimer peaks, maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.3 per cent;
— *reporting threshold*: 0.05 per cent.

Water (*2.5.12*)
Maximum 2.0 per cent, determined on 0.50 g.

ASSAY

Carry out the test protected from light.

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection 10 µL of test solution (b) and reference solution (c).

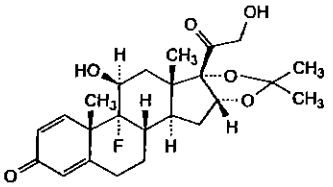
Calculate the percentage content of $C_{30}H_{41}FO_7$ taking into account the assigned content of *triamcinolone hexacetonide CRS*.

STORAGE
Protected from light.

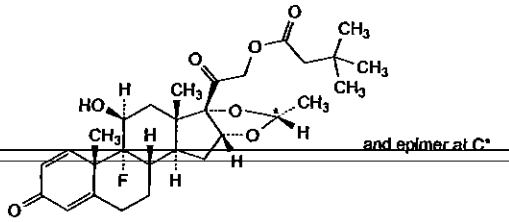
IMPURITIES

Specified impurities *B.*

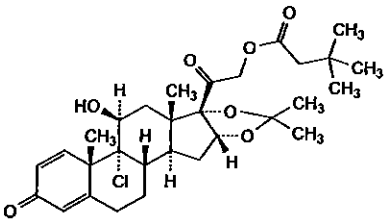
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, C.*



A. 9-fluoro-11β,21-dihydroxy-2′,2′-dimethyl-(16β*H*)-[1,3]dioxolo[4′,5′:16,17]pregna-1,4-diene-3,20-dione (triamcinolone acetonide),



B. (2′*RS*)-9-fluoro-11β-hydroxy-2′-methyl-3,20-dioxo-(16β*H*)-[1,3]dioxolo[4′,5′:16,17]pregna-1,4-dien-21-yl 3,3-dimethylbutanoate,

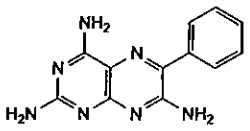


C. 9-chloro-11β-hydroxy-2′,2′-dimethyl-3,20-dioxo-(16β*H*)-[1,3]dioxolo[4′,5′:16,17]pregna-1,4-dien-21-yl 3,3-dimethylbutanoate.

Ph Eur

Triamterene

(Ph. Eur. monograph 0058)



$C_{12}H_{11}N_7$ 253.3 *396-01-0*

Action and use
Sodium channel blocker; potassium-sparing diuretic.

Preparations
Co-triamterzide Tablets
Triamterene Capsules

Ph Eur

DEFINITION
6-Phenylpteridine-2,4,7-triamine.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
Yellow, crystalline powder.

Solubility
Very slightly soluble in water and in ethanol (96 per cent).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *triamterene CRS.*

TESTS

Acidity
Boil 1.0 g with 20 mL of *water R* for 5 min, cool, filter and wash the filter with 3 quantities, each of 10 mL, of *water R*. Combine the filtrate and washings and add 0.3 mL of *phenolphthalein solution R*. Not more than 1.5 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Impurity D
Gas chromatography (*2.2.28*).

Internal standard solution Dilute 0.1 mL of *nitrobenzene R* to 100 mL with *methanol R*. Dilute 1 mL of this solution to 50 mL with *methanol R*.

Test solution Introduce 0.800 g of the substance to be examined into a suitable vial, add 5 mL of *dimethyl sulfoxide R* and heat until the sample is dissolved (do not heat to boiling). Allow to cool. Add 5 mL of cold *methanol R* to enhance the precipitation of triamterene. Filter and wash the filter with 5 mL of *methanol R*. Combine the filtrate and washing, add 2.0 mL of the internal standard solution and dilute to 20.0 mL with *methanol R*.

Reference solution Dissolve 20.0 mg of *benzyl cyanide R* (impurity D) in *methanol R* and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of the solution to 50.0 mL with *methanol R*. To 2.0 mL of this solution add 2.0 mL of the internal standard solution and 5 mL of *dimethyl sulfoxide R* and dilute to 20.0 mL with *methanol R*.

Blank solution Dilute 5 mL of *dimethyl sulfoxide R* to 20 mL with *methanol R*.

Column:
— *material*: fused silica;
— *size*: *l* = 30 m, Ø = 0.25 mm;
— *stationary phase*: *macrogol 20 000 R* (0.5 µm).

Carrier gas *helium for chromatography R.*

Flow rate 1.5 mL/min.

Split ratio 1:15.

Temperature:
— *column*: 170 °C;
— *injection port*: 210 °C;
— *detector*: 230 °C.

Detection Flame ionisation.

Injection 1 µL.

Run time Twice the retention time of the internal standard.

Relative retention With reference to the internal standard (retention time = about 6 min): impurity D = about 1.6.

System suitability Reference solution:
— *resolution*: minimum 2.0 between the peak due to impurity D and the nearest peak due to the solvent (blank solution);
— *signal-to-noise ratio*: minimum 10 for the peak due to impurity D.

Limit:
— *impurity D*: calculate the ratio (*R*) of the area of the peak due to impurity D to the area of the peak due to the internal standard from the chromatogram obtained with the reference solution; from the chromatogram obtained with the test solution, calculate the ratio of the area of the peak due to impurity D to the area of the peak due to the internal standard: this ratio is not greater than *R* (50 ppm).

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 10.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *nitrosotriaminopyrimidine CRS* (impurity A) in the mobile phase and dilute to 100.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (c) Dissolve the contents of a vial of *triamterene impurity B CRS* in 200 µL of *dimethyl sulfoxide R*. Add 5.0 mL of the test solution and dilute to 50.0 mL with the mobile phase. Filter the solution through a membrane filter (nominal pore size 0.45 µm) before injection.

Column:
— *size*: *l* = 0.25 m, Ø = 4.0 mm;
— *stationary phase*: spherical *end-capped octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *butylamine R, acetonitrile R, methanol R, water R* (2:200:200:600 *V/V/V/V*), adjusted to pH 5.3 with *acetic acid R*.

Flow rate 1 mL/min.

Detection Spectrophotometer at 320 nm and at 355 nm.

Injection 50 µL.

Relative retention With reference to triamterene (retention time = about 5 min): impurity A = about 0.6; impurity B = about 0.8; impurity C = about 1.7.

System suitability:
— *resolution*: minimum 1.5 between the peaks due to impurity B and triamterene in the chromatogram obtained with reference solution (c) at 355 nm; if necessary, increase the quantity of *water R* in the mobile phase;
— *signal-to-noise ratio*: minimum 10 for the principal peak in the chromatogram obtained with reference solution (b) at 320 nm.

Limits:
— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 1.8; impurity C = 1.5;
— *impurity A at 320 nm*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (50 ppm);
— *impurities B, C at 355 nm*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *unspecified impurities at 355 nm*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total at 355 nm*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *disregard limit at 355 nm*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

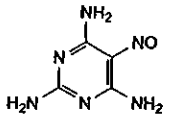
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.150 g in 5 mL of *anhydrous formic acid R* and add 100 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

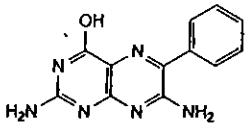
1 mL of *0.1 M perchloric acid* is equivalent to 25.33 mg of $C_{12}H_{11}N_7$.

STORAGE
Protected from light.

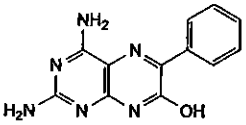
IMPURITIES
Specified impurities *A, B, C, D.*



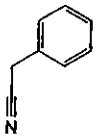
A. 5-nitrosopyrimidine-2,4,6-triamine (nitrosotriaminopyrimidine),



B. 2,7-diamino-6-phenylpteridin-4-ol,



C. 2,4-diamino-6-phenylpteridin-7-ol,

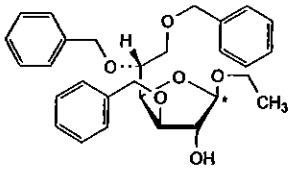


D. phenylacetonitrile (benzyl cyanide).

Ph Eur

Tribenoside

(Ph. Eur. monograph 1740)



and anomer at C*

$C_{29}H_{34}O_6$ 478.6 *10310-32-4*

Action and use
Sclerosing agent.

Ph Eur

DEFINITION
Mixture of α- and β-anomers of ethyl 3,5,6-tri-*O*-benzyl-D-glucofuranoside.

Content
96.0 per cent to 102.0 per cent.

CHARACTERS
Appearance
Yellowish or pale yellow, clear, viscous liquid.

Solubility
Practically insoluble in water, very soluble in acetone, in methanol and in methylene chloride.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *tribenoside CRS.*

TESTS
Solution S
Dissolve 4.00 g in *methanol R* and dilute to 20.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and its absorbance (*2.2.25*) at 420 nm has a maximum of 0.10.

Specific optical rotation (*2.2.7*)
−40.0 to −31.0.

Dilute 2.0 mL of solution S to 20.0 mL with *methanol R*.

Related substances
Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 1.000 g of the substance to be examined in a mixture of 5 volumes of *water R* and 95 volumes of *acetonitrile R* and dilute to 25.0 mL with the same mixture of solvents.

Test solution (b) Dissolve 50.0 mg of the substance to be examined in a mixture of 5 volumes of *water R* and 95 volumes of *acetonitrile R* and dilute to 50.0 mL with the same mixture of solvents.

Reference solution (a) Dilute 25.0 mg of *benzaldehyde CRS* (impurity C) and 30.0 mg of *tribenoside impurity A CRS* to 100.0 mL with *acetonitrile R*. Introduce 20.0 mL of this solution into a 50 mL volumetric flask, add 2.5 mL of *water R* and dilute to 50.0 mL with *acetonitrile R*.

Reference solution (b) Dissolve 50.0 mg of *tribenoside CRS* in a mixture of 5 volumes of *water R* and 95 volumes of *acetonitrile R* and dilute to 50.0 mL with the same mixture of solvents.

Reference solution (c) Dissolve 12.0 mg of *tribenoside impurity D CRS* in a mixture of 5 volumes of *water R* and

95 volumes of *acetonitrile R* and dilute to 100.0 mL with the same mixture of solvents.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:
— *mobile phase A*: 0.1 per cent *V/V* solution of *phosphoric acid R*;
— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 40	55 → 10	45 → 90
40 - 55	10	90

Flow rate 1.3 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL of test solution (a) and reference solutions (a), (b) and (c).

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A and C; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity D.

Relative retentions With reference to the β-anomer of tribenoside (retention time = about 18 min): α-anomer = about 1.1; impurity C = about 0.2; impurity B = about 0.6; impurity D = about 0.8; impurity A = about 1.4.

System suitability Reference solution (b):
— *resolution*: minimum 3.0 between the peaks due to the α-anomer and to the β-anomer of tribenoside.

Limits:
— *impurity A*: not more than 1.7 times the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
— *impurity C*: not more than twice the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.5 per cent); if the area of the peak due to impurity C in the chromatogram obtained with the test solution is greater than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.25 per cent), dilute the test solution to obtain an area equal to or smaller than the area of the peak in the chromatogram obtained with reference solution (a); calculate the content of impurity C taking into account the dilution factor;
— *impurity D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent);
— *any other impurity*: not more than the area of the peak due to impurity A in the chromatogram obtained with reference solution (a) (0.3 per cent);
— *total*: maximum 2.0 per cent;
— *disregard limit*: 0.17 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (a) (0.05 per cent).

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

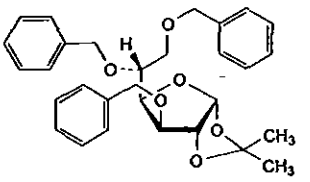
Injection Test solution (b) and reference solution (b).

Calculate the sum of the percentage contents of the α-anomer and the β-anomer of tribenoside taking into account the assigned content of *tribenoside CRS*.

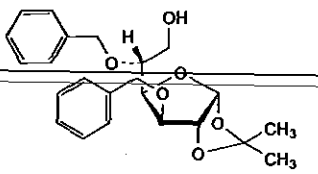
STORAGE

Under nitrogen, in an airtight container.

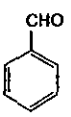
IMPURITIES



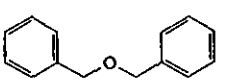
A. 3,5,6-tri-*O*-benzyl-1,2-*O*-(1-methylethylidene)-α-D-glucofuranose,



B. 3,5-di-*O*-benzyl-1,2-*O*-(1-methylethylidene)-α-D-glucofuranose,



C. benzaldehyde,

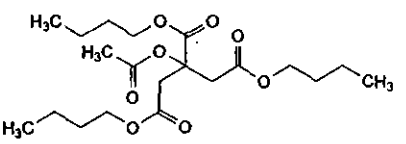


D. dibenzyl ether.

Ph Eur

Tributyl Acetylcitrate

(Ph. Eur. monograph 1770)



$C_{20}H_{34}O_8$ 402.5 *77-90-7*

Action and use

Excipient.

Ph Eur

DEFINITION

Tributyl 2-(acetyloxy)propane-1,2,3-tricarboxylate.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Clear, oily liquid.

Solubility

Not miscible with water, miscible with ethanol (96 per cent) and with methylene chloride.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Preparation Thin films between 2 sodium chloride plates.

Comparison *tributyl acetylcitrate CRS*.

TESTS

Appearance

The substance to be examined is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Acidity

Dilute 10 g with 10 mL of previously neutralised *ethanol (96 per cent) R* and add 0.5 mL of *bromothymol blue solution R2*. Not more than 0.3 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator to blue.

Refractive index (*2.2.6*)

1.442 to 1.445.

Related substances

Gas chromatography (*2.2.28*).

Test solution Dissolve 0.5 g of the substance to be examined in *methylene chloride R* and dilute to 20 mL with the same solvent.

Reference solution (a) Dissolve 50 mg of the substance to be examined and 50 mg of *tributyl citrate R* (impurity A) in *methylene chloride R* and dilute to 20 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of the test solution to 20.0 mL with *methylene chloride R*. Dilute 1.0 mL of this solution to 25.0 mL with *methylene chloride R*.

Reference solution (c) Dissolve the contents of a vial of *tributyl acetylcitrate for peak identification CRS* (containing impurities B and C) in 1 mL of *methylene chloride R*.

Column:

- *material*: fused silica;
- *size*: *l* = 30 m, Ø = 0.25 mm;
- *stationary phase*: *cyanopropyl(25)phenyl(25)methyl(50) polysiloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R*.

Linear velocity 36 cm/s.

Split ratio 1:20.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 7	70 → 210
	7 - 50	210
Injection port		250
Detector		250

Detection Flame ionisation.

Injection 1 µL; inject via an inert, glass-lined injection port using an automatic injection device.

Identification of impurities Use the chromatogram supplied with *tributyl acetylcitrate for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities B and C; use the chromatogram obtained with reference solution (a) to identify the peak due to impurity A.

Relative retention With reference to tributyl acetylcitrate (retention time = about 24 min): impurity B = about 0.70; impurity C = about 0.83; impurity A = about 0.87.

System suitability:

- *resolution*: minimum 2.0 between the peaks due to impurity A and tributyl acetylcitrate in the chromatogram obtained with reference solution (a);
- *repeatability*: maximum relative standard deviation of 5.0 per cent after 6 injections of reference solution (b).

Limits:

- *impurity A*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.6 per cent);
- *impurity C*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent);
- *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
- *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.12*)

Maximum 0.25 per cent, determined on 2.00 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

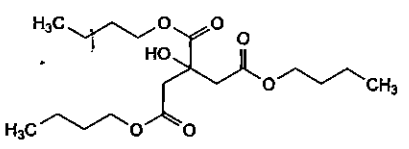
Introduce 1.500 g into a 250 mL borosilicate glass flask. Add 25 mL of *2-propanol R*, 50 mL of *water R*, 25.0 mL of *1 M sodium hydroxide* and a few glass beads. Heat under a reflux condenser for 3 h. Allow to cool. Add 1 mL of *phenolphthalein solution R1* and titrate with *1 M hydrochloric acid*. Carry out a blank titration.

1 mL of *1 M sodium hydroxide* is equivalent to 100.6 mg of $C_{20}H_{34}O_8$.

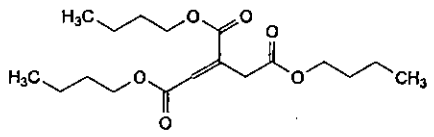
IMPURITIES

Specified impurities *A, B, C.*

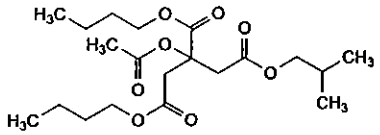
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *D, E.*



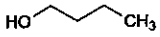
A. tributyl 2-hydroxypropane-1,2,3-tricarboxylate (tributyl citrate),



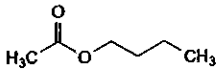
B. tributyl propene-1,2,3-tricarboxylate (tributyl aconitate),



C. 1,2-dibutyl 3-(2-methylpropyl) 2-(acetyloxy)propane1,2,3-tricarboxylate,



D. butan-1-ol,

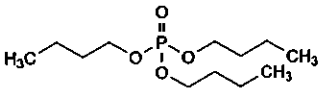


E. butyl acetate.

Ph Eur

Tributyl Phosphate

(Tri-n-butyl Phosphate, Ph. Eur. monograph 1682)



$C_{12}H_{27}O_4P$ 266.3 *126-73-8*

Action and use
Excipient.

Ph Eur

CHARACTERS

Appearance
Clear, colourless or pale yellow liquid.

Solubility
Slightly soluble in water, miscible with ethanol (96 per cent).

bp
About 289 °C, with decomposition.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *tri-n-butyl phosphate CRS.*

TESTS

Appearance
The substance to be examined is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Acidity
Dissolve 50 mL in 50 mL of *ethanol (96 per cent) R* previously adjusted with *0.02 M potassium hydroxide* or *0.02 M hydrochloric acid* to a bluish-green colour, using 0.5 mL of *bromothymol blue solution R1* as indicator. Titrate with *0.02 M potassium hydroxide* to the initial bluish-green coloration. Not more than 0.8 mL of *0.02 M potassium hydroxide* is required.

Related substances
Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution The substance to be examined.

Reference solution Dissolve 10 mg of the substance to be examined and 10 mg of *methyl myristate R* in *methylene chloride R* and dilute to 10 mL with the same solvent.

Column:
— *material*: fused silica;
— *size*: *l* = 30 m, Ø = 0.32 mm;
— *stationary phase*: *methylpolysiloxane R* (5 µm).

Carrier gas *helium for chromatography R.*

Linear velocity 32 cm/s.

Split ratio 65:1.

Temperature:
— *column*: 250 °C;
— *injection port and detector*: 250 °C.

Detection Flame ionisation.

Injection 1 µL.

Run time Twice the retention time of tri-*n*-butyl phosphate.

System suitability Reference solution:
— *resolution*: minimum 10 between the peaks due to tri-*n*-butyl phosphate and methyl myristate.

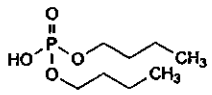
Limits:
— *any impurity*: for each impurity, maximum 0.3 per cent;
— *total*: maximum 0.5 per cent;
— *disregard limit*: 0.01 per cent.

Chlorides (*2.4.4*)
Maximum 200 ppm.
Dissolve 0.25 g in 15 mL of *ethanol (70 per cent V/V) R.* The solution complies with the test. Prepare the reference solution using 10 mL of *chloride standard solution (5 ppm Cl) R* and 5 mL of *anhydrous ethanol R.*

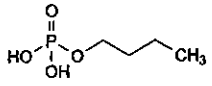
Water (*2.5.32*)
Maximum 0.1 per cent, determined on 1.0 g.

STORAGE
Protected from light.

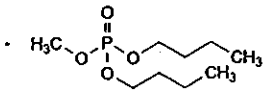
IMPURITIES



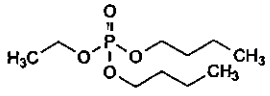
A. dibutyl hydrogen phosphate,



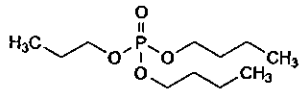
B. butyl dihydrogen phosphate,
C. H_3C-$[CH_2]_3$-OH: butan-1-ol,



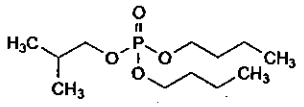
D. dibutyl methyl phosphate,



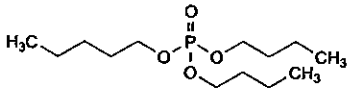
E. dibutyl ethyl phosphate,



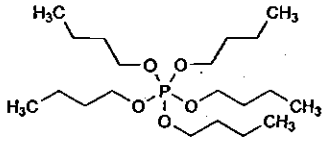
F. dibutyl propyl phosphate,



G. dibutyl 2-methylpropyl phosphate,



H. dibutyl pentyl phosphate,

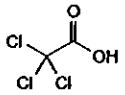


I. pentabutyl phosphate.

Ph Eur

Trichloroacetic Acid

(Ph. Eur. monograph 1967)



$C_2HCl_3O_2$ 163.4 *76-03-9*

Preparation
Trichloroacetic Acid Solution

Ph Eur

DEFINITION
2,2,2-Trichloroacetic acid.

Content
98.0 per cent to 100.5 per cent.

CHARACTERS

Appearance
White or almost white, crystalline mass or colourless crystals, very deliquescent.

Solubility
Very soluble in water, in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION
First identification: A.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison Ph. Eur. reference spectrum of trichloroacetic acid.

B. To 0.5 mL of solution S (see Tests) add 2 mL of *pyridine R* and 5 mL of *strong sodium hydroxide solution R*. Shake vigorously and heat in a water-bath at 60-70 °C for 5 min. The upper layer shows an intense red colour.

C. Solution S is strongly acidic (*2.2.4*).

TESTS

Solution S
Dissolve 2.5 g in *water R* and dilute to 25 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_7 (*2.2.2, Method II*).

Chlorides (*2.4.4*)
Maximum 100 ppm.

Dilute 5 mL of solution S to 15 mL with *water R*.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

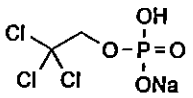
ASSAY
Dissolve 0.150 g in 20 mL of *water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M sodium hydroxide* is equivalent to 16.34 mg of $C_2HCl_3O_2$.

STORAGE
In an airtight container.

Ph Eur

Triclofos Sodium



$C_2H_3Cl_3NaO_4P$ 251.4 *7246-20-0*

Action and use
Hypnotic.

Preparation
Triclofos Oral Solution

DEFINITION
Triclofos Sodium is sodium 2,2,2-trichloroethyl hydrogen orthophosphate. It contains not less than 41.3% and not more than 43.2% of Cl and not less than 97.0% and not more than 102.0% of $C_2H_3Cl_3NaO_4P$, both calculated with reference to the dried substance.

CHARACTERISTICS
A white or almost white powder; hygroscopic.

Freely soluble in *water*; slightly soluble in *ethanol (96%)*; practically insoluble in *ether*.

IDENTIFICATION
A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of triclofos sodium *(RS 350)*.

B. Yields the reactions characteristic of *sodium salts*, Appendix VI.

C. Heat 0.1 g with 1 g of *anhydrous sodium carbonate* to a dull red heat and maintain for 10 minutes, cool, extract the residue with *water* and filter. The filtrate yields the reactions characteristic of *chlorides* and of *phosphates*, Appendix VI.

TESTS

Acidity
pH of a 2% w/v solution, 3.0 to 4.5, Appendix V L.

Clarity of solution
A 2.0% w/v solution is *clear*, Appendix IV A.

Chloride
Dissolve 0.1 g in 50 mL of *water*. 15 mL of the solution complies with the *limit test for chlorides*, Appendix VII (0.17%).

Phosphate
Not more than 1.0%, calculated as PO_4, when determined by the following method. Dissolve 25 mg in 10 mL of *water*, add 4 mL of 1M *sulfuric acid*, 1 mL of *ammonium molybdate solution* and 2 mL of *methylaminophenol—sulfite reagent* and allow to stand for 15 minutes. Add sufficient *water* to produce 25 mL, allow to stand for a further 15 minutes and measure the *absorbance* of a 4 cm layer of the resulting solution at 730 nm, Appendix II B. Calculate the content of phosphate from a calibration curve prepared by treating suitable volumes of a 0.00143% w/v solution of *potassium dihydrogen orthophosphate* in the same manner.

Loss on drying
When dried at 100° at a pressure not exceeding 0.7 kPa for 3 hours, loses not more than 5.0% of its weight. Use 1 g.

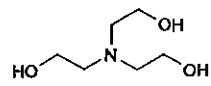
ASSAY

For Cl
Mix 0.25 g with 1 g of *anhydrous sodium carbonate* in a nickel crucible about 3 cm in diameter, fill the crucible completely with *anhydrous sodium carbonate* and invert into a nickel crucible about 4 cm in diameter; cover the smaller crucible with *anhydrous sodium carbonate*, well pressed down, using about 25 g of *anhydrous sodium carbonate* in all. Heat for 30 minutes at a dull red heat, cool, transfer to a 400 mL beaker, add 150 mL of *water* and boil gently for 10 minutes. Filter through absorbent cotton into a 600 mL beaker, washing the residue thoroughly with hot *water*, until about 400 mL of filtrate has been collected. Cool, cautiously add *nitric acid* until the solution is neutral to *litmus paper* and add 3 mL of *nitric acid* in excess. Add 50 mL of *0.1M silver nitrate VS*, allow to stand until precipitation is complete, filter, wash the precipitate with *water* and titrate the combined filtrate and washings with *0.1M ammonium thiocyanate VS* using *ammonium iron(III) sulfate solution R2* as indicator. Each mL of *0.1M silver nitrate VS* is equivalent to 3.545 mg of Cl.

For $C_2H_3Cl_3NaO_4P$
Heat 0.2 g in a Kjeldahl flask with 2 mL of *sulfuric acid* and 2.5 mL of *nitric acid* until brown fumes cease to be evolved, cool, add 1 mL of *nitric acid* and heat again. Continue adding *nitric acid* and heating until brown fumes are no longer evolved and the solution is colourless when cold. Heat until dense, white fumes are evolved, cool, transfer the solution to a flask with the aid of 150 mL of *water*, add 50 mL of *citric—molybdic acid solution* and heat slowly to boiling. Swirling the flask continuously, add 25 mL of *quinoline solution* at first drop wise and then in a steady stream, heat on a water bath for 5 minutes and cool. Filter, wash the precipitate with *water* until free from acid, transfer the precipitate to a flask with the aid of 100 mL of *water*, add 50 mL of *0.5M sodium hydroxide VS* and shake until dissolved. Titrate the excess of alkali with *0.5M hydrochloric acid VS* using *phenolphthalein—thymol blue solution* as indicator. Each mL of *0.5M sodium hydroxide VS* is equivalent to 4.835 mg of $C_2H_3Cl_3NaO_4P$. Correct the result for the content of phosphate, as determined by the test described above; each mg of PO_4 is equivalent to 2.65 mg of $C_2H_3Cl_3NaO_4P$.

Triethanolamine

(Trolamine, Ph. Eur. monograph 1577)



$C_6H_{15}NO_3$ 149.2 *102-71-6*

Action and use
Pharmaceutical aid.

Ph Eur

DEFINITION
2,2′,2″-Nitrilotriethanol.

Content
99.0 per cent *m/m* to 103.0 per cent *m/m* of total bases (anhydrous substance).

CHARACTERS

Appearance
Clear, viscous, colourless or slightly yellow liquid, very hygroscopic.

Solubility
Miscible with water and with ethanol (96 per cent), soluble in methylene chloride.

IDENTIFICATION
First identification: B, C.
Second identification: A, B, D.
A. Relative density (*2.2.5*): 1.120 to 1.130.
B. Refractive index (*2.2.6*): 1.482 to 1.485.
C. Examine the chromatograms obtained in the test for related substances.
Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).
D. To 1 mL add 0.3 mL of *copper sulfate solution R*. A blue colour develops. Add 2.5 mL of *dilute sodium hydroxide solution R* and heat to boiling. The blue colour remains unchanged.

TESTS

Solution S
Dissolve 12 g in *water R* and dilute to 20 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_6 (*2.2.2, Method II*).

Related substances
Gas chromatography (*2.2.28*).
Internal standard solution Dissolve 5.0 g of *3-aminopropanol R* in *water R* and dilute to 100.0 mL with the same solvent.
Test solution Dissolve 10.0 g of the substance to be examined in *water R*. Add 1.0 mL of the internal standard solution and dilute to 100.0 mL with *water R*.
Reference solution (a) Dissolve 1.0 g of *trolamine CRS* in *water R* and dilute to 10.0 mL with the same solvent.
Reference solution (b) Dissolve 0.1 g of *trolamine impurity A CRS*, 0.5 g of *trolamine impurity B CRS* and 0.1 g of *trolamine CRS* in *water R* and dilute to 10.0 mL with the same solvent. To 1.0 mL of this solution add 1.0 mL of the

internal standard solution and dilute to 100.0 mL with *water R*.

Column:
— *material*: fused silica;
— *size*: *l* = 25 m, Ø = 0.25 mm;
— *stationary phase*: *phenyl(5)methyl(95)polysiloxane R* (film thickness 0.50 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1 mL/min.

Split ratio 1:35.

Temperature:

	Time (min)	Temperature (°C)
Column	0	60
	0 - 8.5	60 → 230
	8.5 - 14	230
Injection port		260
Detector		280

Detection Flame ionisation.

Injection 2 µL; if necessary inject a blank solution.

Elution order Impurity A, 3-aminopropanol, impurity B, trolamine.

System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the peaks due to 3-aminopropanol and impurity A.

Limits:
— *impurity A*: calculate the ratio (*R1*) of the area of the peak due to impurity A to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (b); from the chromatogram obtained with the test solution, calculate the ratio of the area of any peak due to impurity A to the area of the peak due to the internal standard: this ratio is not greater than *R1* (0.1 per cent);
— *impurity B*: calculate the ratio (*R2*) of the area of the peak due to impurity B to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (b); from the chromatogram obtained with the test solution, calculate the ratio of the area of any peak due to impurity B to the area of the peak due to the internal standard: this ratio is not greater than *R2* (0.5 per cent);
— *total*: calculate the ratio (*R4*) of the area of the peak due to trolamine to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (b); from the chromatogram obtained with the test solution, calculate the ratio of the sum of the areas of any peaks, apart from the principal peak and the peak due to the internal standard, to the area of the peak due to the internal standard: this ratio is not greater than 10 times *R4* (1.0 per cent);
— *disregard limit*: 0.5 times the ratio of the area of the peak due to trolamine to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (b) (0.05 per cent).

Impurity C

Gas chromatography (*2.2.28*).

Solvent mixture *acetone R, chloroform R* (10:50 *V/V*).

Preparation of solid phase extraction columns

Column A Fill a glass chromatography column (*l* = 400 mm; Ø = 20 mm) fitted with a polytetrafluoroethylene stopcock and a sintered-glass filter (160) (*2.1.2*) with 3 g of *anhydrous sodium sulfate R* and cover with a mixture of 17 g of *kieselguhr for chromatography R* and 3 g of *potassium carbonate R*. Settle the column bed by gently tapping the column.

Column B Fill a glass chromatography column (*l* = 400 mm; Ø = 20 mm) fitted with a polytetrafluoroethylene stopcock and a sintered-glass filter (160) (*2.1.2*) with a slurry of 25 g of *silica gel for chromatography R* (0.063 to 0.200 mm) in the solvent mixture. Apply slight pressure to settle the column and cover the column bed with 5 g of *anhydrous sodium sulfate R*.

Standard solution (a) Dissolve 50 µL of *N-nitrosodiethanolamine R* (impurity C) in *methanol R* and dilute to 50.0 mL with the same solvent. Dilute 100 µL of this solution to 100.0 mL with *methanol R*.

Standard solution (b) Dilute 10.0 mL of standard solution (a) to 50.0 mL with *methanol R*.

Standard solution (c) Dissolve 50 mg of *N-nitrosodiisopropanolamine R* in *methanol R* and dilute to 50.0 mL with the same solvent. Dilute 100 µL of this solution to 100.0 mL with *methanol R*.

Test solution To 2.000 g of the substance to be examined add 200 µL of *methanol R* and 0.5 g of *sulfamic acid R*. Dissolve in 8 mL of *water for chromatography R* and apply the solution to column A. Rinse the vessel twice with 1.5 mL of *water for chromatography R*, applying the rinsings to the column as well. After 15 min of equilibration time elute the column with 100 mL of *ethyl acetate R*, collecting the eluate in a 250 mL distillation flask. Evaporate the eluate to dryness. Take up the residue in 1 mL of the solvent mixture, apply to column B and let it settle. Rinse the flask twice with 2 mL of the solvent mixture, apply the rinsings to the column and let it settle. Wash the column with 100 mL of the solvent mixture and discard. Elute the column with 120 mL of *acetone R*, collecting the eluate in a 250 mL distillation flask. Evaporate the eluate to dryness. Transfer the residue with the aid of a small volume of *acetone R* into a vial and evaporate again to dryness under a stream of *nitrogen R*. Dissolve the residue in 100 µL of *trimethylpentane for chromatography R*, add 100 µL of *N-methyltrimethylsilyl-trifluoroacetamide R* and heat at 70 °C for 1 h.

Reference solution (a) To 2.000 g of the substance to be examined add 200 µL of standard solution (b) and 0.5 g of *sulfamic acid R*. Dissolve in 8 mL of *water for chromatography R*, then proceed exactly as described for the test solution.

Reference solution (b) To 1.0 mL of standard solution (a) add 4.0 mL of standard solution (c) and mix. Transfer 500 µL of the solution to a vial and evaporate to dryness under a stream of *nitrogen R*. Dissolve the residue in 200 µL of *trimethylpentane for chromatography R*, add 200 µL of *N-methyltrimethylsilyl-trifluoroacetamide R* and heat at 70 °C for 1 h.

Reference solution (c) In a vial, evaporate 200 µL of standard solution (b) to dryness under a stream of *nitrogen R*. Dissolve the residue in 100 µL of *trimethylpentane for chromatography R*, add 100 µL of *N-methyltrimethylsilyl-trifluoroacetamide R* and heat at 70 °C for 1 h.

Blank solution In a gas chromatography vial, evaporate 200 µL of *methanol R* to dryness under a stream of *nitrogen R*. Dissolve the residue in 100 µL of *trimethylpentane for chromatography R* add 100 µL of *N-methyltrimethylsilyl-trifluoroacetamide R* and heat at 70 °C for 1 h.

Column:
— *material*: fused silica;
— *size*: *l* = 30 m; Ø = 0.25 mm;
— *stationary phase*: *base-deactivated phenyl(5)methyl(95) polysiloxane R* (film thickness 1 µm).

Carrier gas *helium for chromatography R.*

Flow rate 2 mL/min.

Split ratio 1:10.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 5	180 → 280
	5 - 10	280
Injection port		220

Detection Chemoluminescence:
— dual plasma burner in nitrosamine mode;
— burner temperature: 450 °C;
— oxygen flow rate: 4.4-5.0 mL/min.

Injection 4 µL.

System suitability:
— *resolution*: minimum 1.3 between the peaks due to impurity C and *N*-nitrosodiisopropanolamine in the chromatogram obtained with reference solution (b);
— *recovery*: minimum 50 per cent. The difference between the area of the peak due to impurity C in the chromatogram obtained with reference solution (a) and the area of the corresponding peak in the chromatogram obtained with the test solution is not less than 0.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (c).

Limits:
— *impurity C*: not more than the difference between the area of the peak due to impurity C in the chromatogram obtained with reference solution (a) and the area of the corresponding peak in the chromatogram obtained with the test solution (24).

Water (*2.5.12*)
Maximum 1.0 per cent, determined on 1.000 g.

Open the titration vessel, introduce the substance to be examined directly into the previously titrated solvent. Stopper the flask immediately.

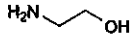
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g. Do not carry out the initial heating on a water-bath.

ASSAY
Dissolve 1.200 g in 75 mL of *carbon dioxide-free water R.* Add 0.3 mL of *methyl red solution R.* Titrate with *1 M hydrochloric acid.*

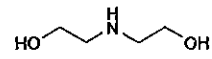
1 mL of *1 M hydrochloric acid* is equivalent to 0.149 g of $C_6H_{15}NO_3$.

STORAGE
In an airtight container, protected from light.

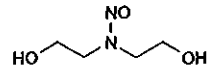
IMPURITIES
Specified impurities *A, B, C.*



A. 2-aminoethanol (ethanolamine),



B. 2,2′-iminodiethanol (diethanolamine),

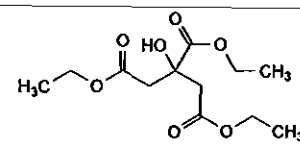


C. 2,2′-(nitrosoimino)diethanol (*N*-nitrosodiethanolamine).

Ph Eur

Triethyl Citrate

(Ph. Eur. monograph 1479)



$C_{12}H_{20}O_7$ 276.3 *77-93-0*

Action and use
Excipient.

Ph Eur

DEFINITION
Triethyl 2-hydroxypropane-1,2,3-tricarboxylate.

Content
98.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS
Appearance
Clear, viscous, colourless or almost colourless, hygroscopic liquid.

Solubility
Soluble in water, miscible with ethanol (96 per cent), slightly soluble in fatty oils.

IDENTIFICATION
First identification: A, B.

Second identification: A, C, D.

A. Refractive index (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of triethyl citrate.*

C. It gives the reaction of esters (*2.3.1*).

D. To 0.5 mL add 5 mL of *ethanol (96 per cent) R* and 4 mL of *dilute sodium hydroxide solution R.* Boil under reflux for about 10 min. 2 mL of the solution gives the reaction of citrates (*2.3.1*).

TESTS
Appearance
The substance to be examined is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Acidity
Dilute 10 g with 10 mL of previously neutralised *ethanol (96 per cent) R,* add 0.5 mL of *bromothymol blue solution R2.* Not more than 0.3 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator to blue.

Refractive index (*2.2.6*)
1.440 to 1.446.

Related substances
Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution Dissolve 1.0 mL of the substance to be examined in *methylene chloride R* and dilute to 50.0 mL with the same solvent.

Reference solution Dissolve 1.0 mL of the substance to be examined and 0.5 mL of *methyl tridecanoate R* in *methylene chloride R*, then dilute to 50.0 mL with the same solvent.

Column:
— *material*: fused silica;
— *size*: *l* = 30 m, Ø = 0.32 mm;
— *stationary phase*: *methylpolysiloxane R* (5 µm).

Carrier gas *helium for chromatography R.*

Linear velocity About 26 cm/s.

Split ratio About 1:50.

Temperature:
— *column*: 200 °C;
— *injection port and detector*: 220 °C.

Detection Flame ionisation.

Injection 1.0 µL.

Run time Twice the retention time of triethyl citrate.

Retention time Triethyl citrate = about 13.6 min.

System suitability Reference solution:
— *resolution*: minimum 1.5 between the peaks due to triethyl citrate and methyl tridecanoate.

Limits:
— *any impurity*: for each impurity, maximum 0.2 per cent;
— *total*: maximum 0.5 per cent;
— *disregard limit*: 0.04 per cent.

Water (*2.5.12*)
Maximum 0.25 per cent, determined on 1.000 g.

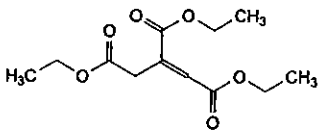
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Introduce 1.500 g into a 250 mL borosilicate-glass flask fitted with a reflux condenser. Add 25 mL of *2-propanol R*, 50 mL of *water R*, 25.0 mL of *1 M sodium hydroxide* and a few glass beads. Heat under a reflux condenser for 1 h. Allow to cool. Add 1 mL of *phenolphthalein solution R1* and titrate with *1 M hydrochloric acid*. Carry out a blank titration.

1 mL of *1 M sodium hydroxide* is equivalent to 92.1 mg of $C_{12}H_{20}O_7$.

STORAGE
In an airtight container.

IMPURITIES

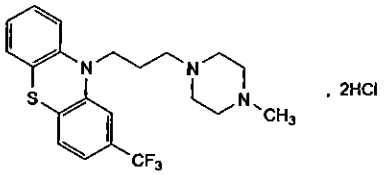


A. triethyl propene-1,2,3-tricarboxylate (triethyl aconitate).

Ph Eur

Trifluoperazine Hydrochloride

(Ph. Eur. monograph 0059)



$C_{21}H_{26}Cl_2F_3N_3S$ 480.4 *440-17-5*

Action and use
Dopamine receptor antagonist; neuroleptic.

Preparation
Trifluoperazine Tablets

Ph Eur

DEFINITION
Trifluoperazine hydrochloride contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 10-[3-(4-methylpiperazin-1-yl)propyl]-2-(trifluoromethyl)-10*H*-phenothiazine dihydrochloride, calculated with reference to the dried substance.

CHARACTERS
A white to pale yellow, crystalline powder, hygroscopic, freely soluble in water, soluble in alcohol.

It melts at about 242 °C, with decomposition.

IDENTIFICATION
A. *Protect the solutions from bright light and measure the absorbances immediately.* Dissolve 50 mg in *0.1 M hydrochloric acid* and dilute to 500 mL with the same acid. Examined between 280 nm and 350 nm, the solution shows an absorption maximum (*2.2.25*) at 305 nm. Dilute 5 mL of the solution to 100 mL with *0.1 M hydrochloric acid*. Examined between 230 nm and 280 nm, this solution shows an absorption maximum at 255 nm. The specific absorbance at this maximum is about 650.

B. It complies with the identification test for phenothiazines by thin-layer chromatography (*2.3.3*): use *trifluoperazine hydrochloride CRS* to prepare the reference solution.

C. Place 0.25 g in a 100 mL separating funnel, add 5 mL of *water R* and 2 mL of *dilute sodium hydroxide solution R*. Shake vigorously with 20 mL of *ether R*. Wash the ether layer with 5 mL of *water R*, add 0.15 g of *maleic acid R* and evaporate the ether. The residue, recrystallised from 30 mL of *alcohol R* and dried, melts (*2.2.14*) at about 192 °C.

D. Dissolve about 0.5 mg in 1 mL of *water R*, add 0.1 mL of *bromine water R* and shake for about 1 min. Add dropwise 1 mL of *sulfuric acid R* with constant, vigorous agitation. A red colour develops.

E. Dissolve about 50 mg in 5 mL of *water R* and add 2 mL of *nitric acid R*. A dark-red colour develops which turns to pale yellow. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS
pH (*2.2.3*)
Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 20 mL with the same solvent. The pH of the solution is 1.6 to 2.5.

Related substances
Carry out the test protected from bright light.

Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel GF_{254} plate R*.

Test solution Dissolve 0.2 g of the substance to be examined in a mixture of 5 volumes of *diethylamine R* and 95 volumes of *methanol R* and dilute to 10 mL with the same mixture of solvents. Prepare immediately before use.

Reference solution Dilute 1 mL of the test solution to 200 mL with a mixture of 5 volumes of *diethylamine R* and 95 volumes of *methanol R*.

Apply to the plate 10 µL of each solution. Develop over a path of 12 cm using a mixture of 10 volumes of *acetone R*, 10 volumes of *diethylamine R* and 80 volumes of *cyclohexane R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Loss on drying (*2.2.32*)
Not more than 1.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 50 mL of *alcohol R* and add 5.0 mL of *0.01 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 48.04 mg of $C_{21}H_{26}Cl_2F_3N_3S$.

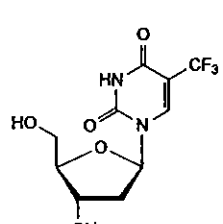
STORAGE

Store in an airtight container, protected from light.

Ph Eur

Trifluridine

(Ph. Eur. monograph 2910)



$C_{10}H_{11}F_3N_2O_5$ 296.2 *70-00-8*

Action and use
Pyrimidine nucleoside analogue; antiviral (herpes viruses).

Preparation
Trifluridine Eye Drops

Ph Eur

DEFINITION

1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-5-(trifluoromethyl)pyrimidine-2,4(1*H*,3*H*)-dione.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Soluble to slightly soluble in water and in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison trifluridine CRS.

TESTS

Specific optical rotation (*2.2.7*)
+ 47 to + 51 (dried substance).

Dissolve 0.300 g in *water R* and dilute to 10.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*). *Carry out the test protected from light. Use the solutions within 12 h.*

Solvent mixture Mobile phase B, mobile phase A (5:95 *V/V*).

Test solution Dissolve 20.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 10.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 20.0 mg of *trifluridine CRS* in the solvent mixture and dilute to 10.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 2 mg of *5-carboxyuracil R* (impurity C) in the solvent mixture and dilute to 100 mL with the solvent mixture. Dilute 1 mL of the solution to 10 mL with the solvent mixture (solution A). Dissolve the contents of a vial of *trifluridine impurity A CRS* in 1 mL of solution A.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: 7.8 g/L solution of *sodium dihydrogen phosphate R* adjusted to pH 6.0 with *ammonia R*;
— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	95	5
3 - 10	95 → 85	5 → 15
10 - 15	85	15

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 263 nm.

Autosampler Set at 4 °C.

Injection 20 µL.

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A and C.

Relative retention With reference to trifluridine (retention time = about 13.8 min): impurity C = about 0.19; impurity A = about 0.21.

System suitability Reference solution (c):
— *resolution*: minimum 1.5 between the peaks due to impurities C and A.

Calculation of percentage contents:
— for each impurity, use the concentration of trifluridine in reference solution (a).

Limits:
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.2 per cent;
— *reporting threshold*: 0.05 per cent.

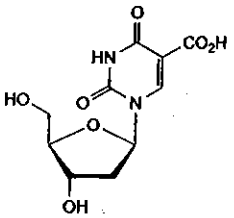
Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

ASSAY
Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

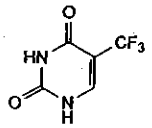
Injection Test solution and reference solution (b).

Calculate the percentage content of $C_{10}H_{11}F_3N_2O_5$ taking into account the assigned content of *trifluridine CRS*.

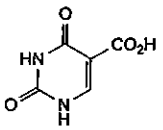
IMPURITIES
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, E.*



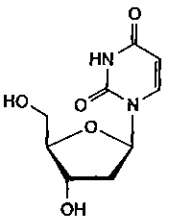
A. 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid,



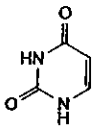
B. 5-(trifluoromethyl)pyrimidine-2,4(1*H*,3*H*)-dione,



C. 2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid (5-carboxyuracil),



D. 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione,

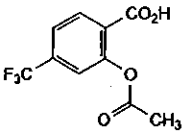


E. pyrimidine-2,4(1*H*,3*H*)-dione (uracil).

Ph Eur

Triflusal

(Ph. Eur. monograph 1377)



$C_{10}H_7F_3O_4$ 248.2 *322-79-2*

Action and use
Thromboxane synthesis inhibitor; antiplatelet drug.

Ph Eur

DEFINITION
2-(Acetyloxy)-4-(trifluoromethyl)benzoic acid.

Content
98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, very soluble in anhydrous ethanol, freely soluble in methylene chloride.

mp
About 118 °C, with decomposition.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *triflusal CRS*.

TESTS
Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.200 g of the substance to be examined in *acetonitrile R* and dilute to 20.0 mL with the same solvent. *Prepare the solution immediately before use.*

Reference solution (a) Dissolve 5.0 mg of *triflusal impurity B CRS* in *acetonitrile R* and dilute to 10.0 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 25.0 mL with *acetonitrile R*.

Reference solution (c) Dissolve 2.5 mg of the substance to be examined in *acetonitrile R*, add 5 mL of reference solution (a) and dilute to 10.0 mL with *acetonitrile R*. *Prepare the solution immediately before use.*

Column:
— *size*: *l* = 0.15 m, Ø = 4.0 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (4-5 µm).

Mobile phase:
— *mobile phase A*: 0.5 per cent *V/V* solution of *phosphoric acid R*;
— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	80 → 30	20 → 70
20 - 25	30	70

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 237 nm.

Injection 10 µL of the test solution and reference solutions (b) and (c).

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity B.

Relative retention With reference to triflusal (retention time = about 11 min): impurity B = about 1.2.

System suitability Reference solution (c):
— *resolution*: minimum 3.0 between the peaks due to triflusal and impurity B.

Limits:
— *impurity B*: not more than 1.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *sum of impurities other than B*: not more than 0.5 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) (0.1 per cent);
— *disregard limit*: 0.25 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo*.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

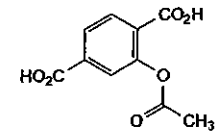
ASSAY
Dissolve 0.200 g in 50 mL of *anhydrous ethanol R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M sodium hydroxide* is equivalent to 24.82 mg of $C_{10}H_7F_3O_4$.

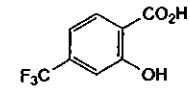
STORAGE
In an airtight container, at a temperature not exceeding 25 °C.

IMPURITIES
Specified impurities *B*.

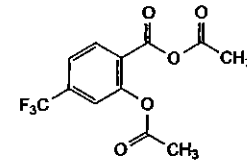
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, C, D.*



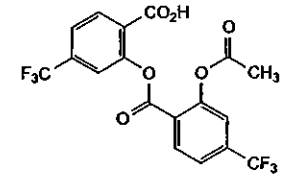
A. 2-(acetyloxy)benzene-1,4-dicarboxylic acid (2-acetoxyterephthalic acid),



B. 2-hydroxy-4-(trifluoromethyl)benzoic acid (4-(trifluoromethyl)salicylic acid),



C. acetic 2-(acetyloxy)-4-(trifluoromethyl)benzoic anhydride,



D. 2-[[2-(acetyloxy)-4-(trifluoromethyl)benzoyl]oxy]-4-(trifluoromethyl)benzoic acid.

Ph Eur

Medium-chain Triglycerides

Fractionated Coconut Oil
(Ph. Eur. monograph 0868)

Action and use.
Excipient.

When Medium-chain Triglycerides are prepared from the endosperm of Cocos nucifera L the title fractionated coconut oil may be used.

Ph Eur

DEFINITION
Mixture of triglycerides of saturated fatty acids, mainly of caprylic (octanoic) acid and of capric (decanoic) acid.
The fatty acids are obtained from the oil extracted from the hard, dried fraction of the endosperm of *Cocos nucifera* L. or from the dried endosperm of *Elaeis guineensis* Jacq.

Content
Minimum 95.0 per cent of saturated fatty acids with 8 and 10 carbon atoms.

CHARACTERS

Appearance
Colourless or slightly yellowish, oily liquid.

Solubility
Practically insoluble in water, miscible with ethanol (96 per cent), with methylene chloride, with light petroleum and with fatty oils.

Relative density
About 0.95.

Refractive index
About 1.446.

IDENTIFICATION
First identification: C, D.
Second identification: A, B, D.
A. Iodine value (*2.5.4*): maximum 1.0.
B. Saponification value (*2.5.6*): 310 to 360.
C. Composition of fatty acids (see Tests).
D. Viscosity (see Tests).

TESTS

Appearance
The substance to be examined is clear (*2.2.1*) and not more intensely coloured than reference solution Y_3 (*2.2.2, Method I*).

Alkaline impurities
Dissolve 2.00 g in a mixture of 1.5 mL of *ethanol (96 per cent) R* and 3.0 mL of *ether R*. Add 0.05 mL of *bromophenol blue solution R*. Not more than 0.15 mL of *0.01 M hydrochloric acid* is required to change the colour of the indicator to yellow.

Viscosity (*2.2.9*)
25 mPa·s to 33 mPa·s.

Acid value (*2.5.1*)
Maximum 0.2.

Hydroxyl value (*2.5.3, Method A*)
Maximum 10.

Peroxide value (*2.5.5, Method A*)
Maximum 1.0.

Composition of fatty acids
Gas chromatography (*2.4.22, Method C*) with the following modifications. Use the mixture of calibrating substances in Table 2.4.22.-2.

Column:
— *material*: fused silica;
— *size*: *l* = 30 m, Ø = 0.32 mm;
— *stationary phase*: *macrogol 20 000 R* (film thickness 0.5 µm).

Carrier gas *helium for chromatography R.*

Flow rate 1.3 mL/min.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 1	70
	1 - 35	70 → 240
	35 - 50	240
Injection port		250
Detector		250

Detection Flame ionisation.

Split ratio 1:100.

Composition of the fatty-acid fraction of the substance:
— *caproic acid*: maximum 2.0 per cent;
— *caprylic acid*: 50.0 per cent to 80.0 per cent;
— *capric acid*: 20.0 per cent to 50.0 per cent;
— *lauric acid*: maximum 3.0 per cent;
— *myristic acid*: maximum 1.0 per cent;
— *fatty acids of chain length greater than or equal to C_{16}*: maximum 1.0 per cent.

Water (*2.5.32*)
Maximum 0.2 per cent, determined on 1.00 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 2.0 g.

STORAGE
In a well-filled container, protected from light.

LABELLING
The label states, where applicable, that the substance is intended for use in parenteral nutrition.

Ph Eur

Triglycerol Diisostearate

(Ph. Eur. monograph 2032)

Ph Eur

DEFINITION
Mixture of polyglycerol diesters of mainly isostearic acid, obtained by esterification of polyglycerol and isostearic acid. The polyglycerol consists mainly of triglycerol.

CHARACTERS

Appearance
Clear, yellowish, viscous liquid.

Solubility
Practically insoluble in water, miscible with ethanol (96 per cent) and with fatty oils.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Preparation Film between 2 plates of *sodium chloride R*.
Comparison *triglycerol diisostearate CRS*.
B. Composition of fatty acids (see Tests).

TESTS

Appearance of solution
The solution is not more intensely coloured than reference solution BY_3 (*2.2.2, Method I*).
Mix 10 mL with 10 mL of *ethanol (96 per cent) R*.

Acid value (*2.5.1*)
Maximum 3.0, determined on 1.0 g.

Hydroxyl value (*2.5.3, Method A*)
180 to 230, determined on 0.25 g.

Iodine value (*2.5.4, Method B*)
Maximum 5.0.

Peroxide value (*2.5.5, Method B*)
Maximum 6.0.

Saponification value (*2.5.6*)
128 to 160.

Composition of fatty acids (*2.4.22, Method B*)
Use the mixture of calibrating substances in Table 2.4.22.-1.

Composition of the fatty-acid fraction of the substance:
- *sum of the contents of the fatty acids eluting between palmitic acid and stearic acid*: minimum 60.0 per cent;
- *sum of the contents of myristic acid, palmitic acid and stearic acid*: maximum 11.0 per cent.

Water (*2.5.12*)
Maximum 0.5 per cent, determined on 2.00 g.

Sulfated ash
Maximum 0.5 per cent, determined on 1.0 g.

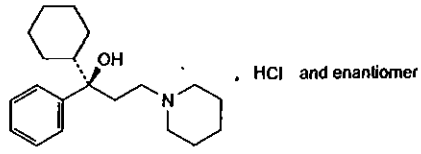
Heat a silica crucible to redness for 30 min, allow to cool in a desiccator and weigh. Evenly distribute 1.00 g of the substance to be examined in the crucible and weigh. Dry at 100-105 °C for 1 h and ignite in a muffle furnace at 600 °C ± 25 °C until the substance is thoroughly charred. Carry out the test for sulfated ash (*2.4.14*) on the residue obtained, starting with "Moisten the substance to be examined...".

STORAGE
In an airtight container, protected from light.

Ph Eur

Trihexyphenidyl Hydrochloride

(Ph. Eur. monograph 1626)



$C_{20}H_{32}ClNO$ 337.9 *52-49-3*

Action and use
Anticholinergic.

Preparation
Trihexyphenidyl Tablets

Ph Eur

DEFINITION
(1*RS*)-1-Cyclohexyl-1-phenyl-3-(piperidin-1-yl)propan-1-ol hydrochloride.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Slightly soluble in water, sparingly soluble in ethanol (96 per cent) and in methylene chloride.

mp
About 250 °C, with decomposition.

IDENTIFICATION
First identification *A, D.*

Second identification *B, C, D.*

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *trihexyphenidyl hydrochloride CRS.*

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 25 mg of the substance to be examined in a mixture of 20 volumes of *methanol R* and 80 volumes of *methylene chloride R* and dilute to 10 mL with the same mixture of solvents.

Reference solution Dissolve 25 mg of *trihexyphenidyl hydrochloride CRS* in a mixture of 20 volumes of *methanol R* and 80 volumes of *methylene chloride R* and dilute to 10 mL with the same mixture of solvents.

Plate *TLC silica gel G plate R.*

Mobile phase *diethylamine R, hexane R* (5:95 *V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with a 0.1 g/L solution of *chloroplatinic acid R* in *hydrochloric acid R* containing 0.4 per cent *V/V* of *hydriodic acid R*.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. Dissolve 0.5 g in 5 mL of warm *methanol R* and make just alkaline to *red litmus paper R* with *sodium hydroxide solution R*. A precipitate is formed which, after recrystallisation from *methanol R*, melts (*2.2.14*) at about 113°C to 115 °C.

D. It gives reaction (a) of chlorides (*2.3.1*).

TESTS
pH (*2.2.3*)
5.2 to 6.2.

Dissolve 0.5 g with heating in 25 mL of *carbon dioxide-free water R*. Cool to room temperature and dilute to 50 mL with *carbon dioxide-free water R*.

Optical rotation (*2.2.7*)
−0.10° to + 0.10°.

Dissolve 1.25 g in a mixture of 20 volumes of *methanol R* and 80 volumes of *methylene chloride R* and dilute to 25.0 mL with the same mixture of solvents.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 200.0 mL with the mobile phase. Dilute 10.0 mL to 50.0 mL with the mobile phase.

Reference solution (b) Dissolve 10.0 mg of *trihexyphenidyl impurity A CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (c) Dilute 1.0 mL of reference solution (b) to 100.0 mL with the mobile phase.

Reference solution (d) To 1 mL of reference solution (b), add 1 mL of the test solution and dilute to 100 mL with the mobile phase.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 200 mL of *water R* with 0.2 mL of *triethylamine R*. Adjust to pH 4.0 with *phosphoric acid R* and add 800 mL of *acetonitrile R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µL.

Run time 3 times the retention time of trihexyphenidyl.

System suitability Reference solution (d):
— *resolution*: minimum 4.0 between the peaks due to trihexyphenidyl and to impurity A.

Limits:
— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 0.5 per cent;
— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

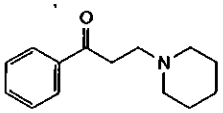
Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.250 g in 50 mL of *ethanol (96 per cent) R* and add 5.0 mL of *0.01 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 33.79 mg of $C_{20}H_{32}ClNO$.

IMPURITIES

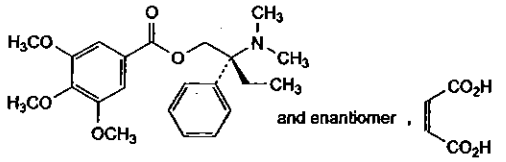


A. 1-phenyl-3-(piperidin-1-yl)propan-1-one.

Ph Eur

Trimebutine Maleate

(Ph. Eur. monograph 2182)



$C_{26}H_{33}NO_9$ 503.5 *34140-59-5*

Action and use
Treatment of irritable bowel syndrome.

Ph Eur

DEFINITION
(2*RS*)-2-(Dimethylamino)-2-phenylbutyl 3,4,5-trimethoxybenzoate (*Z*)-butenedioate.

Content
99.0 per cent to 101.5 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Slightly soluble in water, soluble in acetonitrile, sparingly soluble in acetone, slightly soluble in ethanol (96 per cent).

mp
About 133 °C.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *trimebutine maleate CRS*.

TESTS
Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 0.5 g in *acetone R*, sonicate and dilute to 100 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture Dissolve 0.24 g of *anhydrous sodium dihydrogen phosphate R* in 180 mL of *water R* and adjust to pH 2.5 with *dilute phosphoric acid R*; dilute to 200 mL with *water R*. Add 50 mL of *acetonitrile R* and mix.

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 5 mg of *methyl 3,4,5-trimethoxybenzoate R* (impurity C) in 10 mL of the solvent mixture. Dilute 1 mL of the solution to 20 mL with the solvent mixture.

Reference solution (c) Dissolve the contents of a vial of *trimebutine for system suitability CRS* (containing impurities D and E) in 1 mL of reference solution (b).

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *polar end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 25 °C.

Mobile phase:
— *mobile phase A*: dissolve 3.6 g of *anhydrous sodium dihydrogen phosphate R* in 990 mL of *water for chromatography R* and adjust to pH 3.0 with *phosphoric acid R*; dilute to 1000 mL with *water for chromatography R*;
— *mobile phase B*: *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	78	22
3 - 6.5	78 → 65	22 → 35
6.5 - 15	65 → 60	35 → 40
15 - 35	60	40

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µL of the test solution and reference solutions (a) and (c).

Identification of impurities Use the chromatogram supplied with *trimebutine for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities C, D and E.

Relative retention With reference to trimebutine (retention time = about 12 min): maleic acid = about 0.17;

impurity E = about 0.9; impurity D = about 1.3; impurity C = about 1.4.

System suitability Reference solution (c):
- *resolution*: minimum 1.5 between the peaks due to impurities D and C;
- *peak-to-valley ratio*: minimum 10, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to trimebutine.

Limits:
- *impurity E*: not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.6 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent); disregard any peak due to maleic acid.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

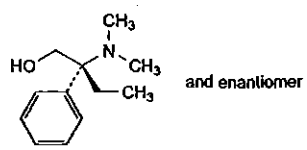
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.300 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 50.35 mg of $C_{26}H_{33}NO_9$.

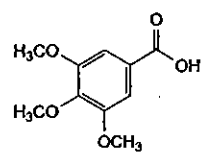
IMPURITIES
Specified impurities *E.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D.*

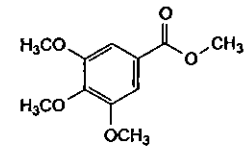


and enantiomer

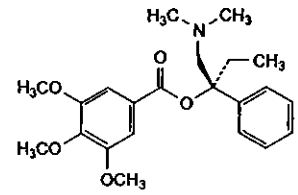
A. (2*RS*)-2-(dimethylamino)-2-phenylbutanol,



B. 3,4,5-trimethoxybenzoic acid,

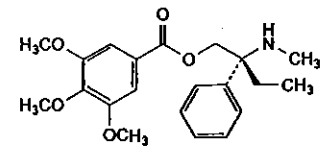


C. methyl 3,4,5-trimethoxybenzoate,



and enantiomer

D. (1*RS*)-1-[(dimethylamino)methyl]-1-phenylpropyl 3,4,5-trimethoxybenzoate,



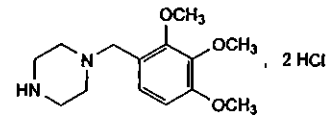
and enantiomer

E. (2*RS*)-2-(methylamino)-2-phenylbutyl 3,4,5-trimethoxybenzoate.

Ph Eur

Trimetazidine Hydrochloride

(Trimetazidine Dihydrochloride, Ph. Eur. monograph 1741)



$C_{14}H_{24}Cl_2N_2O_3$ 339.3 *13171-25-0*

Action and use
Vasodilator.

Ph Eur

DEFINITION
1-(2,3,4-Trimethoxybenzyl)piperazine dihydrochloride.

Content
98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder, slightly hygroscopic.

Solubility
Freely soluble in water, sparingly soluble in alcohol.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of trimetazidine dihydrochloride.*

B. Dissolve 25 mg in 5 mL of *water R*. 2 mL of the solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Dissolve 1.0 g in *water R* and dilute to 10 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.200 g of the substance to be examined in *water R* and dilute to 50.0 mL with the same solvent.

Reference solution (a) Dissolve 20.0 mg of *trimetazidine for system suitability CRS* in *water R* and dilute to 5.0 mL with the same solvent.

Reference solution (b) Dilute 2.0 mL of the test solution to 100.0 mL with *water R*. Dilute 5.0 mL of this solution to 100.0 mL with *water R*.

Reference solution (c) Dilute 25.0 mL of reference solution (b) to 50.0 mL with *water R*.

Column:
- *size*: l = 0.15 m, Ø = 4.6 mm,
- *stationary phase*: spherical *octadecylsilyl silica gel for chromatography R* (5 µm) with a pore size of 10 nm,
- *temperature*: 30 °C.

Mobile phase:
- *mobile phase A*: mix 357 volumes of *methanol R* and 643 volumes of a 2.87 g/L solution of *sodium heptanesulfonate R* adjusted to pH 3.0 with *dilute phosphoric acid R*,
- *mobile phase B*: *methanol R*,

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 50	95 → 75	5 → 25
50 - 52	75 → 95	25 → 5

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 240 nm.

Equilibration For at least 1 h with the mobile phase at the initial composition.

Injection 10 µL.

Relative retention With reference to trimetazidine (retention time = about 25 min): impurity D = about 0.2; impurity C = about 0.4; impurity H = about 0.6; impurities A and I = about 0.9; impurity E = about 0.95; impurity F = about 1.4; impurity B = about 1.8.

System suitability:
- *peak-to-valley ratio*: minimum 3, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the principal peak in the chromatogram obtained with reference solution (a);
- *signal-to-noise ratio*: minimum 10 for the principal peak in the chromatogram obtained with reference solution (c).

Limits:
- *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.55; impurity C = 0.37; impurity F = 0.71;
- *impurities A, B, C, D, E, F, H, I*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *disregard limit*: area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Impurity G

Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.10 g of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 22.6 mg of *piperazine hydrate R* in *methanol R* and dilute to 100 mL with the same solvent. Dilute 10 mL of the solution to 100 mL with *methanol R*.

Plate *TLC silica gel plate R*.

Mobile phase *concentrated ammonia R, alcohol R* (20:80 *V/V*).

Application 10 µL.

Development Over 2/3 of the plate.

Drying At 100-105 °C for 30 min.

Detection Spray with *iodoplatinate reagent R*.

Limit:
- *impurity G*: any spot due to impurity G is not more intense than the spot in the chromatogram obtained with the reference solution (0.1 per cent, expressed as anhydrous piperazine).

Loss on drying (*2.2.32*)

Maximum 2.5 per cent, determined on 1.000 g by drying *in vacuo* at 105 °C at a pressure not exceeding 15 kPa.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.120 g in 50.0 mL of *water R*. Add 1 mL of *nitric acid R* and titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (*2.2.20*).

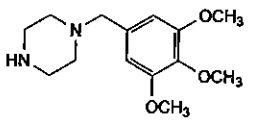
1 mL of *0.1 M silver nitrate* is equivalent to 16.96 mg of $C_{14}H_{24}Cl_2N_2O_3$.

STORAGE

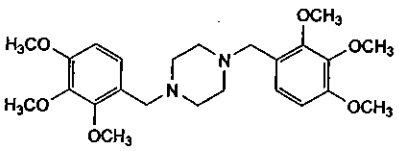
In an airtight container.

IMPURITIES

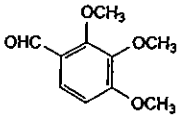
Specified impurities *A, B, C, D, E, F, G, H, I.*



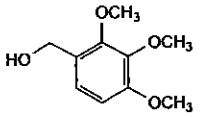
A. 1-(3,4,5-trimethoxybenzyl)piperazine,



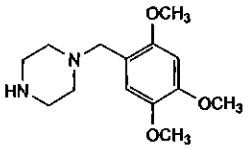
B. 1,4-bis(2,3,4-trimethoxybenzyl)piperazine,



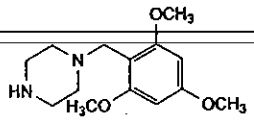
C. 2,3,4-trimethoxybenzaldehyde,



D. (2,3,4-trimethoxyphenyl)methanol,

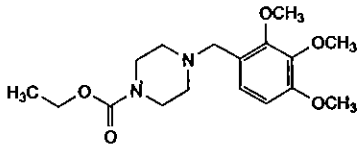


E. 1-(2,4,5-trimethoxybenzyl)piperazine,

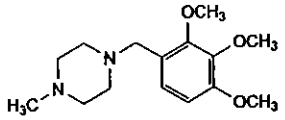


F. 1-(2,4,6-trimethoxybenzyl)piperazine,

G. piperazine,



H. ethyl 4-(2,3,4-trimethoxybenzyl)piperazine-1-carboxylate,

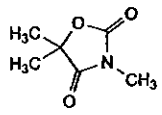


I. 1-methyl-4-(2,3,4-trimethoxybenzyl)piperazine (*N*-methyltrimetazidine).

Ph Eur

Trimethadione

(Ph. Eur. monograph 0440)



$C_6H_9NO_3$ 143.1 *127-48-0*

Action and use
Antiepileptic.

Ph Eur

DEFINITION
3,5,5-Trimethyloxazolidine-2,4-dione.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS
Appearance
Colourless or almost colourless crystals.

Solubility
Soluble in water, very soluble in ethanol (96 per cent).

IDENTIFICATION
First identification: B.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 45 °C to 47 °C, determined without previous drying.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs prepared using 3 mg of substance per 0.4 g of *potassium bromide R*.

Comparison *trimethadione CRS*.

C. To 2 mL of solution S (see Tests) add 1 mL of *barium hydroxide solution R*. A white precipitate is formed, which dissolves on addition of 1 mL of *dilute hydrochloric acid R*.

D. Dissolve 0.3 g in a mixture of 5 mL of *alcoholic potassium hydroxide solution R* and 5 mL of *ethanol (96 per cent) R*. Allow to stand for 10 min. Add 0.05 mL of *phenolphthalein solution R1* and neutralise exactly with *hydrochloric acid R*. Evaporate to dryness on a water-bath and take up the residue with 4 quantities, each of 5 mL, of *ether R*. Filter the combined ether layers and evaporate to dryness. The residue, recrystallised from 5 mL of *toluene R* and dried, melts (*2.2.14*) at about 80 °C.

TESTS
Solution S
Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 40 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity
To 10 mL of solution S add 0.1 mL of *methyl red solution R*. Not more than 0.1 mL of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.00 g by drying in a desiccator over *anhydrous silica gel R* for 6 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Gas chromatography (*2.2.28*).

Internal standard solution Dissolve 0.125 g of *decanol R* in *anhydrous ethanol R* and dilute to 25 mL with the same solvent.

Test solution Dissolve 0.100 g of the substance to be examined in the internal standard solution and dilute to 10.0 mL with the same solution.

Reference solution Dissolve 0.100 g of *trimethadione CRS* in the internal standard solution and dilute to 10.0 mL with the same solution.

Column:
— *material:* stainless steel,
— *size:* *l* = 0.75 m, Ø = 3 mm,
— *stationary phase:* *styrene-divinylbenzene copolymer R* (125-150 µm).

Carrier gas *nitrogen for chromatography R*.

Flow rate 20 mL/min.

Temperature:
— *column*: 210 °C,
— *injection port*: 240 °C,
— *detector*: 270 °C.

Detection Flame ionisation.

Injection 1 µL.

Calculate the content of $C_6H_9NO_3$ from the declared content of *trimethadione CRS*.

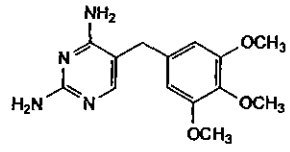
STORAGE

Protected from light.

Ph Eur

Trimethoprim

(Ph. Eur. monograph 0060)



$C_{14}H_{18}N_4O_3$ 290.3 *738-70-5*

Action and use

Dihydrofolate reductase inhibitor; antibacterial.

Preparations

Co-trimoxazole Infusion

Co-trimoxazole Oral Suspension

Paediatric Co-trimoxazole Oral Suspension

Co-trimoxazole Tablets

Co-trimoxazole Dispersible Tablets

Paediatric Co-trimoxazole Tablets

Trimethoprim Oral Suspension

Trimethoprim Tablets

Ph Eur

DEFINITION

5-(3,4,5-Trimethoxybenzyl)pyrimidine-2,4-diamine.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or yellowish-white powder.

Solubility

Very slightly soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: C.

Second identification: A, B, D.

A. Melting point (*2.2.14*): 199 °C to 203 °C.

B. Dissolve about 20 mg in *0.1 M sodium hydroxide* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of this solution to 10.0 mL with *0.1 M sodium hydroxide*. Examined between 230 nm and 350 nm (*2.2.25*), the solution shows an absorption maximum at 287 nm. The specific absorbance at the absorption maximum is 240 to 250.

C. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *trimethoprim CRS*.

D. Dissolve about 25 mg, heating if necessary, in 5 mL of *0.005 M sulfuric acid* and add 2 mL of a 16 g/L solution of *potassium permanganate R* in *0.1 M sodium hydroxide*. Heat to boiling and add to the hot solution 0.4 mL of *formaldehyde R*. Mix, add 1 mL of *0.5 M sulfuric acid*, mix and heat again to boiling. Cool and filter. To the filtrate, add 2 mL of *methylene chloride R* and shake vigorously. The organic layer, examined in ultraviolet light at 365 nm, shows green fluorescence.

TESTS

Appearance of solution

The solution is not more intensely coloured than reference solution BY_7 (*2.2.2, Method II*).

Dissolve 0.5 g in 10 mL of a mixture of 1 volume of *water R*, 4.5 volumes of *methanol R* and 5 volumes of *methylene chloride R*.

Related substances

A. Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 200.0 mL with the mobile phase.

Reference solution (b) Dissolve the contents of a vial of *trimethoprim for system suitability CRS* (containing impurity E) in 1 mL of the mobile phase.

Column:
— *size*: *l* = 0.250 m, Ø = 4.0 mm;
— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 30 volumes of *methanol R* and 70 volumes of a 1.4 g/L solution of *sodium perchlorate R* adjusted to pH 3.6 with *phosphoric acid R*.

Flow rate 1.3 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µL loop injector.

Run time 11 times the retention time of trimethoprim.

Relative retention With reference to trimethoprim (retention time = about 5 min): impurity C = about 0.8; impurity E = about 0.9; impurity A = about 1.5; impurity D = about 2.0; impurity G = about 2.1; impurity B = about 2.3; impurity J = about 2.7; impurity F = about 4.0.

System suitability Reference solution (b):
— *resolution*: minimum 2.5 between the peaks due to impurity E and trimethoprim.

Limits:
— *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.43; impurity E = 0.53; impurity J = 0.66;
— *any impurity*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *total*: not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *disregard limit*: 0.04 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent); disregard any peak

corresponding to impurity H (relative retention = about 10.3).

B. Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 200.0 mL with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *trimethoprim CRS* and 5.0 mg of *trimethoprim impurity B CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase.

Column:
- *size*: l = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *nitrile silica gel for chromatography R* (5 µm) with a specific surface area of 350 m^2/g and a pore diameter of 10 nm.

Mobile phase Dissolve 1.14 g of *sodium hexanesulfonate R* in 600 mL of a 13.6 g/L solution of *potassium dihydrogen phosphate R*; adjust to pH 3.1 with *phosphoric acid R* and mix with 400 mL of *methanol R*.

Flow rate 0.8 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µL loop injector.

Run time 6 times the retention time of trimethoprim.

Relative retention With reference to trimethoprim (retention time = about 4 min): impurity H = about 1.8; impurity I = about 4.9.

System suitability Reference solution (b):
- *resolution*: minimum 2.0 between the peaks due to trimethoprim and impurity B.

Limits:
- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity H = 0.50; impurity I = 0.28;
- *any impurity*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- *total*: not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *disregard limit*: 0.04 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent); disregard any peak due to impurity B (relative retention = about 1.3).

Impurity K

Gas chromatography (*2.2.28*).

Test solution Dissolve 0.500 g of the substance to be examined in 35.0 mL of *citrate buffer solution pH 5.0 R*, add 10.0 mL of *1,1-dimethylethyl methyl ether R*, shake thoroughly and centrifuge for 10 min. Use the upper layer.

Reference solution Dilute 5.0 mL of *hydrochloric acid R* to 50.0 mL with *water R*, add 12.5 mg of *aniline R* and shake thoroughly. Add 10.0 µL of this solution and 10.0 mL of *1,1-dimethylethyl methyl ether R* to 35.0 mL of *citrate buffer solution pH 5.0 R*, shake thoroughly and centrifuge for 10 min. Use the upper layer.

Column:
- *material*: fused silica;
- *size*: l = 30 m, Ø = 0.53 mm;
- *stationary phase*: *methylpolysiloxane R* (film thickness 3 µm).

Carrier gas *helium for chromatography R*.

Flow rate 12 mL/min.

Temperature:
- *column*: 80 °C;
- *injection port*: 230 °C;
- *detector*: 270 °C.

Detection Nitrogen-phosphorus detector.

Injection 3 µL.

Run time 15 min.

System suitability Reference solution:
- *repeatability*: maximum relative standard deviation of 5.0 per cent after 6 injections.

Limit:
- *impurity K*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (5 ppm).

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 29.03 mg of $C_{14}H_{18}N_4O_3$.

IMPURITIES

Test A for related substances

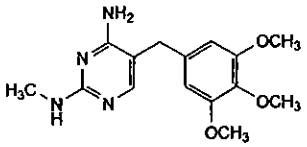
A, B, C, D, E, F, G, H, J.

Test B for related substances

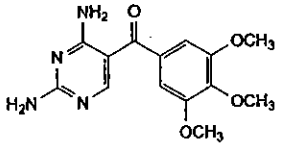
B, H, I.

Test for impurity K

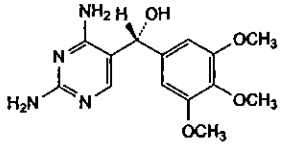
K.



A. N^2-methyl-5-(3,4,5-trimethoxybenzyl)pyrimidine-2,4-diamine,

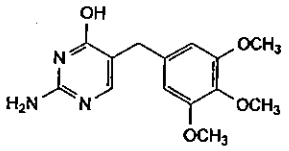


B. (2,4-diaminopyrimidin-5-yl)(3,4,5-trimethoxyphenyl)methanone,

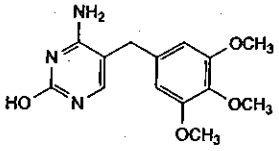


and enantiomer

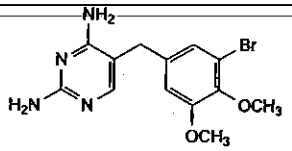
C. (*RS*)-(2,4-diaminopyrimidin-5-yl)(3,4,5-trimethoxyphenyl) methanol,



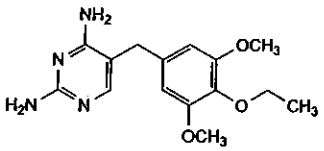
D. 2-amino-5-(3,4,5-trimethoxybenzyl)pyrimidin-4-ol,



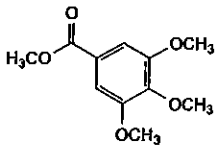
E. 4-amino-5-(3,4,5-trimethoxybenzyl)pyrimidin-2-ol,



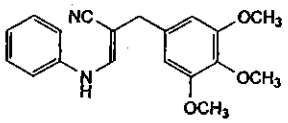
F. 5-(3-bromo-4,5-dimethoxybenzyl)pyrimidine-2,4-diamine,



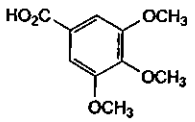
G. 5-(4-ethoxy-3,5-dimethoxybenzyl)pyrimidine-2,4-diamine,



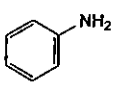
H. methyl 3,4,5-trimethoxybenzoate,



I. 3-(phenylamino)-2-(3,4,5-trimethoxybenzyl)prop-2-enenitrile,



J. 3,4,5-trimethoxybenzoic acid,

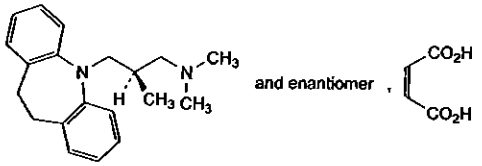


K. aniline.

Ph Eur

Trimipramine Maleate

(Ph. Eur. monograph 0534)



$C_{24}H_{30}N_2O_4$ 410.5 *521-78-8*

Action and use
Monoamine reuptake inhibitor; tricyclic antidepressant.

Preparation
Trimipramine Tablets

Ph Eur

DEFINITION

(2*RS*)-3-(10,11-Dihydro-5*H*-dibenzo[*b*,*f*]azepin-5-yl)-*N*,*N*,2-trimethylpropan-1-amine hydrogen (2*Z*)-but-2-enedioate.

Content
98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Slightly soluble in water and in ethanol (96 per cent).

IDENTIFICATION

A. Melting point (*2.2.14*): 140 °C to 144 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *trimipramine maleate CRS.*

TESTS

Related substances
Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Buffer solution pH 7.7 2.64 g/L solution of *ammonium phosphate R* in *water for chromatography R*; adjust to pH 7.7 with *phosphoric acid R.*

Test solution Dissolve 50 mg of the substance to be examined in the mobile phase and dilute to 100 mL with the mobile phase.

Reference solution (a) Dissolve 5 mg of the substance to be examined and 5 mg of *iminodibenzyl R* (impurity F) in the mobile phase and dilute to 10 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (c) Dissolve the contents of a vial of *trimipramine for peak identification CRS* (containing impurities A, B, C, D and E) in 1 mL of *acetonitrile R1.*

Column:
— *size: l* = 0.125 m, Ø = 4.0 mm;
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *acetonitrile R1,* buffer solution pH 7.7 (38:62 *V/V*).

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µL.

Run time 3 times the retention time of trimipramine.

Identification of impurities Use the chromatogram supplied with *trimipramine for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, C, D and E; doubling of the peak due to impurity E may be observed.

Relative retention With reference to trimipramine (retention time = about 27 min): impurity A = about 0.1; impurity B = about 0.3; impurity C = about 0.4; impurity D = about 0.5; impurity F = about 1.4; impurity E = about 1.5.

System suitability:
- *resolution*: minimum 3.5 between the peaks due to trimipramine and impurity F in the chromatogram obtained with reference solution (a);
- the chromatogram obtained with reference solution (c) is similar to the chromatogram supplied with *trimipramine for peak identification CRS*.

Limits:
- *correction factor*: for the calculation of content, multiply the peak area of impurity F by 0.5;
- *impurity E*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
- *impurity F*: not more than 2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *impurities A, B, C, D*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.350 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 41.05 mg of $C_{24}H_{30}N_2O_4$.

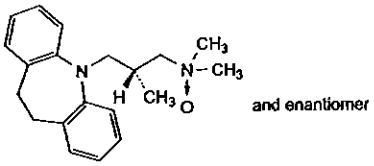
STORAGE

Protected from light.

IMPURITIES

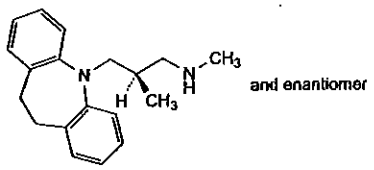
Specified impurities *A, B, C, D, E, F.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) G.



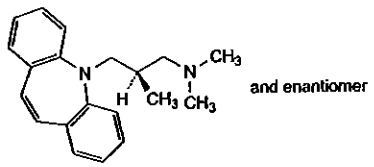
and enantiomer

A. (2*RS*)-3-(10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepin-5-yl)-*N*,*N*,2-trimethylpropan-1-amine *N*-oxide,



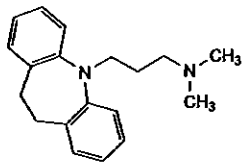
and enantiomer

B. (2*RS*)-3-(10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepin-5-yl)-*N*,2-dimethylpropan-1-amine,

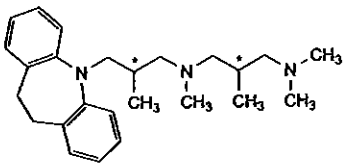


and enantiomer

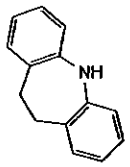
C. (2*RS*)-3-(5*H*-dibenzo[b,f]azepin-5-yl)-*N*,*N*,2-trimethylpropan-1-amine,



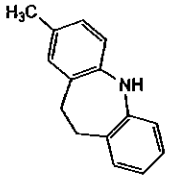
D. imipramine,



E. mixture of the stereoisomers of *N*-[3-(10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepin-5-yl)-2-methylpropyl]-*N*,*N*′,*N*′,2-tetramethylpropane-1,3-diamine,

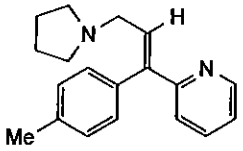


F. 10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine,



G. 2-methyl-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine.

Triprolidine Hydrochloride



,HCl

$C_{19}H_{22}N_2,HCl,H_2O$ 332.9 *6138-79-0*

Action and use
Histamine H_1 receptor antagonist; antihistamine.

Preparation
Triprolidine Tablets

DEFINITION
Triprolidine Hydrochloride is (*E*)-2-(3-pyrrolidin-1-yl-1-*p*-tolylprop1-enyl)pyridine hydrochloride monohydrate.
It contains not less than 98.5% and not more than 101.0% of $C_{19}H_{22}N_2,HCl$, calculated with reference to the anhydrous substance.

CHARACTERISTICS
A white, crystalline powder.

Freely soluble in *water*; freely soluble in *ethanol (96%)* and practically insoluble in *ether*.

IDENTIFICATION
A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of triprolidine hydrochloride *(RS 356)*.

B. Yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS
Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *methanol*.

(1) 1.0% w/v of the substance being examined.

(2) 0.020% w/v of *Z-triprolidine hydrochloride BPCRS*.

(3) 0.010% w/v of the substance being examined.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating silica gel F_{254} (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air, examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

Equal volumes of *butan-2-one* and *dimethylformamide*.

LIMITS

In the chromatogram obtained with solution (1):

any spot corresponding to *Z*-triprolidine is not more intense than the spot in the chromatogram obtained with solution (2);

and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (3).

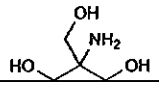
Sulfated ash
Not more than 0.1%, Appendix IX A.

Water
4.5 to 6.0% w/w, Appendix IX C. Use 0.4 g.

ASSAY
Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.25 g dissolved in a mixture of 50 mL of *anhydrous acetic acid* and 0.5 mL of *acetic anhydride* and *crystal violet solution* as indicator. Each mL of *0.1M perchloric acid VS* is equivalent to 15.74 mg of $C_{19}H_{22}N_2$, HCl.

Trometamol

(Ph. Eur. monograph 1053)



$C_4H_{11}NO_3$ 121.1 *77-86-1*

Action and use
Organic amine proton acceptor; alkalinizing agent.

Ph Eur

DEFINITION
Trometamol contains not less than 99.0 per cent and not more than the equivalent of 100.5 per cent of aminomethylidynetri(methanol), calculated with reference to the dried substance.

CHARACTERS
A white or almost white, crystalline powder, or colourless crystals, freely soluble in water, sparingly soluble in alcohol, very slightly soluble in ethyl acetate.

IDENTIFICATION
First identification: B, C.

Second identification: A, B, D.

A. Solution S (see Tests) is strongly alkaline (*2.2.4*).

B. Melting point (*2.2.14*): 168 °C to 174 °C.

C. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *trometamol CRS*.

D. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS
Solution S
Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)
The pH of freshly prepared solution S is 10.0 to 11.5.

Related substances
Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance. Wash the plate with *methanol R* before applying the solutions.

Test solution (a) Dissolve 0.20 g in 1 mL of *water R*, with heating, and dilute to 10 mL with *methanol R*.

Test solution (b) Dilute 1 mL of test solution (a) to 10 mL with *methanol R*.

Reference solution (a) Dissolve 20 mg of *trometamol CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dilute 1 mL of test solution (a) to 100 mL with *methanol R*.

Apply to the plate 10 µL of each solution. Develop over a path of 10 cm using a mixture of 10 volumes of *dilute ammonia R1* and 90 volumes of *2-propanol R*. Dry the plate at 100 °C to 105 °C. Spray with a 5 g/L solution of *potassium permanganate R* in a 10 g/L solution of *sodium carbonate R*. After about 10 min examine in daylight. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.0 per cent).

Chlorides (*2.4.4*)
To 10 mL of solution S add 2.5 mL of *dilute nitric acid R* and dilute to 15 mL with *water R*. The solution complies with the limit test for chlorides (100 ppm).

Iron (*2.4.9*)
Dissolve 1.0 g in *water R* and dilute to 10 mL with the same solvent. The solution complies with the limit test for iron (10 ppm).

Loss on drying (*2.2.32*)
Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

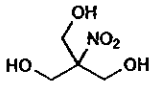
Sulfated ash (*2.4.14*)
Not more than 0.1 per cent, determined on 1.0 g.

Bacterial endotoxins (*2.6.14*)
Less than 0.03 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY
Dissolve 0.100 g in 20 mL of *water R*. Add 0.2 mL of *methyl red solution R*. Titrate with *0.1 M hydrochloric acid* until the colour changes from yellow to red.

1 mL of *0.1 M hydrochloric acid* is equivalent to 12.11 mg of $C_4H_{11}NO_3$.

IMPURITIES

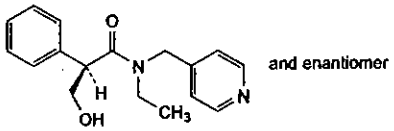


A. nitromethylidynetri(methanol).

Ph Eur

Tropicamide

(Ph. Eur. monograph 1159)



and enantiomer

$C_{17}H_{20}N_2O_2$ 284.4 *1508-75-4*

Action and use
Anticholinergic.

Preparation
Tropicamide Eye Drops

Ph Eur

DEFINITION
(2*RS*)-*N*-Ethyl-3-hydroxy-2-phenyl-*N*-(pyridin-4-ylmethyl) propanamide.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Slightly soluble in water, freely soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION
First identification: C.
Second identification: A, B, D, E.

A. Melting point (*2.2.14*): 95 °C to 98 °C.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 20.0 mg in *0.1 M hydrochloric acid* and dilute to 50.0 mL with the same acid. Dilute 2.0 mL of this solution to 20.0 mL with *0.1 M hydrochloric acid*.

Spectral range 230-350 nm.

Absorption maximum At 254 nm.

Specific absorbance at the absorption maximum 170 to 190.

C. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *tropicamide CRS*.

D. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *methylene chloride R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 10 mg of *tropicamide CRS* in *methylene chloride R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *concentrated ammonia R, methanol R, methylene chloride R* (0.5:5:95 *V/V/V*).

Application 10 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the spot in the chromatogram obtained with the reference solution.

E. Dissolve about 5 mg in 3 mL of a mixture of 9 mL of *acetic anhydride R*, 1 mL of *acetic acid R* and 0.1 g of *citric acid monohydrate R*. Heat on a water-bath for 5-10 min. A reddish-yellow colour is produced.

TESTS
Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 0.1 g in *ethanol (96 per cent) R* and dilute to 10 mL with the same solvent.

Optical rotation (*2.2.7*)
−0.1° to + 0.1°.

Dissolve 2.5 g in *anhydrous ethanol R* and dilute to 25.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in 3 mL of *acetonitrile R1* and dilute to 50.0 mL with *water for chromatography R*.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *water for chromatography R*. Dilute 1.0 mL of this solution to 10.0 mL with *water for chromatography R*.

Reference solution (b) Dissolve 5 mg of *4-[(ethylamino) methyl]pyridine R* (impurity A), 5.0 mg of *tropicamide impurity C CRS* and 5.0 mg of *tropicamide impurity D CRS* in 2 mL of *acetonitrile R1* and dilute to 50.0 mL with *water for chromatography R*. Dilute 1.0 mL of this solution to 10.0 mL with *water for chromatography R*.

Reference solution (c) Dissolve 5 mg of *tropicamide for peak identification CRS* (containing impurity B) in 1.0 mL of *acetonitrile R1* and dilute to 10.0 mL with *water for chromatography R*.

Reference solution (d) To 1 mL of reference solution (b) add 1 mL of reference solution (c).

Reference solution (e) Dilute 1.5 mL of reference solution (b) to 10.0 mL with *water for chromatography R*.

Column:
- *size*: l = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm);
- *temperature*: 40 °C.

Mobile phase Dissolve 0.135 g of *sodium dodecyl sulfate R* and 3.4 mL of *phosphoric acid R* in 950 mL of *water for chromatography R*. Adjust to pH 3.0 with *strong sodium hydroxide solution R* and dilute to 1000 mL with *water for chromatography R*. Mix 73 volumes of this solution with 27 volumes of *acetonitrile R1*.

Flow rate 0.8 mL/min.

Detection Spectrophotometer at 210 nm and at 254 nm.

Injection 15 µL.

Run time 3 times the retention time of tropicamide.

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peak due to impurity B; use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, C and D.

Relative retention With reference to tropicamide (retention time = about 11 min): impurity C = about 0.4; impurity A = about 0.5; impurity D = about 0.8; impurity B = about 2.3.

System suitability Reference solution (d):
- *resolution at 210 nm*: minimum 2.0 between the peaks due to impurities C and A;
- *resolution at 210 nm*: minimum 2.0 between the peaks due to impurities A and D.

Limits:
- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.8; impurity B = 0.6;
- *impurity B at 254 nm*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
- *impurity A at 254 nm*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);
- *impurity C at 210 nm*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (e) (0.15 per cent);
- *impurity D at 210 nm*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (e) (0.15 per cent);
- *unspecified impurities at 254 nm*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *sum of impurities other than C and D at 254 nm*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit at 254 nm*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Chlorides (*2.4.4*)
Maximum 100 ppm.

Dissolve 1.0 g with heating in 8 mL of *acetic acid R*, cool and dilute to 10 mL with the same acid. Dilute 5 mL of this solution to 15 mL with *water R*.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 80 °C at a pressure not exceeding 0.7 kPa for 4 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 50 mL of *anhydrous acetic acid R*. Add 0.2 mL of *naphtholbenzein solution R* and titrate with *0.1 M perchloric acid* until the colour changes from orange to green.

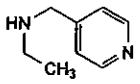
1 mL of *0.1 M perchloric acid* is equivalent to 28.44 mg of $C_{17}H_{20}N_2O_2$.

STORAGE

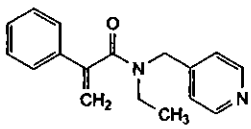
Protected from light.

IMPURITIES

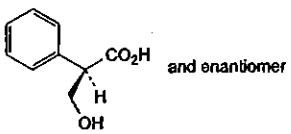
Specified impurities *A, B, C, D.*



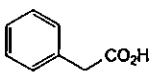
A. *N*-(pyridin-4-ylmethyl)ethanamine,



B. *N*-ethyl-2-phenyl-*N*-(pyridin-4-ylmethyl)propenamide,



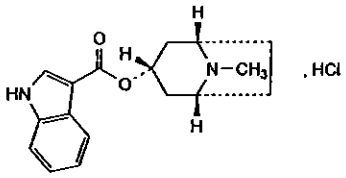
C. (2*RS*)-3-hydroxy-2-phenylpropanoic acid (tropic acid),



D. phenylacetic acid.

Tropisetron Hydrochloride

(Ph. Eur. monograph 2102)



$C_{17}H_{21}ClN_2O_2$ 320.8 *105826-92-4*

Action and use
Serotonin $5HT_3$ receptor antagonist; antiemetic.

Ph Eur

DEFINITION
Hydrochloride of (1*R*,3*r*,5*S*)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl 1*H*-indole-3-carboxylate.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Freely soluble or soluble in water, sparingly soluble in ethanol (96 per cent), very slightly soluble in methylene chloride.

IDENTIFICATION
First identification: B, D.
Second identification: A, C, D.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 50 mg in *methanol R* and dilute to 25.0 mL with the same solvent. Dilute 1.0 mL of this solution to 100.0 mL with *methanol R*.

Spectral range 220-360 nm.

Absorption maxima At 228 nm and 282 nm.

Absorbance ratio A_{228}/A_{282} = 1.3 to 1.4.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *tropisetron hydrochloride CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 5 mg of the substance to be examined in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 5 mL with the same mixture of solvents.

Reference solution Dissolve 5 mg of *tropisetron hydrochloride CRS* in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 5 mL with the same mixture of solvents.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *anhydrous formic acid R, water R, methanol R, methylene chloride R* (2:2:30:70 *V/V/V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying In cold air.

Detection A Examine in ultraviolet light at 254 nm.

Detection B Spray first with a solution prepared as follows: dissolve 0.85 g of *bismuth subnitrate R* in a mixture of 10 mL of *acetic acid R* and 40 mL of *water R*; to 5 mL of this solution add 5 mL of a 400 g/L solution of *potassium iodide R* and dilute to 100 mL with *water R*. Then spray with *strong hydrogen peroxide solution R*.

Results For both detection methods, the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

D. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution B_7 (*2.2.2, Method II*).

Dissolve 1.00 g in *water R* and dilute to 20 mL with the same solvent.

Impurity A
Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.200 g of the substance to be examined in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 5.0 mL with the same mixture of solvents.

Reference solution (a) Dissolve 5.0 mg of *tropine CRS* (impurity A) in a mixture of equal volumes of *methanol R* and *methylene chloride R* and dilute to 25.0 mL with the same mixture of solvents.

Reference solution (b) Dilute 1.0 mL of the test solution to 20.0 mL with a mixture of equal volumes of *methanol R* and *methylene chloride R*. To 0.1 mL of this solution add 1.0 mL of reference solution (a).

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *ammonia R, methanol R, methylene chloride R* (5:40:60 *V/V/V*).

Application 10 µL.

Development Over 2/3 of the plate.

Drying In a current of cold air.

Detection Dip the plate in *potassium iodobismuthate solution R1*.

System suitability Reference solution (b):
— the chromatogram shows 2 clearly separated spots.

Limit:
— *impurity A*: any spot due to impurity A is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.100 g of the substance to be examined in mobile phase A and dilute to 20.0 mL with mobile phase A.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

Reference solution (b) Dissolve 5.0 mg of *tropisetron impurity B CRS* and 5 mg of *ethyl indole-3-carboxylate CRS* in mobile phase A and dilute to 20.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 50.0 mL with mobile phase A.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: *triethylamine R, acetonitrile R, water R, methanol R* (0.3:35:400:565 *V/V/V/V*);

— *mobile phase B*: *triethylamine R, acetonitrile R, water R, methanol R* (0.3:100:100:800 *V/V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 14	100	0
14 - 32	100 → 0	0 → 100
32 - 36	0	100
36 - 37	0 → 100	100 → 0
37 - 52	100	0

Flow rate 2 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µL.

Relative retention With reference to tropisetron (retention time = about 22 min): impurity B = about 0.05; ethyl indole-3-carboxylate = about 0.2.

System suitability Reference solution (b):

— *resolution*: minimum 4 between the peaks due to impurity B and ethyl indole-3-carboxylate.

Limits:

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

N,N-Dimethylaniline

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 250 mg of the substance to be examined in mobile phase A and dilute to 5.0 mL with mobile phase A.

Reference solution Dissolve 10.0 mg of *N,N-dimethylaniline R* in mobile phase A and dilute to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 100.0 mL with mobile phase A.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: *triethylamine R, acetonitrile R, water R, methanol R* (0.3:35:400:565 *V/V/V/V*);

— *mobile phase B*: *triethylamine R, acetonitrile R, water R; methanol R* (0.3:100:100:800 *V/V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	100	0
10 - 11	100 → 0	0 → 100
11 - 30	0	100
30 - 31	0 → 100	100 → 0
31 - 50	100	0

Flow rate 1 mL/min.

Detection Spectrophotometer at 248 nm.

Injection 20 µL.

Limit:

— *N,N-dimethylaniline*: not more than the area of the principal peak in the chromatogram obtained with the reference solution (20 ppm).

Loss on drying (*2.2.32*)

Maximum 0.3 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

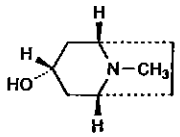
ASSAY

Dissolve 0.250 g in 10 mL of *anhydrous acetic acid R* and add 70 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

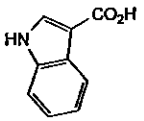
1 mL of *0.1 M perchloric acid* is equivalent to 32.08 mg of $C_{17}H_{21}ClN_2O_2$.

IMPURITIES

Specified impurities *A, B.*



A. (1*R*,3*r*,5*S*)-8-methyl-8-azabicyclo[3.2.1]octan-3-ol (tropine),

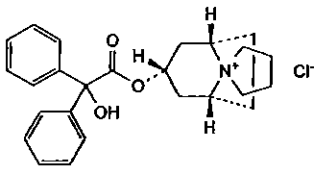


B. 1*H*-indole-3-carboxylic acid.

Ph Eur

Trospium Chloride

(Ph. Eur. monograph 1798)



$C_{25}H_{30}ClNO_3$ 428.0 *10405-02-4*

Action and use

Anticholinergic.

Preparations

Trospium Chloride Prolonged-release Capsules

Trospium Chloride Tablets

Ph Eur

DEFINITION

(1*R*,3*r*,5*S*)-3-[(Hydroxydiphenylacetyl)oxy]spiro[8-azoniabicyclo[3.2.1]octane-8,1′-pyrrolidinium] chloride.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Very soluble in water, freely soluble in methanol, practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *trospium chloride CRS.*

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S

Dissolve 3.0 g in *carbon dioxide-free water R* and dilute to 30 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_7 (*2.2.2, Method II*).

pH (*2.2.3*)

5.0 to 7.0.

Dilute 5 mL of solution S to 50 mL with *carbon dioxide-free water R.*

Impurity C

Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.20 g of the substance to be examined in 2.0 mL of *methanol R.*

Reference solution (a) Dissolve 1.0 mg of *trospium impurity C CRS* in 2.0 mL of *methanol R.*

Reference solution (b) Dilute 1.0 mL of test solution to 10.0 mL with *methanol R.* To 50 µL of this solution add 1 mL of reference solution (a).

Plate *TLC silica gel plate R.*

Mobile phase *glacial acetic acid R, hydrochloric acid R, acetonitrile R* (1:3.5:45 *V/V/V*).

Application 10 µL as bands.

Development Over 2/3 of the plate.

Drying In a current of warm air until the odour of acetic acid is no longer perceptible.

Detection Spray with *potassium iodobismuthate solution R* and subsequently with a 5 g/L solution of *sodium nitrite R.*

System suitability Reference solution (b):

— the chromatogram shows 2 clearly visible and separated zones.

Limit:

— *impurity C*: any zone due to impurity C is not more intense than the zone in the chromatogram obtained with reference solution (a) (0.5 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 30.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dissolve 6.0 mg of *trospium impurity A CRS* in the mobile phase and dilute to 20.0 mL with the mobile phase.

Reference solution (b) Dissolve 7.5 mg of *trospium impurity B CRS* in the mobile phase and dilute to 5.0 mL with the mobile phase.

Reference solution (c) Dilute a mixture of 0.3 mL of the test solution, 1.0 mL of reference solution (a) and 1.0 mL of reference solution (b) to 100.0 mL with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase Mix 1 volume of *triethylamine R* and 3 volumes of *phosphoric acid R* with 700 volumes of *water R* and add 300 volumes of *acetonitrile R.*

Flow rate 1 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µL.

Run time 3 times the retention time of trospium.

Relative retention With reference to trospium (retention time = about 10 min): impurity B = about 0.7; impurity A = about 1.9.

System suitability Reference solution (c):

— *resolution*: minimum 3 between the peaks due to impurity B and trospium.

Limits:

— *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *impurity A*: not more than 3 times the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.3 per cent);

— *unspecified impurities*: for each impurity, not more than 0.2 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (c) (0.10 per cent);

— *total*: not more than twice the area of the peak due to impurity B in the chromatogram obtained with reference solution (c) (1.0 per cent);

— *disregard limit*: 0.1 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (c) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in 50 mL of *water R.* Titrate with *0.1 M silver nitrate,* determining the end-point potentiometrically (*2.2.20*).

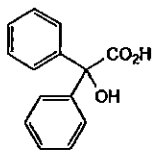
1 mL of *0.1 M silver nitrate* is equivalent to 42.80 mg of $C_{25}H_{30}ClNO_3$.

STORAGE

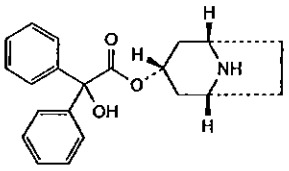
Protected from light.

IMPURITIES

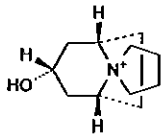
Specified impurities *A, B, C.*



A. hydroxydiphenylacetic acid (benzilic acid),



B. (1*R*,3*r*,5*S*)-8-azabicyclo[3.2.1]oct-3-yl hydroxydiphenylacetate,

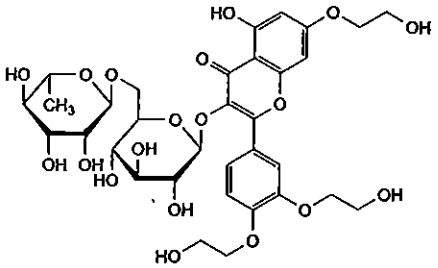


C. (1*R*,3*r*,5*S*)-3-hydroxyspiro[8-azoniabicyclo[3.2.1] octane8,1′-pyrrolidinium].

Ph Eur

Troxerutin

(Ph. Eur. monograph 2133)



$C_{33}H_{42}O_{19}$ 743 *7085-55-4*

Action and use
Bioflavonoid.

Ph Eur

DEFINITION
Mixture of *O*-hydroxyethylated derivatives of rutoside containing minimum 80 per cent of 2-[3,4-bis(2-hydroxyethoxy)phenyl]-3-[[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-7-(2-hydroxyethoxy)-4*H*-1-benzopyran-4-one (tris(hydroxyethyl) rutin).

Content
95.0 per cent to 105.0 per cent (dried substance).

CHARACTERS
Appearance
Yellowish-green, crystalline powder, hygroscopic.

Solubility
Freely soluble in water, slightly soluble in ethanol (96 per cent) and practically insoluble in methylene chloride.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *troxerutin CRS*.

B. Examine the chromatograms obtained in the test for composition.

Results The principal peak in the chromatogram obtained with the test solution is similar in position and size to the principal peak in the chromatogram obtained with the reference solution (a).

TESTS
Composition
Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution Dissolve 10.0 mg of the substance to be examined in the mobile phase, if necessary using an ultrasonic bath and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dissolve 10.0 mg of *troxerutin CRS* in the mobile phase, if necessary using an ultrasonic bath and dilute to 10.0 mL with the mobile phase.

Reference solution (b) Dilute 1 mL of reference solution (a) to 10 mL with the mobile phase. Dilute 1 mL of this solution to 100 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm,
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 20 volumes of *acetonitrile R* and 80 volumes of a 15.6 g/L solution of *sodium dihydrogen phosphate R* adjusted to pH 4.4 with *dilute phosphoric acid R* or *dilute sodium hydroxide solution R*.

Flow rate 0.5 mL/min.

Detection Spectrophotometer at 350 nm.

Injection 10 µL.

Run time Twice the retention time of the main compound of troxerutin (tris(hydroxyethyl)rutin).

Relative retention With reference to tris(hydroxyethyl)rutin (retention time = about 25 min): tetrakis (hydroxyethyl)rutin = about 0.5; mono(hydroxyethyl) rutin = about 0.8; bis(hydroxyethyl)rutin = about 1.1.

System suitability Reference solution (a):
— *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to bis(hydroxyethyl) rutin and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to tris(hydroxyethyl)rutin;
— *signal-to-noise ratio*: minimum 10 for the principal peak in the chromatogram obtained with reference solution (b).

Limits:
— *principal peak*: minimum 80 per cent,
— *any other peak*: for each peak, maximum 5 per cent, except for 1 peak which can be maximum 10 per cent.
— *disregard limit*: area of the principal peak in the chromatogram obtained with reference solution (b).

Ethylene oxide
Head-space gas chromatography (*2.2.28*).

Test solution To 1.00 g of the substance to be examined in a vial, add 1.0 mL of *water R*. Mix to obtain a homogeneous solution. Heat at 70 °C for 45 min.

Reference solution To 1.00 g of the substance to be examined in a vial, add 50 µL of *ethylene oxide solution R4* and 950 µL of *water R* and close tightly. Mix to obtain a homogeneous solution. Heat at 70 °C for 45 min.

Column:
— *material*: fused silica,
— *size*: *l* = 30 m, Ø = 0.32 mm,
— *stationary phase*: *cyanopropyl(7)phenyl(7)methyl(86) polysiloxane R* (film thickness 1 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1.1 mL/min.

Static head-space conditions which may be used:
— *equilibration temperature*: 70 °C,
— *equilibration time*: 45 min,

— *transfer line temperature*: 110 °C,
— *pressurisation time*: 2 min,
— *injection time*: 12 s.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 5	40
	5 - 18	40 → 200
Injection port		150
Detector		250

Detection Flame ionisation.

Injection 1.0 mL.

The peak due to ethylene oxide is identified by injecting solutions of ethylene oxide of increasing concentration.

Determine the content of ethylene oxide (ppm) in the substance to be examined using the following expression:

$$\frac{A_1 \times m_1}{(A_2 \times m_2) - (A_1 \times m_3)}$$

A_1 = area of the peak due to ethylene oxide in the chromatogram obtained with the test solution,
A_2 = area of the peak due to ethylene oxide in the chromatogram obtained with the reference solution,
m_1 = mass of ethylene oxide in the reference solution, in micrograms,
m_2 = mass of the substance to be examined in the test solution, in grams,
m_3 = mass of the substance to be examined in the reference solution, in grams.

Limit:
— *ethylene oxide*: maximum 1 ppm.

Loss on drying (*2.2.32*)
Maximum 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Sulfated ash (*2.4.14*)
Maximum 0.4 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 100.0 mL of *water R*. Dilute 10.0 mL of this solution to 100.0 mL with *water R*. Dilute 10.0 mL to 100.0 mL with *water R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 350 nm.

Calculate the percentage content of $C_{33}H_{42}O_{19}$ taking the specific absorbance to be 250.

STORAGE

In an airtight container, protected from light.

Ph Eur

Trypsin

(Ph. Eur. monograph 0694)

9002-07-7

Action and use
Proteolytic enzyme.

Ph Eur

DEFINITION

Trypsin is a proteolytic enzyme obtained by the activation of trypsinogen extracted from the pancreas of mammals. It has an activity of not less than 0.5 microkatal per milligram, calculated with reference to the dried substance. In solution, it has maximum enzymic activity at pH 8; the activity is reversibly inhibited at pH 3, the pH at which it is most stable.

PRODUCTION

The animals from which trypsin is derived must fulfil the requirements for the health of animals suitable for human consumption.

The method of manufacture is validated to demonstrate that the product, if tested, would comply with the following test.

Histamine (*2.6.10*)
Not more than 1 µg of histamine base per 0.2 microkatal of trypsin activity. Use a 10 g/L solution of the substance to be examined in *0.0015 M borate buffer solution pH 8.0 R* inactivated by heating on a water-bath for 30 min. Carry out dilutions with a 9 g/L solution of *sodium chloride R*.

CHARACTERS

Appearance
White or almost white, crystalline or amorphous powder, hygroscopic if amorphous.

Solubility
Sparingly soluble in water.

IDENTIFICATION

A. Dilute 1 mL of solution S (see Tests) to 100 mL with *water R*. In a depression in a white spot-plate, mix 0.1 mL of this solution with 0.2 mL of *tosylarginine methyl ester hydrochloride solution R*. A reddish-violet colour develops within 3 min.

B. Dilute 0.5 mL of solution S to 5 mL with *water R*. Add 0.1 mL of a 20 g/L solution of *tosyl-lysyl-chloromethane hydrochloride R*. Adjust to pH 7.0, shake for 2 h and dilute to 50 mL with *water R*. In one of the depressions of a white spot-plate, mix 0.1 mL of this solution with 0.2 mL of *tosylarginine methyl ester hydrochloride solution R*. No reddish-violet colour develops within 3 min.

TESTS

Solution S
Dissolve 0.10 g in *carbon dioxide-free water R* and dilute to 10.0 mL with the same solvent.

Appearance of solution
Solution S is not more opalescent than reference suspension III (*2.2.1*).

pH (*2.2.3*)
3.0 to 6.0 for solution S.

Specific absorbance (*2.2.25*)
13.5 to 16.5, determined at the absorption maximum at 280 nm; maximum 7.0, determined at the absorption minimum at 250 nm.

Dissolve 30.0 mg in *0.001 M hydrochloric acid* and dilute to 100.0 mL with the same acid.

Chymotrypsin
Test solution To 1.8 mL of *buffer solution pH 8.0 R* add 7.4 mL of *water R* and 0.5 mL of *0.2 M acetyltyrosine ethyl ester R*. While shaking the solution, add 0.3 mL of solution S and start a timer. After exactly 5 min, measure the pH (*2.2.3*).

Reference solution Prepare in the same manner as the test solution, replacing solution S by 0.3 mL of a 0.5 g/L solution of *chymotrypsin BRP*, and measure the pH (*2.2.3*) exactly 5 min after adding the chymotrypsin.

The pH of the test solution is higher than that of the reference solution.

Loss on drying (*2.2.32*)
Not more than 5.0 per cent, determined on 0.500 g by drying at 60 °C at a pressure not exceeding 0.67 kPa for 2 h.

Microbial contamination
TAMC: acceptance criterion 10^4 CFU/g (*2.6.12*).
TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).
Absence of *Escherichia coli* (*2.6.13*).
Absence of *Salmonella* (*2.6.13*).

ASSAY

The activity of trypsin is determined by comparing the rate at which it hydrolyses *benzoylarginine ethyl ester hydrochloride R* with the rate at which *trypsin BRP* hydrolyses the same substrate in the same conditions.

Apparatus Use a reaction vessel of about 30 mL capacity provided with:
— a device that will maintain a temperature of 25.0 ± 0.1 °C;
— a stirring device (for example, a magnetic stirrer);
— a lid with holes for the insertion of electrodes, the tip of the burette, a tube for the admission of nitrogen and the introduction of reagents.

An automatic or manual titration device may be used. For the latter, the burette is graduated in 0.005 mL and the pH meter is provided with a wide-range scale and glass-silver-silver chloride or other suitable electrodes.

Test solution Dissolve a suitable quantity of the substance to be examined in *0.001 M hydrochloric acid* and dilute to 25.0 mL with the same acid in order to obtain a solution containing approximately 700 nanokatals per millilitre.

Reference solution Dissolve a suitable quantity of *trypsin BRP* in *0.001 M hydrochloric acid* and dilute to 25.0 mL with the same acid in order to obtain a solution containing approximately 700 nanokatals per millilitre.

Store the solutions at 0-5 °C. Warm 1 mL of each solution to about 25 °C over 15 min and use 50 µL of each solution for each titration. Carry out the titration in an atmosphere of nitrogen. Transfer 10.0 mL of *0.0015 M borate buffer solution pH 8.0 R* to the reaction vessel and, while stirring, add 1.0 mL of a freshly prepared 6.86 g/L solution of *benzoylarginine ethyl ester hydrochloride R*. When the temperature is steady at 25.0 ± 0.1 °C (after about 5 min) adjust to pH 8.0 exactly with *0.1 M sodium hydroxide*. Add 50 µL of the test solution and start a timer. Maintain at pH 8.0 by the addition of *0.1 M sodium hydroxide*, the tip of the microburette being immersed in the solution; note the volume added every 30 s. Follow the reaction for 8 min. Calculate the volume of *0.1 M sodium hydroxide* used per second. Carry out a titration in the same manner using the reference solution and calculate the volume of *0.1 M sodium hydroxide* used per second.

Calculate the activity in microkatals per milligram using the following expression:

$$\frac{m' \times V}{m \times V'} \times A$$

m	=	mass of the substance to be examined, in milligrams;
m'	=	mass of *trypsin BRP*, in milligrams;
V	=	volume of *0.1 M sodium hydroxide* used per second by the test solution;
V'	=	volume of *0.1 M sodium hydroxide* used per second by the reference solution;
A	=	activity of *trypsin BRP*, in microkatals per milligram.

STORAGE

In an airtight container, protected from light, at a temperature of 2 °C to 8 °C.

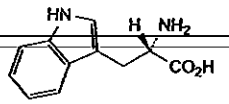
LABELLING

The label states:
— the activity in microkatals per milligram;
— for the amorphous substance, that it is hygroscopic.

Ph Eur

Tryptophan

(Ph. Eur. monograph 1272)



$C_{11}H_{12}N_2O_2$ 204.2 *73-22-3*

Ph Eur

DEFINITION

(2*S*)-2-Amino-3-(1*H*-indol-3-yl)propanoic acid.
Product of fermentation or of protein hydrolysis.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline or amorphous powder.

Solubility
Sparingly soluble in water, slightly soluble in ethanol (96 per cent). It dissolves in dilute solutions of mineral acids and alkali hydroxides.

IDENTIFICATION

First identification: A, B.
Second identification: A, C, D.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *tryptophan CRS.*

C. Thin-layer chromatography (*2.2.27*).
Solvent mixture *glacial acetic acid R, water R* (50:50 *V/V*).
Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 50 mL with the solvent mixture.
Reference solution Dissolve 10 mg of *tryptophan CRS* in the solvent mixture and dilute to 50 mL with the solvent mixture.
Plate *TLC silica gel plate R.*
Mobile phase *glacial acetic acid R, water R, butanol R* (20:20:60 *V/V/V*).
Application 5 µL.
Development Over 2/3 of the plate.
Drying In air.
Detection Spray with *ninhydrin solution R* and heat at 105 °C for 15 min.
Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve about 20 mg in 10 mL of *water R*. Add 5 mL of *dimethylaminobenzaldehyde solution R6* and 2 mL of *hydrochloric acid R1*. Heat on a water-bath. A purple-blue colour develops.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Dissolve 0.1 g in *1 M hydrochloric acid* and dilute to 10 mL with the same acid.

Specific optical rotation (*2.2.7*)

−33.0 to −30.0 (dried substance).

Dissolve 0.25 g in *water R*, heating on a water-bath if necessary, and dilute to 25.0 mL with the same solvent.

Ninhydrin-positive substances

Amino acid analysis (*2.2.56*). For analysis, use Method 1.

The concentrations of the test solution and the reference solutions may be adapted according to the sensitivity of the equipment used. The concentrations of all solutions are adjusted so that the system suitability requirements described in general chapter *2.2.46* are fulfilled, keeping the ratios of concentrations between all solutions as described.

Solution A *dilute hydrochloric acid R1* or a sample preparation buffer suitable for the apparatus used.

Test solution Dissolve 30.0 mg of the substance to be examined in solution A and dilute to 50.0 mL with solution A.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with solution A. Dilute 2.0 mL of this solution to 10.0 mL with solution A.

Reference solution (b) Dissolve 30.0 mg of *proline R* in solution A and dilute to 100.0 mL with solution A. Dilute 1.0 mL of the solution to 250.0 mL with solution A.

Reference solution (c) Dilute 6.0 mL of *ammonium standard solution (100 ppm NH_4) R* to 50.0 mL with solution A. Dilute 1.0 mL of this solution to 100.0 mL with solution A.

Reference solution (d) Dissolve 30 mg of *isoleucine R* and 30 mg of *leucine R* in solution A and dilute to 50.0 mL with solution A. Dilute 1.0 mL of the solution to 200.0 mL with solution A.

Blank solution Solution A.

Inject suitable, equal amounts of the test, blank and reference solutions into the amino acid analyser. Run a program suitable for the determination of physiological amino acids.

System suitability Reference solution (d):
- *resolution*: minimum 1.5 between the peaks due to isoleucine and leucine.

Calculation of percentage contents:
- for any ninhydrin-positive substance detected at 570 nm, use the concentration of tryptophan in reference solution (a);
- for any ninhydrin-positive substance detected at 440 nm, use the concentration of proline in reference solution (b); if a peak is above the reporting threshold at both wavelengths, use the result obtained at 570 nm for quantification.

Limits:
- *any ninhydrin-positive substance*: for each impurity, maximum 0.2 per cent;
- *total*: maximum 0.5 per cent;
- *reporting threshold*: 0.05 per cent.

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Impurity A and other related substances

Liquid chromatography (*2.2.29*). *Prepare the standard, test and reference solutions immediately before use.*

Buffer solution pH 2.3 Dissolve 3.90 g of *sodium dihydrogen phosphate R* in 1000 mL of *water R*. Add about 700 mL of a 2.9 g/L solution of *phosphoric acid R* and adjust to pH 2.3 with the same acid solution.

Solvent mixture *acetonitrile R, water R* (10:90 *V/V*).

Standard solution Dissolve 10.0 mg of *N-acetyltryptophan R* in the solvent mixture and dilute to 100.0 mL with the solvent mixture. Dilute 2.0 mL of this solution to 100.0 mL with the solvent mixture.

Test solution (a) Dissolve 0.10 g of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Test solution (b) Dissolve 0.10 g of the substance to be examined in the standard solution and dilute to 10.0 mL with the standard solution.

Reference solution (a) Dissolve the contents of a vial of *1,1'-ethylidenebistryptophan CRS* (impurity A) in 1.0 mL of the solvent mixture.

Reference solution (b) Dissolve the contents of a vial of *1,1'-ethylidenebistryptophan CRS* (impurity A) in 1.0 mL of the standard solution.

Reference solution (c) Dilute 0.5 mL of reference solution (a) to 5.0 mL with the solvent mixture.

Column:
- *size*: l = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase:
- *mobile phase A*: *acetonitrile R*, buffer solution pH 2.3 (115:885 *V/V*);
- *mobile phase B*: *acetonitrile R*, buffer solution pH 2.3 (350:650 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	100	0
10 - 45	100 → 0	0 → 100
45 - 65	0	100

Flow rate 0.7 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µL of test solutions (a) and (b) and reference solutions (b) and (c).

Retention time Tryptophan = about 8 min; *N*-acetyltryptophan = about 29 min; impurity A = about 34 min.

System suitability:
- *resolution*: minimum 8.0 between the peaks due to *N*-acetyltryptophan and impurity A in the chromatogram obtained with reference solution (b); if necessary, adjust the time programme for the elution gradient (an increase in the duration of elution with mobile phase A produces longer retention times and a better resolution);
- *signal-to-noise ratio*: minimum 15 for the principal peak in the chromatogram obtained with reference solution (c);

— *symmetry factor*: maximum 3.5 for the peak due to impurity A in the chromatogram obtained with reference solution (b);
— in the chromatogram obtained with test solution (a) there is no peak with the same retention time as *N*-acetyltryptophan (in such case correct the area of the *N*-acetyltryptophan peak).

Limits Test solution (b):
— *impurity A*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (10 ppm);
— *sum of impurities with a retention time less than that of tryptophan*: not more than 0.6 times the area of the peak due to *N*-acetyltryptophan in the chromatogram obtained with reference solution (b) (100 ppm);
— *sum of impurities with a retention time greater than that of tryptophan and up to 1.8 times the retention time of N-acetyltryptophan*: not more than 1.9 times the area of the peak due to *N*-acetyltryptophan in the chromatogram obtained with reference solution (b) (300 ppm);
— *disregard limit*: 0.02 times the area of the peak due to *N*-acetyltryptophan in the chromatogram obtained with reference solution (b); disregard the peak due to *N*-acetyltryptophan.

Chlorides (*2.4.4*)
Maximum 200 ppm.

Dissolve 0.25 g in 3 mL of *dilute nitric acid R* and dilute to 15 mL with *water R*. The solution, without any further addition of nitric acid, complies with the test.

Sulfates (*2.4.13*)
Maximum 300 ppm.

Dissolve 0.5 g in a mixture of 5 volumes of *dilute hydrochloric acid R* and 25 volumes of *distilled water R*, and dilute to 15 mL with the same mixture of solvents.

Ammonium
Amino acid analysis (*2.2.56*) as described in the test for ninhydrin-positive substances with the following modifications.

Injection Test solution, reference solution (c) and blank solution.

Limit:
— *ammonium at 570 nm*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.02 per cent), taking into account the peak due to ammonium in the chromatogram obtained with the blank solution.

Iron (*2.4.9*)
Maximum 20 ppm.

In a separating funnel, dissolve 0.50 g in 10 mL of *dilute hydrochloric acid R*. Shake with 3 quantities, each of 10 mL, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 mL of *water R* and shake for 3 min. Examine the aqueous layer.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

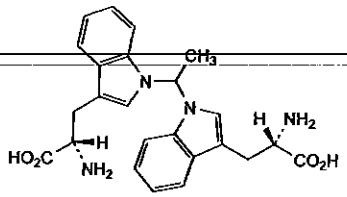
ASSAY
Dissolve 0.150 g in 3 mL of *anhydrous formic acid R*. Add 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 mL of *0.1 M perchloric acid* is equivalent to 20.42 mg of $C_{11}H_{12}N_2O_2$.

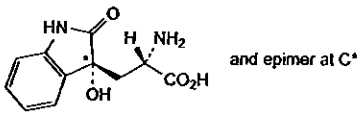
STORAGE
Protected from light.

IMPURITIES
Specified impurities *A.*

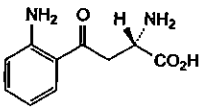
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D, E, F, G, H, I, J, K, L.*



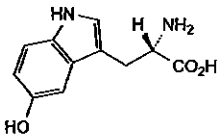
A. 3,3′-[ethane-1,1-diylbis(1*H*-indol-1,3-diyl)]bis[(2*S*)-2-aminopropanoic acid] (ethane-1,1-diylbistryptophan),



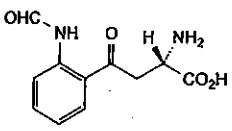
B. (2*S*)-2-amino-3-[(3*RS*)-3-hydroxy-2-oxo-2,3-dihydro-1*H*-indol-3-yl]propanoic acid (dioxyindolylalanine),



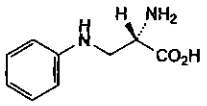
C. (2*S*)-2-amino-4-(2-aminophenyl)-4-oxobutanoic acid (kynurenine),



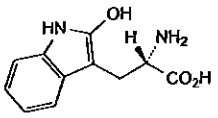
D. (2*S*)-2-amino-3-(5-hydroxy-1*H*-indol-3-yl)propanoic acid (5-hydroxytryptophan),



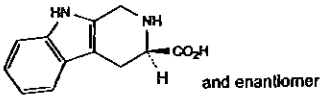
E. (2*S*)-2-amino-4-[2-(formylamino)phenyl]-4-oxobutanoic acid (*N*-formylkynurenine),



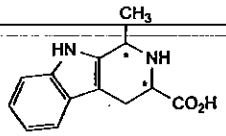
F. (2*S*)-2-amino-3-(phenylamino)propanoic acid (3-phenylaminoalanine),



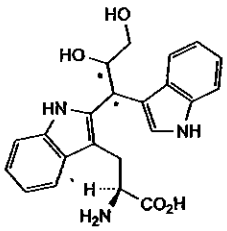
G. (2*S*)-2-amino-3-(2-hydroxy-1*H*-indol-3-yl)propanoic acid (2-hydroxytryptophan),



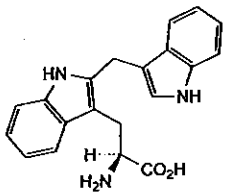
H. (3*RS*)-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole-3-carboxylic acid,



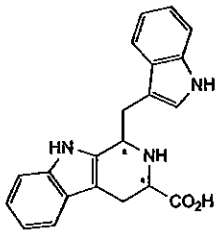
I. 1-methyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole-3-carboxylic acid,



J. (2*S*)-2-amino-3-[2-[2,3-dihydroxy-1-(1*H*-indol-3-yl) propyl]-1*H*-indol-3-yl]propanoic acid,



K. (2*S*)-2-amino-3-[2-(1*H*-indol-3-ylmethyl)-1*H*-indol-3-yl] propanoic acid,

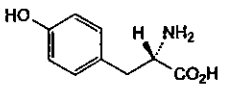


L. 1-(1*H*-indol-3-ylmethyl)-1,2,3,4-tetrahydro-9*H*-pyrido [3,4-*b*]indole-3-carboxylic acid.

Ph Eur

Tyrosine

(Ph. Eur. monograph 1161)



$C_9H_{11}NO_3$ 181.2 *60-18-4*

Action and use
Amino acid.

Ph Eur

DEFINITION

(2*S*)-2-Amino-3-(4-hydroxyphenyl)propanoic acid.

Product of fermentation or of protein hydrolysis.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder or colourless crystals.

Solubility
Very slightly soluble in water, practically insoluble in ethanol (96 per cent). It dissolves in dilute mineral acids and in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification: A, B.

Second identification: A, C, D, E.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *tyrosine CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in 1 mL of *dilute ammonia R2* and dilute to 50 mL with *water R.*

Reference solution Dissolve 10 mg of *tyrosine CRS* in 1 mL of *dilute ammonia R2* and dilute to 50 mL with *water R.*

Plate *TLC silica gel plate R.*

Mobile phase *concentrated ammonia R1, propanol R* (30:70 *V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *ninhydrin solution R* and heat at 105 °C for 15 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. To about 50 mg add 1 mL of *dilute nitric acid R.* A dark red colour is produced within 15 min.

E. Dissolve about 30 mg in 2 mL of *dilute sodium hydroxide solution R.* Add 3 mL of a freshly prepared mixture of equal volumes of a 100 g/L solution of *sodium nitrite R* and a solution of 0.5 g of *sulfanilic acid R* in a mixture of 6 mL of *hydrochloric acid R1* and 94 mL of *water R.* An orange-red colour is produced.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

Dissolve 0.5 g in *dilute hydrochloric acid R* and dilute to 20 mL with the same acid.

Specific optical rotation (*2.2.7*)

−12.3 to −11.0 (dried substance).

Dissolve 1.25 g in a mixture of equal volumes of *dilute hydrochloric acid R* and *water R* and dilute to 25.0 mL with the same mixture of solvents.

Ninhydrin-positive substances

Amino acid analysis (*2.2.56*). For analysis, use Method 1.

The concentrations of the test solution and the reference solutions may be adapted according to the sensitivity of the equipment used. The concentrations of all solutions are adjusted so that the system suitability requirements described in general chapter *2.2.46* are fulfilled, keeping the ratios of concentrations between all solutions as described.

Solution A *dilute hydrochloric acid R1* or a sample preparation buffer suitable for the apparatus used.

Test solution Dissolve 30.0 mg of the substance to be examined in solution A and dilute to 50.0 mL with solution A.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with solution A. Dilute 2.0 mL of this solution to 10.0 mL with solution A.

Reference solution (b) Dissolve 30.0 mg of *phenylalanine R* (impurity A) in solution A and dilute to 100.0 mL with solution A. Dilute 1.0 mL of the solution to 250.0 mL with solution A.

Reference solution (c) Dissolve 30.0 mg of *proline R* in solution A and dilute to 100.0 mL with solution A. Dilute 1.0 mL of the solution to 250.0 mL with solution A.

Reference solution (d) Dilute 6.0 mL of *ammonium standard solution (100 ppm NH_4) R* to 50.0 mL with solution A. Dilute 1.0 mL of this solution to 100.0 mL with solution A.

Reference solution (e) Dissolve 30 mg of *isoleucine R* and 30 mg of *leucine R* in solution A and dilute to 50.0 mL with solution A. Dilute 1.0 mL of the solution to 200.0 mL with solution A.

Blank solution Solution A.

Inject suitable, equal amounts of the test, blank and reference solutions into the amino acid analyser. Run a program suitable for the determination of physiological amino acids.

System suitability Reference solution (e):

— *resolution*: minimum 1.5 between the peaks due to isoleucine and leucine.

Calculation of percentage contents:

— for impurity A, use the concentration of impurity A in reference solution (b);

— for any ninhydrin-positive substance detected at 570 nm, use the concentration of tyrosine in reference solution (a);

— for any ninhydrin-positive substance detected at 440 nm, use the concentration of proline in reference solution (c); if a peak is above the reporting threshold at both wavelengths, use the result obtained at 570 nm for quantification.

Limits:

— *impurity A at 570 nm*: maximum 0.5 per cent;

— *any ninhydrin-positive substance*: for each impurity, maximum 0.2 per cent;

— *total*: maximum 0.6 per cent;

— *reporting threshold*: 0.05 per cent.

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Chlorides (*2.4.4*)

Maximum 200 ppm.

Dissolve 0.25 g in 3 mL of *dilute nitric acid R* and dilute to 15 mL with *water R*. The solution, without further addition of nitric acid, complies with the test.

Sulfates (*2.4.13*)

Maximum 300 ppm.

Dissolve, with gentle heating, 0.5 g in 5 mL of *dilute hydrochloric acid R* and dilute to 15 mL with *distilled water R*.

Ammonium

Amino acid analysis (*2.2.56*) as described in the test for ninhydrin-positive substances with the following modifications.

Injection Test solution, reference solution (d) and blank solution.

Limit:

— *ammonium at 570 nm*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.02 per cent), taking into account the peak due to ammonium in the chromatogram obtained with the blank solution.

Iron (*2.4.9*)

Maximum 10 ppm.

In a separating funnel, dissolve 1.0 g in 10 mL of *dilute hydrochloric acid R*. Shake with 3 quantities, each of 10 mL, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 mL of *water R* and shake for 3 min. Use the aqueous layer.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 5 mL of *anhydrous formic acid R*. Add 30 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 18.12 mg of $C_9H_{11}NO_3$.

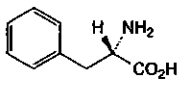
STORAGE

Protected from light.

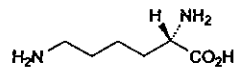
IMPURITIES

Specified impurities *A.*

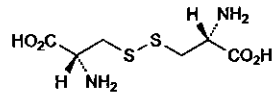
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C.*



A. (2*S*)-2-amino-3-phenylpropanoic acid (phenylalanine),



B. (2*S*)-2,6-diaminohexanoic acid (lysine),

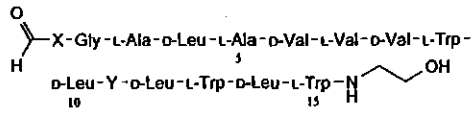


C. 3,3′-disulfanediylbis[(2*R*)-2-aminopropanoic acid] (cystine).

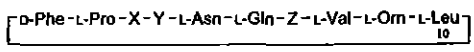
Ph Eur

Tyrothricin

(Ph. Eur. monograph 1662)



Gramicidin	Mol. formula	M_r	X	Y
A1	$C_{99}H_{140}N_{20}O_{17}$	1882	L-Val	L-Trp
A2	$C_{100}H_{142}N_{20}O_{17}$	1896	L-Ile	L-Trp
C1	$C_{97}H_{139}N_{19}O_{18}$	1859	L-Val	L-Tyr
C2	$C_{98}H_{141}N_{19}O_{18}$	1873	L-Ile	L-Tyr



Tyrocidin	Mol. formula	M_r	X	Y	Z
A	$C_{66}H_{88}N_{13}O_{13}$	1271	L-Phe	D-Phe	L-Tyr
B	$C_{68}H_{89}N_{14}O_{13}$	1311	L-Trp	D-Phe	L-Tyr
C	$C_{70}H_{90}N_{15}O_{13}$	1350	L-Trp	D-Trp	L-Tyr
D	$C_{72}H_{91}N_{18}O_{12}$	1373	L-Trp	D-Trp	L-Trp
E	$C_{66}H_{88}N_{13}O_{12}$	1255	L-Phe	D-Phe	L-Phe

Action and use

Polypeptide antibacterial.

Ph Eur

DEFINITION

Mixture of antimicrobial linear and cyclic polypeptides, isolated from the fermentation broth of *Brevibacillus brevis* Dubos. It consists mainly of gramicidins and tyrocidins as described above; other related compounds may be present in smaller amounts.

Potency

180 IU/mg to 280 IU/mg (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, soluble in ethanol (96 per cent) and in methanol.

IDENTIFICATION

First identification: B.

Second identification: A.

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 5 mg of the substance to be examined in 4.0 mL of *ethanol (96 per cent) R*.

Reference solution Dissolve 5 mg of *tyrothricin CRS* in 4.0 mL of *ethanol (96 per cent) R*.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *methanol R, butanol R, water R, acetic acid R, butyl acetate R* (2.5:7.5:12:20:40 *V/V/V/V/V*).

Application 1 µL.

Development Over 2/3 of the plate.

Drying In a current of warm air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spots or groups of principal spots in the chromatogram obtained with the test solution are similar in position and size to the principal spots or groups of principal spots in the chromatogram obtained with the reference solution. The upper group corresponds to gramicidins, the lower group to tyrocidins.

Detection B Spray with *dimethylaminobenzaldehyde solution R2*. Heat the plate in a current of warm air until the spots appear.

System suitability Reference solution:

— the chromatogram shows 2 clearly separated spots or groups of spots.

Results B The principal spots or groups of principal spots in the chromatogram obtained with the test solution are similar in position, colour and size to the principal spots or groups of principal spots in the chromatogram obtained with the reference solution. The upper group corresponds to gramicidins, the lower group to tyrocidins.

B. Composition (see Tests).

TESTS

Composition

Liquid chromatography (*2.2.29*): use the normalisation procedure. *Prepare the solutions immediately before use.*

Test solution Dissolve 5 mg of the substance to be examined in 2 mL of *methanol R* and dilute to 5.0 mL with the mobile phase.

Reference solution (a) Dissolve 5 mg of *tyrothricin CRS* in 2 mL of *methanol R* and dilute to 5.0 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 50.0 mL with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm),

— *temperature*: 60 °C.

Mobile phase 0.79 g/L solution of *ammonium sulfate R*, *methanol R* (25:75 *V/V*).

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 25 µL.

Run time 6 times the retention time of gramicidin A1.

Use the chromatogram obtained with reference solution (a) and the chromatogram supplied with *tyrothricin CRS* to identify the peaks due to gramicidin A1, gramicidin A2 and the tyrocidins.

Relative retention With reference to gramicidin A1 (retention time = about 10 min): gramicidin C1 = about 0.8;

gramicidin C2 = about 0.9; gramicidin A2 = about 1.1; tyrocidins = about 1.5 to 6.

System suitability Reference solution (a):
— *peak-to-valley ratio*: minimum 3.0, where H_p = height above the baseline of the peak due to gramicidin A2 and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to gramicidin A1.

Limits:
— *sum of gramicidins*: 25 per cent to 50 per cent,
— *sum of tyrocidins*: 50 per cent to 70 per cent,
— *total*: minimum 85 per cent,
— *disregard limit*: the sum of the areas of the peaks due to gramicidins in the chromatogram obtained with reference solution (b).

Loss on drying (*2.2.32*)
Maximum 4.0 per cent, determined on 1.000 g by drying under high vacuum at 60 °C for 3 h.

Sulfated ash (*2.4.14*)
Maximum 1.5 per cent, determined on 1.0 g.

ASSAY

Carry out the microbiological assay of antibiotics (*2.7.2*) using the turbidimetric method. Use *gramicidin CRS* as the reference substance.

Test solution Prepare a solution of tyrothricin containing about the same amount of gramicidin as the corresponding solution of *gramicidin CRS* i.e. 5 times more concentrated.

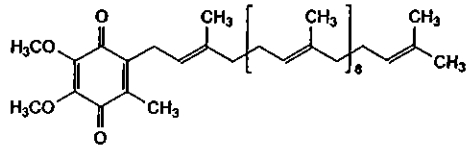
STORAGE

In an airtight container, protected from light.

Ph Eur

Ubidecarenone

(Ph. Eur. monograph 1578)



$C_{59}H_{90}O_4$ 863 *303-98-0*

Action and use
Co-enzyme in mitochondrial electron transport.

Ph Eur

DEFINITION

2-[(*all-E*)-3,7,11,15,19,23,27,31,35,39-Decamethyltetraconta-2,6,10,14,18,22,26,30,34,38-decaenyl]-5,6-dimethoxy-3-methylbenzene-1,4-dione.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
Yellow or orange, hygroscopic, crystalline powder.

Solubility
Practically insoluble in water, soluble in acetone, very slightly soluble in anhydrous ethanol.

It gradually decomposes and darkens on exposure to light.

mp
About 48 °C.

Carry out all operations protected from light.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *ubidecarenone CRS*.

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Related substances
Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Test solution Dissolve 25.0 mg of the substance to be examined in 25.0 mL of *anhydrous ethanol R* by heating at about 50 °C for 2 min. Allow to cool.

Reference solution (a) Dissolve 25.0 mg of *ubidecarenone CRS* in 25.0 mL of *anhydrous ethanol R* by heating at about 50 °C for 2 min. Allow to cool.

Reference solution (b) Dissolve 2 mg of *ubidecarenone impurity D CRS* in 2.0 mL of the test solution by heating at about 50 °C for 2 min. Allow to cool. Dilute 1.0 mL of this solution to 50.0 mL with *anhydrous ethanol R*.

Reference solution (c) Dilute 1.0 mL of the test solution to 100.0 mL with *anhydrous ethanol R*. Dilute 5.0 mL of this solution to 10.0 mL with *anhydrous ethanol R*.

Column:
— *size*: *l* = 0.2 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 30 °C.

Mobile phase *anhydrous ethanol R, methanol R2* (20:80 *V/V*).

Flow rate 1.7 mL/min.

Detection Spectrophotometer at 275 nm.

Injection 10 µL of the test solution and reference solutions (b) and (c).

Run time Twice the retention time of ubidecarenone.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity D.

Relative retention With reference to ubidecarenone (retention time = about 14 min): impurity D = about 0.7.

System suitability Reference solution (b):
— *resolution*: minimum 6.5 between the peaks due to impurity D and ubidecarenone.

Calculation of percentage contents:
— for each impurity, use the concentration of ubidecarenone in reference solution (c).

Limits:
— *impurity D*: maximum 0.3 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.6 per cent,
— *reporting threshold*: 0.05 per cent.

Impurity F
Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Test solution Dissolve 25.0 mg of the substance to be examined in 25.0 mL of *hexane R*.

Reference solution (a) Dissolve the contents of a vial of *ubidecarenone for system suitability CRS* (containing impurity F) in 1.0 mL of *hexane R*.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *hexane R*.

Column:
— *size*: *l* = 0.25 m, Ø = 4.0 mm;
— *stationary phase*: *silica gel for chromatography R* (7 µm).

Mobile phase *ethyl acetate R, hexane R* (3:97 *V/V*).

Flow rate 2 mL/min.

Detection Spectrophotometer at 275 nm.

Injection 20 µL.

Run time 1.2 times the retention time of ubidecarenone.

Identification of impurities Use the chromatogram supplied with *ubidecarenone for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peak due to impurity F.

Relative retention With reference to ubidecarenone (retention time = about 10 min): impurity F = about 0.85.

System suitability Reference solution (a):
— *resolution*: minimum 1.5 between the peaks due to impurity F and ubidecarenone.

Limit:
— *impurity F*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent).

Water (*2.5.12*)
Maximum 0.2 per cent, determined on 3.00 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{59}H_{90}O_4$ taking into account the assigned content of *ubidecarenone CRS*.

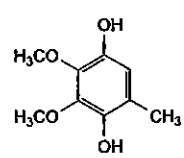
STORAGE

In an airtight container, protected from light.

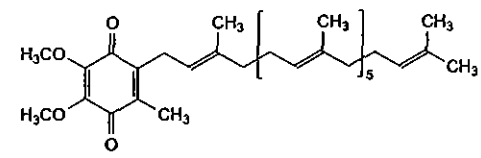
IMPURITIES

Specified impurities *D, F.*

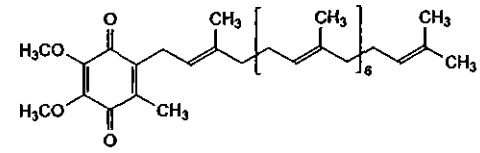
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, E, G.*



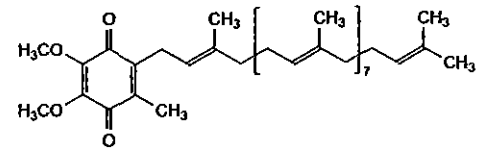
A. 2,3-dimethoxy-5-methylbenzene-1,4-diol,



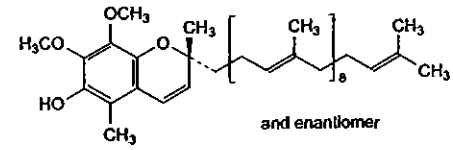
B. 2-[(*all-E*)-3,7,11,15,19,23,27-heptamethyloctadocosa-2,6,10,14,18,22,26-heptaenyl]-5,6-dimethoxy-3-methylbenzene-1,4-dione (ubiquinone-7),



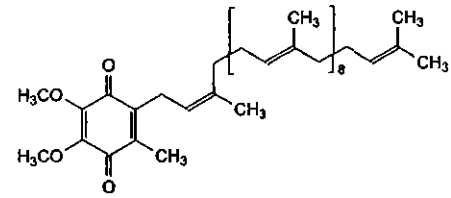
C. 5,6-dimethoxy-3-methyl-2-[(*all-E*)-3,7,11,15,19,23,27,31-octamethyldotriaconta-2,6,10,14,18,22,26,30-octaenyl]benzene-1,4-dione (ubiquinone-8),



D. 5,6-dimethoxy-3-methyl-2-[(*all-E*)-3,7,11,15,19,23,27,31,35-nonamethylhexatriaconta-2,6,10,14,18,22,26,30,34-nonaenyl]benzene-1,4-dione (ubiquinone-9),



E. (2*RS*)-7,8-dimethoxy-2,5-dimethyl-2-[(*all-E*)-4,8,12,16,20,24,28,32,36-nonamethylheptatriaconta-3,7,11,15,19,23,27,31,35-nonaenyl]-2*H*-1-benzopyran-6-ol (ubicromenol),



F. 2-[(2*Z*,6*E*,10*E*,14*E*,18*E*,22*E*,26*E*,30*E*,34*E*)-3,7,11,15,19,23,27,31,35,39-decamethyltetraconta-2,6,10,14,18,22,26,30,34,38-decaenyl]-5,6-dimethoxy-3-methylbenzene-1,4-dione (ubidecarenone (*Z*)-isomer),

G. unknown structure.

Undecenoic Acid

(Undecylenic Acid, Ph. Eur. monograph 0461)

$C_{11}H_{20}O_2$ 184.3 *112-38-9*

Ph Eur

DEFINITION
Undec-10-enoic acid.

Content
97.0 per cent to 102.0 per cent.

CHARACTERS
Appearance
White or very pale yellow, crystalline mass or colourless or pale yellow liquid.

Solubility
Practically insoluble in water, freely soluble in ethanol (96 per cent) and in fatty and essential oils.

IDENTIFICATION
A. Refractive index (*2.2.6*): 1.447 to 1.450, determined at 25 ± 0.5 °C.

B. Freezing point (*2.2.18*): 21 °C to 24 °C.

C. To 2.0 g add 2 mL of freshly distilled *aniline R* and boil under a reflux condenser for 10 min. Allow to cool and add 30 mL of *ether R*. Shake with 3 quantities, each of 20 mL, of *dilute hydrochloric acid R* and then with 20 mL of *water R*. Evaporate the organic layer to dryness on a water-bath. After recrystallising twice from *ethanol (70 per cent V/V) R* and drying *in vacuo* for 3 h, the residue melts (*2.2.14*) at 66 °C to 68 °C.

D. Dissolve 0.1 g in a mixture of 2 mL of *dilute sulfuric acid R* and 5 mL of *glacial acetic acid R*. Add dropwise 0.25 mL of *potassium permanganate solution R*. The colour of the potassium permanganate is discharged.

TESTS
Peroxide value (*2.5.5, Method A*)
Maximum 10.

Fixed and mineral oils
To 1.0 g add 5 mL of *sodium carbonate solution R* and 25 mL of *water R* and boil for 3 min. The hot solution is not more opalescent than reference suspension II (*2.2.1*).

Water-soluble acids
To 1.0 g add 20 mL of *water R* heated to 35-45 °C and shake for 2 min. Cool and filter the aqueous layer through a moistened filter. To 10 mL of the filtrate add 0.1 mL of *phenolphthalein solution R*. Not more than 0.1 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Degree of unsaturation
Dissolve 85.0 mg in a mixture of 5 mL of *dilute hydrochloric acid R* and 30 mL of *glacial acetic acid R*. Using 0.05 mL of *indigo carmine solution R1* as indicator, added towards the end of the titration, titrate with *0.0167 M bromide-bromate* until the colour changes from blue to yellow. 8.9 mL to 9.4 mL of *0.0167 M bromide-bromate* is required. Carry out a blank titration.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 0.50 g.

ASSAY
Dissolve 0.750 g in 10 mL of *ethanol (96 per cent) R*. Titrate with *0.5 M sodium hydroxide* using 0.1 mL of *phenolphthalein solution R* as indicator, until a pink colour is obtained.

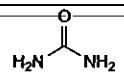
1 mL of *0.5 M sodium hydroxide* is equivalent to 92.14 mg of $C_{11}H_{20}O_2$.

STORAGE
In a non-metallic container, protected from light.

Ph Eur

Urea

(Ph. Eur. monograph 0743)



CH_4N_2O 60.1 *57-13-6*

Action and use
Keratolytic.

Preparation
Urea Cream

Ph Eur

DEFINITION
Carbamide.

Content
98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder or transparent crystals, slightly hygroscopic.

Solubility
Very soluble in water, soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

IDENTIFICATION
First identification: A, B.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 132 °C to 135 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison urea CRS.

C. Dissolve 0.1 g in 1 mL of *water R*. Add 1 mL of *nitric acid R*. A white, crystalline precipitate is formed.

D. Heat 0.5 g in a test tube until it liquefies and the liquid becomes turbid. Cool, dissolve in a mixture of 1 mL of *dilute sodium hydroxide solution R* and 10 mL of *water R* and add 0.05 mL of *copper sulfate solution R*. A reddish-violet colour is produced.

TESTS
Solution S
Dissolve 10.0 g in *water R* and dilute to 50 mL with the same solvent.

Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

To 2.5 mL of solution S add 7.5 mL of *water R*.

Alkalinity
To 2.5 mL of solution S add 7.5 mL of *water R*, 0.1 mL of *methyl red solution R* and 0.4 mL of *0.01 M hydrochloric acid*. The solution is red to orange.

Biuret
Maximum 0.1 per cent.

To 10 mL of solution S add 5 mL of *water R*, 0.5 mL of a 5 g/L solution of *copper sulfate pentahydrate R* and 0.5 mL of *strong sodium hydroxide solution R*. Allow to stand for 5 min. Any reddish-violet colour in the solution is not more intense than that in a standard prepared at the same time in the same manner using 10 mL of a 0.2 g/L solution of *biuret R*.

Ammonium (*2.4.1*)
Maximum 500 ppm, determined on 0.1 mL of solution S.

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 1 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.2000 g in *water R* and dilute to 50.0 mL with the same solvent. Introduce 1.0 mL of the solution into a combustion flask. Add 4 g of a powdered mixture of 100 g of *dipotassium sulfate R*, 5 g of *copper sulfate pentahydrate R* and 2.5 g of *selenium R*, and 3 glass beads. Wash any adhering particles from the neck into the flask with 5 mL of *sulfuric acid R*, allowing it to run down the sides of the flask, and mix the contents by rotation. Close the mouth of the flask loosely, for example by means of a glass bulb with a short stem, to avoid excessive loss of sulfuric acid. Heat gradually at first, then increase the temperature until there is vigorous boiling with condensation of sulfuric acid in the neck of the flask; take precautions to prevent the upper part of the flask from becoming overheated. Continue the heating for 30 min. Cool, dissolve the solid material by cautiously adding to the mixture 25 mL of *water R*, cool again and place in a steam-distillation apparatus. Add 30 mL of *strong sodium hydroxide solution R* and distil immediately by passing steam through the mixture. Collect the distillate in 15 mL of a 40 g/L solution of *boric acid R* to which has been added 0.2 mL of *methyl red mixed solution R* and enough *water R* to cover the tip of the condenser. Towards the end of the distillation, lower the receiver so that the tip of the condenser is above the surface of the acid. Take precautions to prevent any water on the outer surface of the condenser from reaching the contents of the receiver. Titrate the distillate with *0.01 M sulfuric acid*.

1 mL of *0.01 M sulfuric acid* is equivalent to 0.6006 mg of CH_4N_2O.

STORAGE
In an airtight container.

Ph Eur

Urofollitropin

(Ph. Eur. monograph 0958)

97048-13-0

Action and use
Follicle-stimulating hormone.

Preparation
Urofollitropin Injection

Ph Eur

DEFINITION
Dried preparation containing menopausal gonadotrophin obtained from the urine of post-menopausal women. It has follicle-stimulating activity and no or virtually no luteinising activity.

Potency
Minimum 90 IU of follicle-stimulating hormone (hFSH) per milligram.

Ratio of the number of units of luteinising hormone (interstitial-cell-stimulating hormone) [hLH(ICSH)] to the number of units of follicle-stimulating hormone Maximum 1/60.

PRODUCTION
It is prepared by suitable collection and extraction procedures followed by purification steps.

The method of preparation includes steps that have been shown to remove and/or inactivate extraneous agents. In addition, the process is designed to minimise microbial contamination.

CHARACTERS

Appearance
Almost white or slightly yellowish powder.

Solubility
Soluble in water.

IDENTIFICATION
When administered to immature female rats as prescribed in the assay, it causes enlargement of the ovaries.

TESTS

Residual luteinising activity
The International Units of FSH and LH are the activities contained in stated amounts of the International Standard of human urinary follicle-stimulating hormone and luteinising hormone (interstitial-cell-stimulating hormone) which consists of a mixture of a freeze-dried extract of urine of post-menopausal women with lactose. The equivalence in International Units of the International Standard is stated by the World Health Organization.

Use immature female rats approximately 21 days old and having masses such that the difference between the heaviest and the lightest rat is not more than 10 g. Assign the rats at random to 4 equal groups of at least 6 animals. If sets of 4 litter mates are available, assign one litter mate at random from each set to each group and mark according to litter.

Inject subcutaneously into each rat 50 IU of *serum gonadotrophin R* on the first day and 25 IU of *chorionic gonadotrophin R* on the fourth day, each in 0.5 mL of *phosphate-albumin buffered saline pH 7.2 R*.

Choose 3 doses of the reference preparation such that the smallest dose produces a depletion of the ovarian ascorbic acid content in all the rats and the largest dose does not produce a maximal depletion in all the rats. Use doses in geometric progression; as an initial approximation, total doses

of 0.5 IU, 1.0 IU and 2.0 IU may be tried although the dose to be used will depend on the sensitivity of the animals.

Choose a dose of the preparation to be examined expected to contain 60 *X* IU of follicle-stimulating hormone (hFSH), in which *X* = the number of International Units of hLH in the middle dose of the reference preparation.

Dissolve separately the total quantities of the preparation to be examined and of the reference preparation in 1.0 mL of *phosphate-albumin buffered saline pH 7.2 R*. Inject into a tail vein to each separate group of rats 6 days after the injection of chorionic gonadotrophin. Exactly 4 h after the injection, euthanise the rats and remove the ovaries from each animal. Remove any extraneous fluid and tissue from the ovaries and weigh the ovaries immediately.

Treat the combined ovaries from each rat separately, as follows. Crush and homogenise within 2 min in a freshly prepared 25 g/L solution of *metaphosphoric acid R* at a temperature of 4 °C and dilute to 7 mL with the same solution. Allow the homogenate to stand for 30 min at 4 °C and centrifuge at 4 °C at approximately 2500 *g*. Filter the supernatant, if necessary, through a 0.22 µm filter.

Prepare a fresh solution consisting of a mixture of 2 mL of a 45.3 g/L solution of *sodium acetate R* adjusted to pH 7 with *acetic acid R*, 3 mL of *water R* and 2 mL of *dichlorophenolindophenol standard solution R*. Mix 2 mL of this solution with 2 mL of the clear supernatant. 30 s after mixing, measure the absorbance (*2.2.25*) of the solution at the maximum at about 520 nm. Use as reference a solution with a known content of *ascorbic acid CRS* in a 25 g/L solution of *metaphosphoric acid R*, treated by the same process.

Calculate the amount of ascorbic acid from the ascorbic acid standard curve obtained and express in milligrams per 0.1 g of ovary to obtain the ascorbic acid content of the ovaries. Calculate the mean and its variance of the ascorbic acid content of the ovaries of the rats treated with the preparation to be examined.

For each dose-group of the reference preparation, plot the mean ascorbic acid content of the ovaries as a function of the logarithm of the dose and analyse the regression of the ascorbic acid content on the logarithm of the dose injected, using standard methods of analysis (the method of least squares).

The test is not valid unless:

- the slope constant *b* is significant at the 0.05 significance level;
- for the groups treated with the reference preparation, the sum of squares due to linear regression is equal to at least 95 per cent of the total sum of squares of the ascorbic acid content;
- the within-group variance of the ascorbic acid content of the group receiving the preparation to be examined is not significantly different at the 0.05 significance level from the within-group variance of the ascorbic acid content of the groups receiving the reference preparation.

The mean ascorbic acid content of the ovaries of the rats treated with the preparation to be examined is not significantly lower than that of the rats treated with the middle dose of the reference preparation (calculated from the regression equation) at the 0.05 significance level.

Water (*2.5.32*)
Maximum 5.0 per cent.

Bacterial endotoxins (*2.6.14, Method C*)
Less than 0.40 IU per International Unit of urofollitropin, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

The follicle-stimulating activity of urofollitropin is estimated by comparing under given conditions its effect in enlarging the ovaries of immature rats treated with chorionic gonadotrophin with the same effect of the International Standard preparation of human urinary follicle-stimulating hormone and luteinising hormone or of a reference preparation calibrated in International Units.

The International Units of FSH and LH are the activities contained in stated amounts of the International Standard of human urinary follicle-stimulating hormone and luteinising hormone (interstitial-cell-stimulating hormone) which consists of a mixture of a freeze-dried extract of urine of post-menopausal women with lactose. The equivalence in International Units of the International Standard is stated by the World Health Organization.

Use immature female rats of the same strain, 19-28 days old, differing in age by not more than 3 days and having masses such that the difference between the heaviest and the lightest rat is not more than 10 g. Assign the rats at random to 6 equal groups of at least 5 animals. If sets of 6 litter mates are available, assign one litter mate from each set to each group and mark according to litter.

Choose 3 doses of the reference preparation and 3 doses of the preparation to be examined such that the smallest dose produces a positive response in some of the rats and the largest dose does not produce a maximal response in all the rats. Use doses in geometric progression and as an initial approximation total doses of 1.5 IU, 3.0 IU and 6.0 IU may be tried although the dose will depend on the sensitivity of the animals used, which may vary widely.

Dissolve separately the total quantities of the preparation to be examined and of the reference preparation corresponding to the daily doses to be used in sufficient *phosphate-albumin buffered saline pH 7.2 R* such that the daily dose is administered in a volume of about 0.5 mL. The buffer solution shall contain in the daily dose not less than 14 IU of chorionic gonadotrophin to ensure complete luteinisation. Add a suitable antimicrobial preservative such as 4 g/L of phenol or 0.02 g/L of thiomersal. Store the solutions at 5 ± 3 °C.

Inject subcutaneously into each rat the daily dose allocated to its group. Repeat the injection of each dose 24 h and 48 h after the first injection. About 24 h after the last injection, euthanise the rats and remove the ovaries from each animal. Remove any extraneous fluid and tissue from the ovaries and weigh the 2 combined ovaries of each animal immediately. Calculate the results by the usual statistical methods (for example, *5.3*) using the mass of the 2 combined ovaries as the response (a suitable correction of the organ mass with reference to the mass of the animal from which it was taken may be applied; an analysis of covariance may be used).

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency.

The confidence limits ($P = 0.95$) of the estimated potency are not less than 64 per cent and not more than 156 per cent of the stated potency.

STORAGE
In an airtight container, protected from light, at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-evident container.

LABELLING
The label states:
- the number of International Units of follicle-stimulating hormone per container;
- the potency, in International Units of follicle-stimulating hormone per milligram.

Ph Eur

Urokinase

(Ph. Eur. monograph 0695)

9039-53-6

Action and use
Plasminogen activator; fibrinolytic; enzyme.

Ph Eur

DEFINITION
Enzyme, obtained from human urine, that activates plasminogen. It consists of a mixture of low-molecular-mass (LMM) (M_r 33 000) and high-molecular-mass (HMM) (M_r 54 000) forms, the high-molecular-mass form being predominant.

Potency
Minimum 70 000 IU per milligram of protein.

PRODUCTION
It is prepared by suitable collection and extraction procedures followed by purification steps.

The method of preparation includes steps that have been shown to remove and/or inactivate extraneous agents. In addition, the process is designed to minimise microbial contamination and vasoactive substances.

CHARACTERS
Appearance
White or almost white, amorphous powder.

Solubility
Soluble in water.

IDENTIFICATION
A. Place separately in 2 haemolysis tubes 0.5 mL of citrated human plasma and 0.5 mL of citrated bovine plasma and maintain in a water-bath at 37 °C. To each tube add 0.1 mL of a solution containing a quantity of the substance to be examined equivalent to 1000 IU/mL in *phosphate buffer solution pH 7.4 R* and 0.1 mL of a solution containing a quantity of *human thrombin R* equivalent to 20 IU/mL in *phosphate buffer solution pH 7.4 R*. Shake immediately. In both tubes, a clot forms and lyses within 30 min.

B. Carry out identification by a suitable immunodiffusion test.

TESTS
Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 10 mg in 10 mL of *water R*.

Thromboplastic contaminants
Test solutions Dissolve suitable quantities of the substance to be examined in *barbital buffer solution pH 7.4 R* to obtain solutions with activities of 5000 IU/mL, 2500 IU/mL, 1250 IU/mL, 625 IU/mL and 312 IU/mL.

To each of 6 haemolysis tubes 1 cm in internal diameter add 0.1 mL of *citrated rabbit plasma R*. Allocate the test solutions 1 to each of 5 of the tubes; add to each tube 0.1 mL of the solution allocated to it and to the 6th tube add 0.1 mL of *barbital buffer solution pH 7.4 R* (blank). Incubate the tubes at 25 ± 0.5 °C for 5 min and add 0.1 mL of a 3.675 g/L solution of *calcium chloride R*. Measure with a stop-watch the coagulation time for each tube. Plot the shortening of the recalcification time (clotting time of the blank minus clotting time measured) against log concentration in International Units. Extrapolate the best-fitting straight line through the 5 points until it reaches the log-concentration axis.

The urokinase activity at the intersection point, which represents the limit concentration for coagulant activity (zero coagulant activity), is not less than 150 IU/mL.

Molecular fractions
Size-exclusion chromatography (*2.2.30*).

Test solution Dissolve about 1 mg in 1.0 mL of *0.02 M phosphate buffer solution pH 8.0 R*. Prepare immediately before use.

Column:
- *size:* l = 0.9 m, Ø = 16 mm;
- *stationary phase: cross-linked dextran for chromatography R3*;
- *temperature:* 5 °C.

Mobile phase 17.5 g/L solution of *sodium chloride R* in *0.02 M phosphate buffer solution pH 8.0 R*.

Flow rate 0.1 mL/min.

Apply the test solution to the head of the column rinsing twice with 0.5 mL portions of the buffer and carry out the elution. The eluate may be collected in fractions of 1 mL. Measure the absorbance (*2.2.25*) of the eluate at the maximum at 280 nm and plot the individual values on a graph. Draw perpendicular lines towards the axis of the abscissae from the minima before the HMM peak, between the HMM and the LMM peaks, and after the LMM peak, thus identifying the fractions to be considered in calculating the HMM/LMM activity ratio. Pool the HMM fractions and, separately, the LMM fractions. Determine separately the urokinase activity in International Units of each of the fraction pools by the method prescribed under Assay.
The ratio of the urokinase activity in the HMM fraction pool to that in the LMM fraction pool is not less than 2.0.

Total protein
Determine the nitrogen content, using 10 mg, by the method of sulfuric acid digestion (*2.5.9*) and calculate the quantity of protein by multiplying by 6.25.

ASSAY
The potency of urokinase is determined by comparing its capacity to activate plasminogen to form plasmin with the same capacity of a reference preparation of urokinase calibrated in International Units; the formation of plasmin is measured by the determination of the lysis time of a fibrin clot under given conditions.

The International Unit is the activity contained in a stated amount of the International Reference Preparation, which consists of freeze-dried urokinase with lactose.
The equivalence in International Units of the International Reference Preparation is stated by the World Health Organization.

Unless otherwise prescribed, use *phosphate buffer solution pH 7.4 R* containing 30 g/L of *bovine albumin R* for the preparation of the solutions and dilutions used in the assay.

Test solution Prepare a solution of the substance to be examined expected to have an activity of 1000 IU/mL.

Reference solution Prepare a solution of a reference preparation having an activity of 1000 IU/mL.

Keep the test solution and the reference solution in iced water and use within 6 h. Prepare 3 serial 1.5-fold dilutions of the reference preparation such that the longest clot-lysis time is less than 20 min and the shortest clot-lysis time is greater than 3 min. Prepare 3 similar dilutions of the test solution. Keep the solutions in iced water and use within 1 h. Use 24 tubes 8 mm in diameter. Label the tubes T_1, T_2 and T_3 for the dilutions of the test solution and S_1, S_2 and S_3 for the dilutions of the reference solution, allocating 4 tubes to each dilution. Place the tubes in iced water. Into each tube, introduce 0.2 mL of the appropriate dilution, 0.2 mL of *phosphate buffer solution pH 7.4 R* containing 30 g/L of *bovine albumin R* and 0.1 mL of a solution of *human thrombin R* having an activity of not less than 20 IU/mL. Place the tubes in a water-bath at 37 °C and allow to stand for 2 min to attain temperature equilibrium. Using an automatic pipette, introduce into the bottom of the 1st tube 0.5 mL of a 10 g/L solution of *bovine euglobulins R*, ensuring mixing. At intervals of 5 s, introduce successively into the remaining tubes 0.5 mL of a 10 g/L solution of *bovine euglobulins R*. Using a stop-watch, measure for each tube the time in seconds that elapses between the addition of the euglobulins solution and the lysis of the clot. Plot the logarithms of the lysis times for the substance to be examined and for the reference preparation against the logarithms of the concentration and calculate the activity of the substance to be examined using the usual statistical methods.

The estimated potency is not less than 90 per cent and not more than 111 per cent of the stated potency.
The confidence limits ($P = 0.95$) of the estimated potency are not less than 80 per cent and not more than 125 per cent of the stated potency.

STORAGE

In an airtight container, protected from light, at a temperature not exceeding 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-evident container.

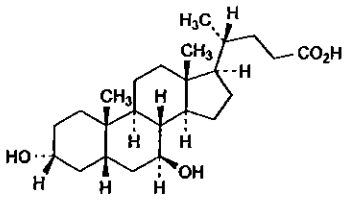
LABELLING

The label states the potency in International Units per milligram of protein.

Ph Eur

Ursodeoxycholic Acid

(Ph. Eur. monograph 1275)



$C_{24}H_{40}O_4$ 392.6 *128-13-2*

Action and use

Bile acid; treatment of gallstones.

Preparations

Ursodeoxycholic Acid Capsules
Ursodeoxycholic Acid Tablets
Ursodeoxycholic Acid Oral Suspension

Ph Eur

DEFINITION

3α,7β-Dihydroxy-5β-cholan-24-oic acid.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water, freely soluble in ethanol (96 per cent), slightly soluble in acetone, practically insoluble in methylene chloride.

mp

About 202 °C.

IDENTIFICATION

First identification: A.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *ursodeoxycholic acid CRS.*

B. Examine the chromatograms obtained in the test for impurity C.

Results The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve about 10 mg in 1 mL of *sulfuric acid R*. Add 0.1 mL of *formaldehyde solution R* and allow to stand for 5 min. Add 5 mL of *water R*. The suspension obtained is greenish-blue.

TESTS

Specific optical rotation (*2.2.7*)

+ 58.0 to + 62.0 (dried substance).

Dissolve 0.500 g in *anhydrous ethanol R* and dilute to 25.0 mL with the same solvent.

Impurity C

Thin-layer chromatography (*2.2.27*).

Solvent mixture *water R, acetone R* (10:90 *V/V*).

Test solution (a) Dissolve 0.40 g of the substance to be examined in the solvent mixture and dilute to 10 mL with the solvent mixture.

Test solution (b) Dilute 1 mL of test solution (a) to 10 mL with the solvent mixture.

Reference solution (a) Dissolve 40 mg of *ursodeoxycholic acid CRS* in the solvent mixture and dilute to 10 mL with the solvent mixture.

Reference solution (b) Dissolve 20 mg of *lithocholic acid CRS* (impurity C) in the solvent mixture and dilute to 10.0 mL with the solvent mixture (solution A). Dilute 2.0 mL of this solution to 100.0 mL with the solvent mixture.

Reference solution (c) To 5 mL of solution A add 10 mg of *chenodeoxycholic acid CRS* (impurity A) and dilute to 50 mL with the solvent mixture.

Plate *TLC silica gel plate R.*

Mobile phase *glacial acetic acid R, acetone R, methylene chloride R* (1:30:60 *V/V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying At 120 °C for 10 min.

Detection Spray immediately with a 47.6 g/L solution of *phosphomolybdic acid R* in a mixture of 1 volume of *sulfuric acid R* and 20 volumes of *glacial acetic acid R* and heat at 120 °C until blue spots appear on a lighter background.

System suitability Reference solution (c):
— the chromatogram shows 2 clearly separated principal spots.

Limit Test solution (a):
— *impurity C*: any spot due to impurity C is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.1 per cent).

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture *methanol R*, mobile phase (10:90 *V/V*).

Test solution Dissolve 60 mg of the substance to be examined in the solvent mixture and dilute to 20.0 mL with the solvent mixture.

Reference solution (a) Dissolve the contents of a vial of *ursodeoxycholic acid for system suitability CRS* (containing impurities A and H) in 1.0 mL of the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 40 °C ± 1 °C.

Mobile phase Mix 30 volumes of *acetonitrile R*, 37 volumes of a 0.78 g/L solution of *sodium dihydrogen phosphate R* adjusted to pH 3 with *phosphoric acid R*, and 40 volumes of *methanol R*.

Flow rate 0.8 mL/min.

Detection Refractometer at 35 ± 1 °C.

Injection 150 µL.

Run time 4 times the retention time of ursodeoxycholic acid.

Identification of impurities Use the chromatogram supplied with *ursodeoxycholic acid for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A and H.

Relative retention With reference to ursodeoxycholic acid (retention time = about 14 min): impurity H = about 0.9; impurity A = about 2.8.

System suitability Reference solution (a):
— *resolution*: minimum 1.5 between the peaks due to impurity H and ursodeoxycholic acid.

Limits:
— *impurity A*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than 15 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

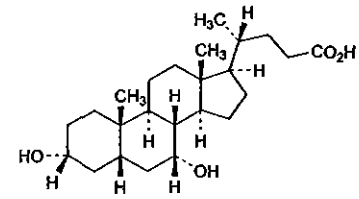
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.350 g in 50 mL of *ethanol (96 per cent) R*, previously neutralised to 0.2 mL of *phenolphthalein solution R*. Add 50 mL of *water R* and titrate with *0.1 M sodium hydroxide* until a pink colour is obtained.

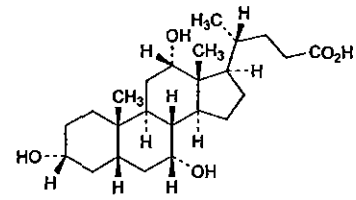
1 mL of *0.1 M sodium hydroxide* is equivalent to 39.26 mg of $C_{24}H_{40}O_4$.

IMPURITIES
Specified impurities *A, C.*

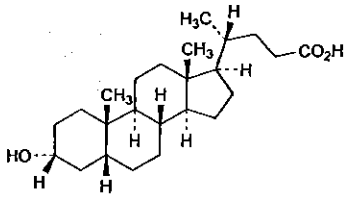
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, D, E, F, G, H, I.*



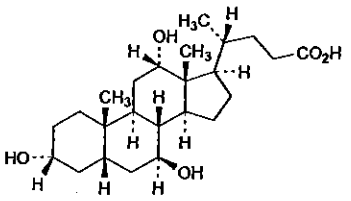
A. 3α,7α-dihydroxy-5β-cholan-24-oic acid (chenodeoxycholic acid),



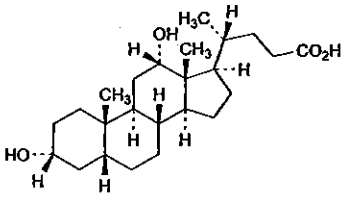
B. 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid (cholic acid),



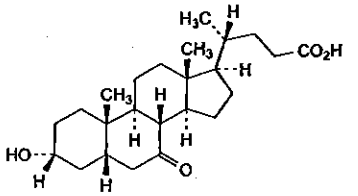
C. 3α-hydroxy-5β-cholan-24-oic acid (lithocholic acid),



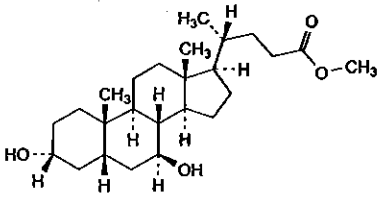
D. 3α,7β,12α-trihydroxy-5β-cholan-24-oic acid (ursocholic acid),



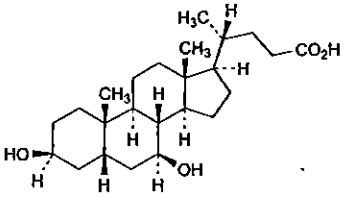
E. 3α,12α-dihydroxy-5β-cholan-24-oic acid (deoxycholic acid),



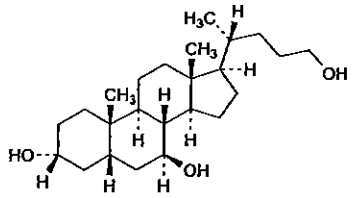
F. 3α-hydroxy-7-oxo-5β-cholan-24-oic acid,



G. methyl 3α,7β-dihydroxy-5β-cholan-24-oate,



H. 3β,7β-dihydroxy-5β-cholan-24-oic acid,

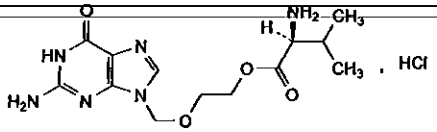


I. 5β-cholane-3α,7β,24-triol.

Ph Eur

Valaciclovir Hydrochloride

(Ph. Eur. monograph 1768)



$C_{13}H_{21}ClN_6O_4$ 360.8 *124832-27-5*

Action and use
Purine nucleoside analogue; antiviral (herpesviruses).

Preparation
Valaciclovir Tablets

Ph Eur

DEFINITION

2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl L-valinate hydrochloride.

Content
95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Freely soluble in water, slightly soluble in anhydrous ethanol.

It shows polymorphism (*5.9*).

IDENTIFICATION

Carry out either tests A, B, C, E or tests A, B, D, E.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison anhydrous valaciclovir hydrochloride CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *anhydrous ethanol R* and evaporate to dryness in a desiccator, under high vacuum, over *diphosphorus pentoxide R*. Record new spectra using the residues.

B. It gives reaction (a) of chlorides (*2.3.1*).

C. It complies with the limit for impurity R given in test A for related substances.

D. Optical rotation (*2.2.7*): laevorotatory.

Dissolve 2.50 g in *water R* and dilute to 50.0 mL with the same solvent.

E. Water (see Tests).

TESTS

Impurities E, F and G
Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.250 g of the substance to be examined in 2 mL of *water R* and dilute to 5.0 mL with *ethanol (96 per cent) R*.

Reference solution (a) Dissolve 5 mg of *valaciclovir impurity D CRS*, 5.0 mg of *valaciclovir impurity E CRS*, 5.0 mg of *valaciclovir impurity G CRS* and 8.4 mg of *valaciclovir impurity F para-toluenesulfonate CRS* in a mixture of 2 mL of *water R* and 6 mL of *ethanol (96 per cent) R*, and dilute to 10.0 mL with *ethanol (96 per cent) R*.

Reference solution (b) Dilute 3.0 mL of reference solution (a) to 10.0 mL with *ethanol (96 per cent) R*.

Reference solution (c) Dilute 2.0 mL of reference solution (a) to 10.0 mL with *ethanol (96 per cent) R*.

Reference solution (d) Dilute 0.5 mL of reference solution (a) to 10.0 mL with *ethanol (96 per cent) R*.

Plate ~~*TLC silica gel F_{254} plate R (2-10 µm).*~~

Pretreatment Wash the plate with *methanol R* until the solvent front has migrated over at least 4/5 of the plate; allow the plate to dry.

Mobile phase *concentrated ammonia R, tetrahydrofuran R, methanol R, methylene chloride R* (3:12:34:54 *V/V/V/V*); use freshly prepared mobile phase.

Application 4 µL of the test solution and reference solutions (b), (c) and (d).

Development Over 4/5 of the plate.

Drying In a current of air.

Detection Examine in ultraviolet light at 254 nm for impurities E and G; spray with a 0.1 g/L solution of *fluorescamine R* in *ethylene chloride R* and examine in ultraviolet light at 365 nm for impurity F.

Retardation factors Impurity A = about 0; impurity B = about 0.2; valaciclovir = about 0.3; impurity C = about 0.5; impurity D = about 0.6; impurity E = about 0.7; impurity F = about 0.75; impurity G = about 0.79; impurity C is masked by the leading edge of the spot due to valaciclovir; impurities F and G may co-elute, but this does not adversely affect their quantification because they are visualised differently.

System suitability The chromatograms obtained with reference solutions (b), (c) and (d) each show 3 clearly separated spots when examined under ultraviolet light at 254 nm, due to impurities D, E and G.

Limits:

— *impurity E*: any spot due to impurity E is not more intense than the corresponding spot in the chromatogram obtained with reference solution (c) (0.2 per cent);
— *impurity F*: any spot due to impurity F is not more intense than the corresponding spot in the chromatogram obtained with reference solution (b) (0.3 per cent calculated as hydrochloride salt);
— *impurity G*: any spot due to impurity G is not more intense than the corresponding spot in the chromatogram obtained with reference solution (d) (0.05 per cent).

Related substances

A. Impurities A, B, I and R. Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution Dissolve 50.0 mg of the substance to be examined in a 0.5 per cent *V/V* solution of *hydrochloric acid R* and dilute to 100.0 mL with the same solution.

Reference solution (a) Dissolve 2.5 mg of *valaciclovir for system suitability CRS* (containing impurities A, B, C, D, H, I, J, M and R) in a 0.5 per cent *V/V* solution of *hydrochloric acid R* and dilute to 5.0 mL with the same solution.

Reference solution (b) Dissolve 50.0 mg of *anhydrous valaciclovir hydrochloride CRS* in a 0.5 per cent *V/V* solution of *hydrochloric acid R* and dilute to 100.0 mL with the same solution.

Reference solution (c) Dilute 3.0 mL of the test solution to 100.0 mL with a 0.5 per cent *V/V* solution of *hydrochloric acid R*. Dilute 1.0 mL of this solution to 100.0 mL with a 0.5 per cent *V/V* solution of *hydrochloric acid R*.

Column:

— *size*: l = 0.15 m, Ø = 4.0 mm;
— *stationary phase*: *crown-ether silica gel for chiral separation R* (5 µm);
— *temperature*: 10 °C.

Mobile phase *perchloric acid R, methanol R, water for chromatography R* (0.5:5:95 *V/V/V*).

Flow rate 0.75 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µL of the test solution and reference solutions (a) and (c).

Run time 1.5 times the retention time of valaciclovir.

Identification of impurities Use the chromatogram supplied with *valaciclovir for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A + B, C + R, D, I and M.

Relative retention With reference to valaciclovir (retention time = about 21 min): impurities A and B = about 0.2; impurity I = about 0.4; impurities C and R = about 0.6; impurity D = about 0.7; impurity M = about 1.3.

System suitability Reference solution (a):

— *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurities C and R.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurities A and B by 0.7;
— *impurity R*: maximum 3.0 per cent; for the calculation, subtract the content of impurity C as determined in test B for related substances from the content of the coeluting impurities C and R as determined in this test;
— *sum of impurities A and B*: maximum 2.0 per cent;
— *impurity I*: maximum 0.2 per cent;
— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.03 per cent); disregard any peaks due to impurities other than A + B, C + R or I.

B. Liquid chromatography (*2.2.29*): use the normalisation procedure. *Use the solutions within 24 h of preparation.*

Solvent mixture *ethanol (96 per cent) R, water R* (20:80 *V/V*).

Test solution Dissolve 40 mg of the substance to be examined in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Reference solution (a) Dissolve 2.5 mg of *valaciclovir for system suitability CRS* (containing impurities A, B, C, D, H, I, J, M and R) in the solvent mixture and dilute to 5.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 20.0 mL with the solvent mixture.

Column:
- — *size*: *l* = 0.25 m, Ø = 4.6 mm;
- — *stationary phase*: *end-capped phenylhexylsilyl silica gel for chromatography R* (5 µm);
- — *temperature*: 15 °C.

Mobile phase:
- — *mobile phase A*: *trifluoroacetic acid R, water for chromatography R* (0.2:100 *V/V*);
- — *mobile phase B*: *trifluoroacetic acid R, methanol R2* (0.2:100 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	90	10
5 - 35	90 → 60	10 → 40

Flow rate 0.8 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µL.

Identification of impurities Use the chromatogram supplied with *valaciclovir for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D, H, I, J and M.

Relative retention With reference to valaciclovir (retention time = about 19 min): impurity A = about 0.3; impurity B = about 0.4; impurity H = about 0.5; impurity C = about 1.06; impurity I = about 1.09; impurity D = about 1.2; impurity J = about 1.3; impurity M = about 1.6.

System suitability Reference solution (a):
- — *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to valaciclovir;
- — the chromatogram obtained is similar to the chromatogram supplied with *valaciclovir for system suitability CRS*.

Limits:
- — *impurity M*: maximum 1.5 per cent;
- — *impurity D*: maximum 0.5 per cent;
- — *impurity C*: maximum 0.3 per cent;
- — *impurities H, J*: for each impurity, maximum 0.10 per cent;
- — *unspecified impurities*: for each impurity, maximum 0.05 per cent;
- — *disregard limit*: 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.03 per cent); disregard the peaks due to impurities A, B and I.

Limit:
- — *total for tests A and B*: maximum 5.0 per cent.

Chloride

9.4 to 9.9 per cent (anhydrous and solvent-free substance).

Dissolve 0.350 g in 100 mL of *water R* and add 0.2 mL of *nitric acid R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M silver nitrate*. Use a silver indicator electrode and a silver-silver chloride reference electrode or a combined silver electrode. Discard the result from the first titration, which is used to condition the electrodes. Carry out a blank titration.

1 mL of *0.1 M silver nitrate* is equivalent to 3.543 mg of Cl.

Water (*2.5.12*)

Maximum 2.0 per cent, determined on 0.250 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in test A for related substances with the following modification.

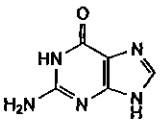
Injection Test solution and reference solution (b).

Calculate the percentage content of $C_{13}H_{21}ClN_6O_4$ taking into account the assigned content of *anhydrous valaciclovir hydrochloride CRS*.

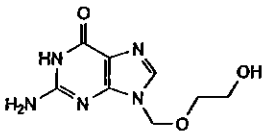
IMPURITIES

Specified impurities *A, B, C, D, E, F, G, H, I, J, M, R.*

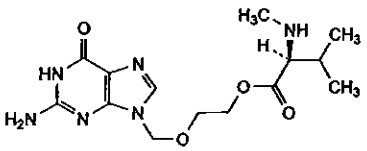
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *K, L, N, O, P, Q.*



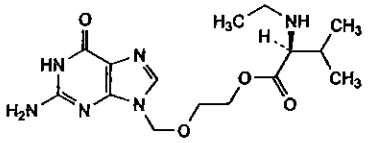
A. 2-amino-1,9-dihydro-6*H*-purin-6-one (guanine),



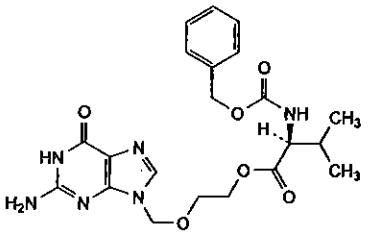
B. 2-amino-9-[(2-hydroxyethoxy)methyl]-1,9-dihydro-6*H*-purin-6-one (aciclovir),



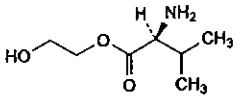
C. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy] ethyl *N*-methyl-L-valinate,



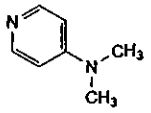
D. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy] ethyl *N*-ethyl-L-valinate,



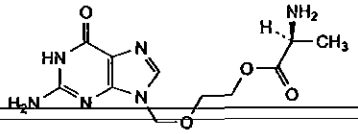
E. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy] ethyl *N*-[(benzyloxy)carbonyl]-L-valinate,



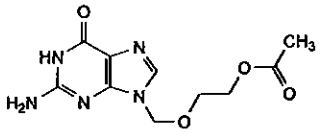
F. 2-hydroxyethyl L-valinate,



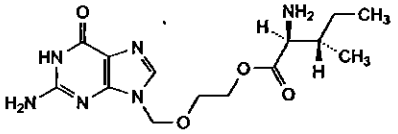
G. *N,N*-dimethylpyridin-4-amine,



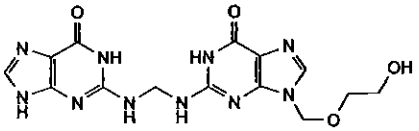
H. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy] ethyl L-alaninate,



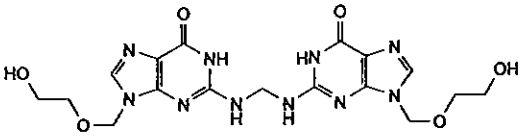
I. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy] ethyl acetate,



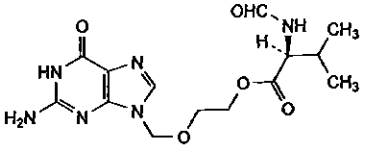
J. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy] ethyl L-isoleucinate,



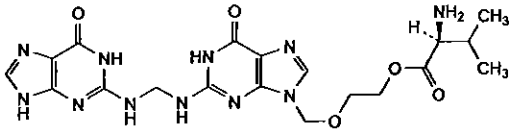
K. 9-[(2-hydroxyethoxy)methyl]-2-[[[(6-oxo-6,9-dihydro-1*H*-purin-2-yl)amino]methyl]amino]-1,9-dihydro-6*H*-purin-6-one,



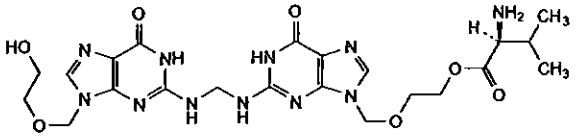
L. 2,2′-(methylenediazanediyl)bis[9-[(2-hydroxyethoxy) methyl]-1,9-dihydro-6*H*-purin-6-one],



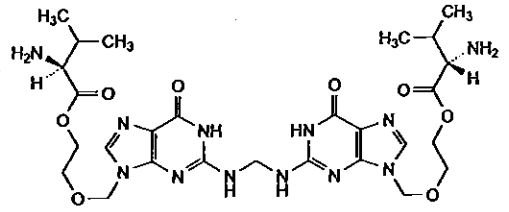
M. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy] ethyl *N*-formyl-L-valinate,



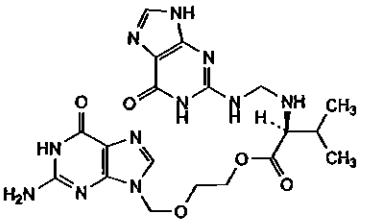
N. 2-[[6-oxo-2-[[[(6-oxo-6,9-dihydro-1*H*-purin-2-yl)amino] methyl]amino]-1,6-dihydro-9*H*-purin-9-yl]methoxy]ethyl L-valinate,



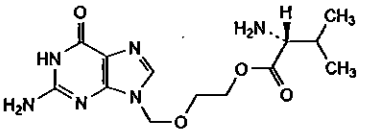
O. 2-[[2-[[[[9-[(2-hydroxyethoxy)methyl]-6-oxo-6,9-dihydro-1*H*-purin-2-yl]amino]methyl]amino]-6-oxo-1,6-dihydro-9*H*-purin-9-yl]methoxy]ethyl L-valinate,



P. [methylenebis[azanediyl(6-oxo-1,6-dihydro-9*H*-purine-2,9-diyl)methyleneoxyethan-2,1-diyl]] di-L-valinate,



Q. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy] ethyl *N*-[[(6-oxo-6,9-dihydro-1*H*-purin-2-yl)amino] methyl]-L-valinate,



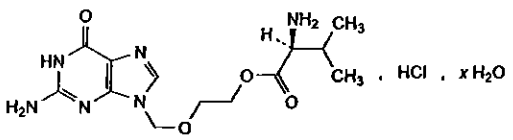
R. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy] ethyl D-valinate.

Ph Eur

Valaciclovir Hydrochloride Hydrate

Valaciclovir Hydrochloride, Hydrated

(Ph. Eur. monograph 2751)



$C_{13}H_{21}ClN_6O_4,xH_2O$ 360.8 *1218948-84-5*

(anhydrous substance)

Action and use

Purine nucleoside analogue; antiviral (herpesviruses).

Preparation

Valaciclovir Tablets

Ph Eur

DEFINITION

2-[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl L-valinate hydrochloride hydrate.

Content

95.0 per cent to 102.0 per cent (anhydrous substance).

It contains a variable quantity of water.

CHARACTERS

Appearance

White or almost white powder, hygroscopic.

Solubility

Freely soluble in water, very slightly soluble in anhydrous ethanol, practically insoluble in acetonitrile.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Dissolve the substance to be examined in the minimum volume of *water R*, evaporate to dryness at room temperature and record the spectrum using the residue.

Comparison Repeat the operations using *anhydrous valaciclovir hydrochloride CRS*.

B. It complies with the limit for impurity R (see test A for related substances).

C. Water (see Tests).

D. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Impurities G and S

Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.250 g of the substance to be examined in 2 mL of *water R* and dilute to 5.0 mL with *ethanol (96 per cent) R*.

Reference solution Dissolve 5.0 mg of *valaciclovir impurity G CRS* and 5.0 mg of *valaciclovir impurity S CRS* in a mixture of 2 mL of *water R* and 6 mL of *ethanol (96 per cent) R* and dilute to 10.0 mL with *ethanol (96 per cent) R*. Dilute 0.5 mL of the solution to 10.0 mL with *ethanol (96 per cent) R*.

Plate *TLC silica gel F_{254} plate R* (2-10 µm).

Pretreatment Wash the plate with *methanol R* until the solvent front has migrated over at least 4/5 of the plate; allow to dry in air.

Mobile phase *concentrated ammonia R, tetrahydrofuran R, methanol R, methylene chloride R* (3:12:34:54 *V/V/V/V*); use freshly prepared mobile phase.

Application 4 µL.

Development Over 4/5 of the plate.

Drying In a current of air.

Detection Examine in ultraviolet light at 254 nm.

Retardation factors Valaciclovir = about 0.3; impurity S = about 0.7; impurity G = about 0.8.

System suitability The chromatogram obtained with the reference solution shows 2 clearly separated spots due to impurities S and G.

Limits:

— *impurity G*: any spot due to impurity G is not more intense than the corresponding spot in the chromatogram obtained with the reference solution (0.05 per cent);

— *impurity S*: any spot due to impurity S is not more intense than the corresponding spot in the chromatogram obtained with the reference solution (0.05 per cent).

Related substances

A. Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution Dissolve 50.0 mg of the substance to be examined in a 0.5 per cent *V/V* solution of *hydrochloric acid R* and dilute to 100.0 mL with the same solution.

Reference solution (a) Dissolve 2.5 mg of *valaciclovir for system suitability CRS* (containing impurities A, B, C, D, H, M and R) in a 0.5 per cent *V/V* solution of *hydrochloric acid R* and dilute to 5.0 mL with the same solution.

Reference solution (b) Dissolve 50.0 mg of *anhydrous valaciclovir hydrochloride CRS* in a 0.5 per cent *V/V* solution of *hydrochloric acid R* and dilute to 100.0 mL with the same solution.

Reference solution (c) Dilute 3.0 mL of the test solution to 100.0 mL with a 0.5 per cent *V/V* solution of *hydrochloric acid R*. Dilute 1.0 mL of this solution to 100.0 mL with a 0.5 per cent *V/V* solution of *hydrochloric acid R*.

Column:

— *size*: *l* = 0.15 m, Ø = 4.0 mm;

— *stationary phase*: *crown-ether silica gel for chiral separation R* (5 µm);

— *temperature*: 10 °C.

Mobile phase *perchloric acid R, methanol R, water for chromatography R* (0.5:5:95 *V/V/V*).

Flow rate 0.75 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µL of the test solution and reference solutions (a) and (c).

Run time 1.5 times the retention time of valaciclovir.

Identification of impurities Use the chromatogram supplied with *valaciclovir for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A + B, C + R, D and M.

Relative retention With reference to valaciclovir (retention time = about 17 min): impurities A and B = about 0.2; impurities C and R = about 0.6; impurity D = about 0.7; impurity M = about 1.3.

System suitability Reference solution (a):

— *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of

the curve separating this peak from the peak due to impurities C and R.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurities A and B by 0.7;
— *impurity R*: maximum 3.0 per cent; for the calculation, subtract the content of impurity C as determined in test B for related substances from the content of the coeluting impurities C and R as determined in this test;
— *sum of impurities A and B*: maximum 2.0 per cent;
— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.03 per cent); disregard any peaks due to impurities other than A + B and C + R.

B. Liquid chromatography (*2.2.29*): use the normalisation procedure. ~~*Use the solutions within 24 h of preparation.*~~

Solvent mixture *ethanol (96 per cent) R, water R* (20:80 *V/V*).

Test solution Dissolve 80 mg of the substance to be examined in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Reference solution (a) Dissolve 1.6 mg of *valaciclovir for system suitability CRS* (containing impurities A, B, C, D, H, M and R) in the solvent mixture and dilute to 2.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 20.0 mL with the solvent mixture.

Reference solution (c) Dissolve 2 mg of *valaciclovir impurity P CRS* in the solvent mixture and dilute to 25.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 100.0 mL with the solvent mixture.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped phenylhexylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 15 °C.

Mobile phase:

— *mobile phase A*: *trifluoroacetic acid R, water for chromatography R* (0.2:100 *V/V*);
— *mobile phase B*: *trifluoroacetic acid R, methanol R2* (0.2:100 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	90	10
5 - 35	90 → 60	10 → 40
35 - 45	60	40

Flow rate 0.8 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µL.

Identification of impurities Use the chromatogram supplied with *valaciclovir for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D, H and M; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity P.

Relative retention With reference to valaciclovir (retention time = about 20 min): impurity A = about 0.3; impurity B = about 0.4; impurity H = about 0.5; impurity C = about 1.06; impurity D = about 1.2; impurity M = about 1.6; impurity P = about 2.0.

System suitability Reference solution (a):

— *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to valaciclovir.

Limits:

— *impurity M*: maximum 0.6 per cent;
— *impurity D*: maximum 0.3 per cent;
— *impurity C*: maximum 0.2 per cent;
— *impurities H, P*: for each impurity, maximum 0.10 per cent;
— *unspecified impurities*: for each impurity, maximum 0.05 per cent;
— *disregard limit*: 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.03 per cent); disregard the peaks due to impurities A and B.

Limit:

— *total for tests A and B*: maximum 4.0 per cent.

Water (*2.5.12*)

4.5 per cent to 11.0 per cent, determined on 0.100 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in test A for related substances with the following modification.

Injection Test solution and reference solution (b).

Calculate the percentage content of $C_{13}H_{21}ClN_6O_4$ taking into account the assigned content of *anhydrous valaciclovir hydrochloride CRS*.

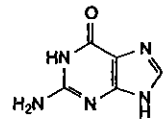
STORAGE

In an airtight container.

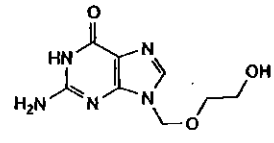
IMPURITIES

Specified impurities *A, B, C, D, G, H, M, P, R, S.*

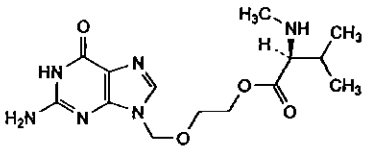
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *I, J, N.*



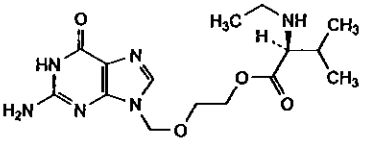
A. 2-amino-1,9-dihydro-6*H*-purin-6-one (guanine),



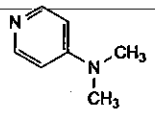
B. 2-amino-9-[(2-hydroxyethoxy)methyl]-1,9-dihydro-6*H*-purin-6-one (aciclovir),



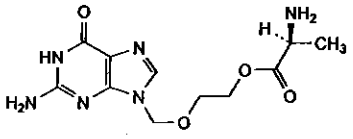
C. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy] ethyl *N*-methyl-L-valinate,



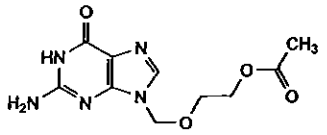
D. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy] ethyl *N*-ethyl-L-valinate,



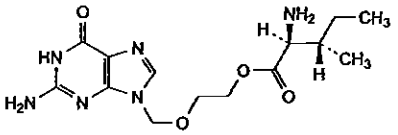
G. *N*,*N*-dimethylpyridin-4-amine,



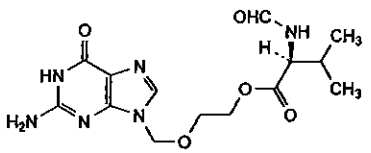
H. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy] ethyl L-alaninate,



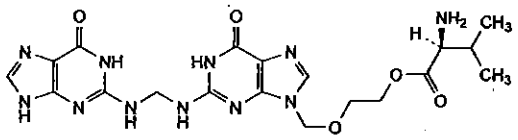
I. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy] ethyl acetate,



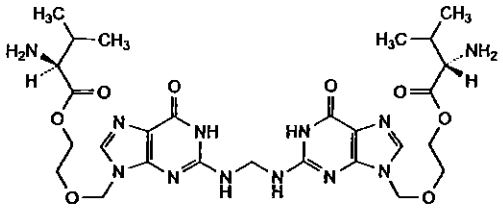
J. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy] ethyl L-isoleucinate,



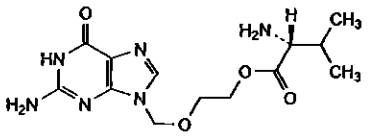
M. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy] ethyl *N*-formyl-L-valinate,



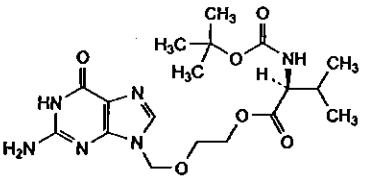
N. 2-[[6-oxo-2-[[[(6-oxo-6,9-dihydro-1*H*-purin-2-yl)amino] methyl]amino]-1,6-dihydro-9*H*-purin-9-yl]methoxy]ethyl L-valinate,



P. [methylenebis[azanediyl(6-oxo-1,6-dihydro-9*H*-purine-2,9-diyl)methyleneoxyethan-2,1-diyl]] di-L-valinate,



R. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy] ethyl D-valinate,

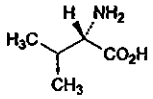


S. 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy] ethyl *N*-(*tert*-butoxycarbonyl)-L-valinate.

Ph Eur

Valine

(Ph. Eur. monograph 0796)



$C_5H_{11}NO_2$ 117.1 *72-18-4*

Action and use
Amino acid.

Ph Eur

DEFINITION

(2*S*)-2-Amino-3-methylbutanoic acid.

Product of fermentation or of protein hydrolysis.

Content
98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder or colourless crystals.

Solubility
Soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: A, B.

Second identification: A, C.

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison valine CRS.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in a 10.3 g/L solution of *hydrochloric acid R* and dilute to 50 mL with the same solution.

Reference solution Dissolve 10 mg of *valine CRS* in a 10.3 g/L solution of *hydrochloric acid R* and dilute to 50 mL with the same solution.

Plate *TLC silica gel plate R.*

Mobile phase *glacial acetic acid R, water R, butanol R* (20:20:60 *V/V/V*)

Application 5 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *ninhydrin solution R* and heat at 105 °C for 15 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Solution S

Dissolve 2.5 g in *water R* and dilute to 100 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*)

+ 26.5 to + 29.0 (dried substance).

Dissolve 2.00 g in *hydrochloric acid R1* and dilute to 25.0 mL with the same acid.

Ninhydrin-positive substances

Amino acid analysis (*2.2.56*). For analysis, use Method 1.

The concentrations of the test solution and the reference solutions may be adapted according to the sensitivity of the equipment used. The concentrations of all solutions are adjusted so that the system suitability requirements described in general chapter *2.2.46* are fulfilled, keeping the ratios of concentrations between all solutions as described.

Solution A *dilute hydrochloric acid R1* or a sample preparation buffer suitable for the apparatus used.

Test solution Dissolve 30.0 mg of the substance to be examined in solution A and dilute to 50.0 mL with solution A.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with solution A. Dilute 2.0 mL of this solution to 10.0 mL with solution A.

Reference solution (b) Dissolve 30.0 mg of *proline R* in solution A and dilute to 100.0 mL with solution A. Dilute 1.0 mL of the solution to 250.0 mL with solution A.

Reference solution (c) Dilute 6.0 mL of *ammonium standard solution (100 ppm NH_4) R* to 50.0 mL with solution A. Dilute 1.0 mL of this solution to 100.0 mL with solution A.

Reference solution (d) Dissolve 30 mg of *isoleucine R* (impurity B) and 30 mg of *leucine R* (impurity C) in solution A and dilute to 50.0 mL with solution A. Dilute 1.0 mL of the solution to 200.0 mL with solution A.

Reference solution (e) Dissolve 30.0 mg of *isoleucine R* (impurity B) in solution A and dilute to 100.0 mL with solution A. Dilute 1.0 mL of the solution to 250.0 mL with solution A.

Blank solution Solution A.

Inject suitable, equal amounts of the test, blank and reference solutions into the amino acid analyser. Run a program suitable for the determination of physiological amino acids.

System suitability Reference solution (d):

— *resolution*: minimum 1.5 between the peaks due to impurities B and C.

Calculation of percentage contents:

— for impurity B, use the concentration of impurity B in reference solution (e);

— for any ninhydrin-positive substance detected at 570 nm, use the concentration of valine in reference solution (a);

— for any ninhydrin-positive substance detected at 440 nm, use the concentration of proline in reference solution (b); if a peak is above the reporting threshold at both wavelengths, use the result obtained at 570 nm for quantification.

Limits:

— *impurity B at 570 nm*: maximum 0.4 per cent;

— *any ninhydrin-positive substance*: for each impurity, maximum 0.2 per cent;

— *total*: maximum 1.0 per cent;

— *reporting threshold*: 0.05 per cent.

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Chlorides (*2.4.4*)

Maximum 200 ppm.

Dilute 10 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)

Maximum 300 ppm.

Dissolve 0.5 g in *distilled water R* and dilute to 15 mL with the same solvent.

Ammonium

Amino acid analysis (*2.2.56*) as described in the test for ninhydrin-positive substances with the following modifications.

Injection Test solution, reference solution (c) and blank solution.

Limit:

— *ammonium at 570 nm*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.02 per cent), taking into account the peak due to ammonium in the chromatogram obtained with the blank solution.

Iron (*2.4.9*)

Maximum 10 ppm.

In a separating funnel, dissolve 1.0 g in 10 mL of *dilute hydrochloric acid R*. Shake with 3 quantities, each of 10 mL, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 mL of *water R* and shake for 3 min. Use the aqueous layer.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 3 mL of *anhydrous formic acid R*. Add 30 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 11.71 mg of $C_5H_{11}NO_2$.

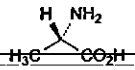
STORAGE

Protected from light.

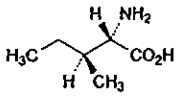
IMPURITIES

Specified impurities B.

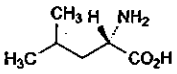
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) A, C.



A. (2*S*)-2-aminopropanoic acid (alanine),



B. (2*S*,3*S*)-2-amino-3-methylpentanoic acid (isoleucine),

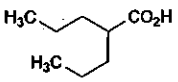


C. (2*S*)-2-amino-4-methylpentanoic acid (leucine).

Ph Eur

Valproic Acid

(Ph. Eur. monograph 1378)



$C_8H_{16}O_2$ 144.2 *99-66-1*

Action and use

Antiepileptic.

Ph Eur

DEFINITION

2-Propylpentanoic acid.

Content

99.0 per cent to 101.0 per cent.

CHARACTERS

Appearance

Colourless or very slightly yellow, clear liquid, slightly viscous.

Solubility

Very slightly soluble in water, miscible with ethanol (96 per cent) and with methylene chloride. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *valproic acid CRS.*

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5 (*2.2.2, Method II*).

Dissolve 2.0 g in *dilute sodium hydroxide solution R* and dilute to 10 mL with the same alkaline solution.

Related substances

Gas chromatography (*2.2.28*).

Test solution Dissolve 0.500 g of the substance to be examined in *heptane R* and dilute to 100.0 mL with the same solvent.

Reference solution (a) Dissolve 5 mg of *valproic acid for system suitability CRS* (containing impurity K) in 1.0 mL of *heptane R.*

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *heptane R.*

Column:

— *material:* wide-bore fused silica;
— *size:* *l* = 30 m, Ø = 0.53 mm;
— *stationary phase: macrogol 20 000 2-nitroterephthalate R* (film thickness 0.5 µm).

Carrier gas *helium for chromatography R.*

Flow rate 8 mL/min.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 5	80
	5 - 15	80 → 150
	15 - 28.3	150 → 190
	28.3 - 30	190
Injection port		220
Detector		220

Detection Flame ionisation.

Injection 1 µL.

Relative retention With reference to valproic acid (retention time = about 17 min): impurity K = about 0.97.

System suitability Reference solution (a):

— *resolution:* minimum 2.0 between the peaks due to impurity K and valproic acid.

Limits:

— *impurity K:* not more than 0.15 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
— *unspecified impurities:* for each impurity, not more than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent);
— *total:* not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
— *disregard limit:* 0.03 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.03 per cent).

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 25 mL of *ethanol (96 per cent) R.* Add 2 mL of *water R.* Titrate with *0.1 M sodium hydroxide,* determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M sodium hydroxide* is equivalent to 14.42 mg of $C_8H_{16}O_2$.

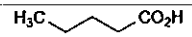
STORAGE

In an airtight container.

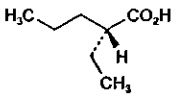
IMPURITIES

Specified impurities K.

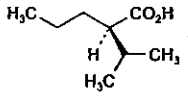
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) A, B, C, D, E, F, G, H, I, J, L.



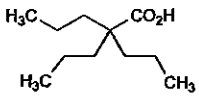
A. pentanoic acid (valeric acid),



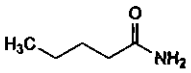
B. (2*RS*)-2-ethylpentanoic acid,



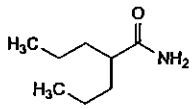
C. (2*RS*)-2-(1-methylethyl)pentanoic acid,



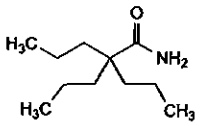
D. 2,2-dipropylpentanoic acid,



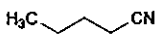
E. pentanamide (valeramide),



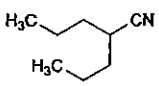
F. 2-propylpentanamide,



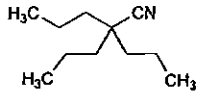
G. 2,2-dipropylpentanamide,



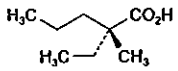
H. pentanenitrile (valeronitrile),



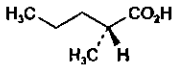
I. 2-propylpentanenitrile,



J. 2,2-dipropylpentanenitrile,



K. (2*RS*)-2-ethyl-2-methylpentanoic acid,

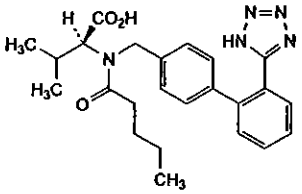


L. (2*RS*)-2-methylpentanoic acid.

Ph Eur

Valsartan

(Ph. Eur. monograph 2423)



$C_{24}H_{29}N_5O_3$ 435.5 *137862-53-4*

Action and use

Angiotensis II (AT_1) receptor antagonist.

Preparations

Valsartan Capsules

Valsartan Tablets

Ph Eur

DEFINITION

(2*S*)-3-Methyl-2-[pentanoyl[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]amino]butanoic acid.

Content

99.0 per cent to 101.0 per cent (anhydrous substance).

PRODUCTION

As *N*-nitrosamines are classified as probable human carcinogens, their presence in valsartan should be avoided or limited as much as possible. For this reason, manufacturers of valsartan for human use are expected to perform an assessment of the risk of *N*-nitrosamine formation and contamination during their manufacturing process; if this assessment identifies a potential risk, the manufacturing process should be modified to minimise contamination and a control strategy implemented to detect and control *N*-nitrosamine impurities in valsartan. The general chapter *2.5.42. N-Nitrosamines in active substances* is available to assist manufacturers.

CHARACTERS

Appearance

White or almost white, hygroscopic powder.

Solubility

Practically insoluble in water, freely soluble in anhydrous ethanol, sparingly soluble in methylene chloride.

IDENTIFICATION

Carry out either tests A, B or tests A, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *valsartan CRS.*

B. Enantiomeric purity (see Tests).

C. Specific optical rotation (*2.2.7*): −69.0 to −64.0 (anhydrous substance).

Dissolve 0.200 g in *methanol R* and dilute to 20.0 mL with the same solvent.

TESTS

Enantiomeric purity

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 5 mg of *valsartan for peak identification CRS* (containing impurity A) in the mobile phase and dilute to 5 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase.

Column:
- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *cellulose derivative of silica gel for chiral separation R* (5 µm).

Mobile phase *trifluoroacetic acid R, 2-propanol R, hexane R* (0.1:15:85 *V/V/V*).

Flow rate 0.8 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 10 µL.

Run time 1.5 times the retention time of valsartan.

Identification of impurities Use the chromatogram supplied with *valsartan for peak identification CRS* and the chromatogram obtained with reference solution (a) to identify the peak due to impurity A.

Relative retention With reference to valsartan (retention time = about 13 min): impurity A = about 0.6.

System suitability Reference solution (a):
- *resolution*: minimum 2.0 between the peaks due to impurity A and valsartan.

Limit:
- *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve the contents of a vial of *valsartan for system suitability CRS* (containing impurity C) in 1 mL of the mobile phase.

Column:
- *size*: *l* = 0.125 m, Ø = 3.0 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *glacial acetic acid R, acetonitrile R1, water for chromatography R* (1:500:500 *V/V/V*).

Flow rate 0.4 mL/min.

Detection Spectrophotometer at 225 nm.

Injection 10 µL.

Run time 6 times the retention time of valsartan.

Identification of impurities Use the chromatogram supplied with *valsartan for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity C.

Relative retention With reference to valsartan (retention time = about 5 min): impurity C = about 0.8.

System suitability Reference solution (b):
- *resolution*: minimum 3.0 between the peaks due to impurity C and valsartan.

Limits:
- *impurity C*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*)

Maximum 2.0 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.170 g in 70 mL of *2-propanol R*. Titrate with *0.1 M tetrabutylammonium hydroxide in 2-propanol*, determining the endpoint potentiometrically (*2.2.20*). Perform all operations under nitrogen.

1 mL of *0.1 M tetrabutylammonium hydroxide in 2-propanol* is equivalent to 21.78 mg of $C_{24}H_{29}N_5O_3$.

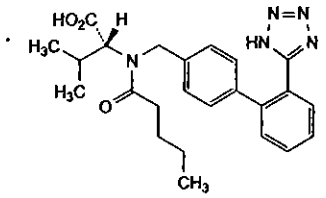
STORAGE

In an airtight container.

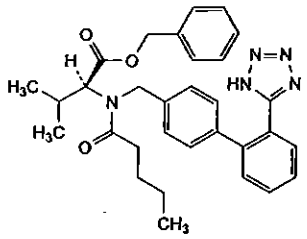
IMPURITIES

Specified impurities *A, C.*

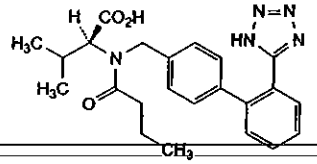
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B.*



A. (2*R*)-3-methyl-2-[pentanoyl[[2′-(1*H*-tetrazol-5-yl) biphenyl-4-yl]methyl]amino]butanoic acid,



B. benzyl (2*S*)-3-methyl-2-[pentanoyl[[2′-(1*H*-tetrazol-5-yl) biphenyl-4-yl]methyl]amino]butanoate,

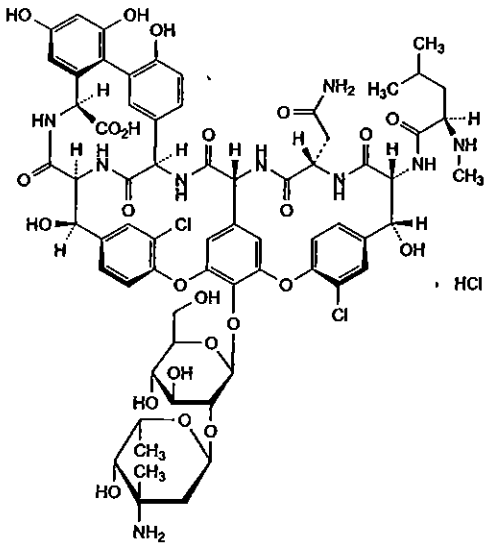


C. (2*S*)-2-[butanoyl[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl] methyl]amino]-3-methylbutanoic acid.

Ph Eur

Vancomycin Hydrochloride

(Ph. Eur. monograph 1058)



$C_{66}H_{76}Cl_3N_9O_{24}$ 1486

Action and use
Glycopeptide antibacterial.

Preparations
Vancomycin Capsules
Vancomycin Eye Drops
Vancomycin Infusion
Vancomycin Oral Solution

Ph Eur

DEFINITION
Monohydrochloride of (3*S*,6*R*,7*R*,8*M*,18*M*,22*R*,23*S*,26*S*,30a*M*,36*R*,38a*R*)-3-(2-amino-2-oxoethyl)-44-[[2-*O*-(3-amino-2,3,6-trideoxy-3-*C*-methyl-α-L-*lyxo*-hexopyranosyl)-β-D-glucopyranosyl]oxy]-10,19-dichloro-7,22,28,30,32-pentahydroxy-6-[(2*R*)-4-methyl-2-(methylamino)pentanamido]-2,5,24,38,39-pentaoxo-2,3,4,5,6,7,23,24,25,26,36,37,38,38a-tetradecahydro-22*H*-23,36-(azanomethano)-8,11:18,21-dietheno-13,16:31,35-dimetheno-1*H*,13*H*-[1,6,9] oxadiazacyclohexadecino[4,5-*m*][10,2,16] benzoxadiazacyclotetracosine-26-carboxylic acid (vancomycin B hydrochloride).

Substance produced by certain strains of *Amycolatopsis orientalis*.

Potency
Minimum 1050 IU/mg (anhydrous substance).

CHARACTERS
Appearance
White or almost white, very hygroscopic powder.

Solubility
~~Freely soluble in water, practically insoluble in ethanol~~ (96 per cent).

IDENTIFICATION
A. Examine the chromatograms obtained in the test for vancomycin B and related substances.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS
Appearance of solution
The solution is clear (*2.2.1*) and its absorbance (*2.2.25*) at 450 nm is not greater than 0.10, and its absorbance at 370 nm is not greater than 0.65.

Dissolve 2.50 g in *water R* and dilute to 25.0 mL with the same solvent.

pH (*2.2.3*)
2.5 to 4.5.

Dissolve 0.50 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Vancomycin B and related substances
Liquid chromatography (*2.2.29*): use the normalisation procedure.

Solution A Dissolve 7.0 g of *tris(hydroxymethyl) aminomethane R* in approximately 950 mL of *water for chromatography R*. Measure the temperature of the solution. Considering the temperature dependency of the buffer, adjust the pH to 8.0-8.3 using a 20 per cent *V/V* solution of *glacial acetic acid R* in *water for chromatography R* and dilute to 1000 mL with *water for chromatography R*.

Test solution Dissolve 20.0 mg of the substance to be examined in *water R* and dilute to 5.0 mL with the same solvent.

Reference solution (a) Dissolve 6 mg of *vancomycin for system suitability CRS* (containing impurities A, C, F, H, I, J, K and M) in 1.5 mL of *water R*.

Reference solution (b) In order to prepare impurities B, D, E, G and L *in situ*, expose 4 mg of *vancomycin for system suitability CRS* (containing impurities A, C, F, H, I, J, K and M) to 80-100 per cent relative humidity at 42 ± 2 °C for at least 7 days. Add 1 mL of *water R* and dissolve the sample completely using sonication.

Reference solution (c) Dilute 1.0 mL of reference solution (a) to 100.0 mL with a 0.1 per cent *V/V* solution of *acetic acid R*. Dilute 1.0 mL of this solution to 10.0 mL with a 0.1 per cent *V/V* solution of *acetic acid R*.

Column:
— *size*: *l* = 0.15 m, Ø = 2.1 mm;
— *stationary phase*: *end-capped, charged surface, ethylene-bridged octadecylsilyl silica gel for chromatography (hybrid material) R* (1.7 µm);
— *temperature*: 40 ± 2 °C.

Mobile phase:
— *mobile phase A*: *acetonitrile R, methanol R*, solution A (3:4:93 *V/V/V*);
— *mobile phase B*: *acetonitrile R, methanol R*, solution A (10:40:50 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 7	88	12
7 - 21	88 → 75	12 → 25
21 - 35	75 → 25	25 → 75
35 - 37	25	75

Flow rate 0.30 ± 0.02 mL/min.

Detection Spectrophotometer at 280 nm.

Autosampler Set at 5 °C.

Injection 2 µL.

Identification of peaks Use the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, C, F, H, I, J, K and M; use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B, D, E, G and L.

Relative retention With reference to vancomycin B (retention time = about 19 min): impurity E = about 0.37; impurity L = about 0.66; impurity B = about 0.70; impurity A = about 0.76; impurity F = about 0.82; impurity G = about 0.90; impurity H = about 0.94; impurity M = about 1.11; impurity I = about 1.14; impurity J = about 1.20; impurity D = about 1.24; impurity K = about 1.50; impurity C = about 1.86.

System suitability:
— *resolution*: minimum 1.5 and maximum 4.0 between the peaks due to impurities G and H, and minimum 1.5 and maximum 5.0 between the peaks due to impurities L and B in the chromatogram obtained with reference solution (b). If the resolution between the peaks due to impurities G and H is greater than 4.0, adjust the pH of solution A to a higher value. If the resolution between the peaks due to impurities L and B is greater than 5.0, adjust the pH of solution A to a lower value;
— *signal-to-noise ratio*: minimum 10 for the peak due to vancomycin B in the chromatogram obtained with reference solution (c).

Limits:
— *vancomycin B*: minimum 91.0 per cent;
— *impurities A, H*: for each impurity, maximum 3.0 per cent;
— *sum of impurities B and E*: maximum 2.0 per cent;
— *impurity J*: maximum 1.6 per cent;
— *impurities D, F, M*: for each impurity, maximum 1.5 per cent;
— *impurities G, I, K*: for each impurity, maximum 1.2 per cent;
— *impurity C*: maximum 1.0 per cent;
— *any other impurity eluting before vancomycin B*: for each impurity, maximum 0.8 per cent, and not more than 5 such impurities exceed 0.30 per cent;
— *any other impurity eluting after vancomycin B*: for each impurity, maximum 0.8 per cent, and not more than 3 such impurities exceed 0.30 per cent;
— *total of impurities*: maximum 9.0 per cent;
— *reporting threshold*: 0.10 per cent.

Water (*2.5.12*)
Maximum 5.0 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*)
Maximum 1.0 per cent, determined on 1.00 g.

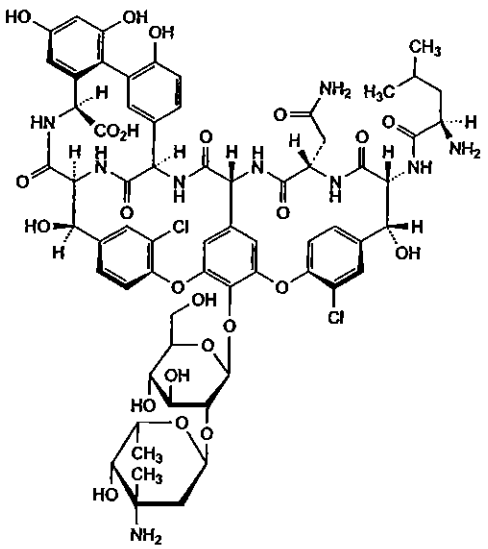
ASSAY
Carry out the microbiological assay of antibiotics (*2.7.2*). Use *vancomycin hydrochloride CRS* as the reference substance.

STORAGE
In an airtight container, protected from light at a temperature of 2 °C to 8 °C. If the substance is sterile, the container is also sterile and tamper-evident.

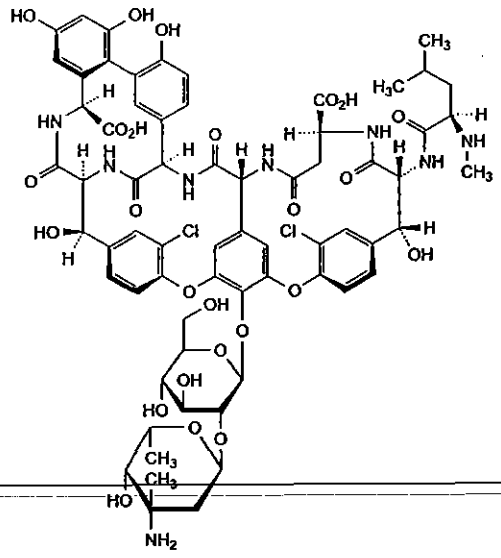
LABELLING
The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

IMPURITIES
Specified impurities *A, B, C, D, E, F, G, H, I, J, K, M.*

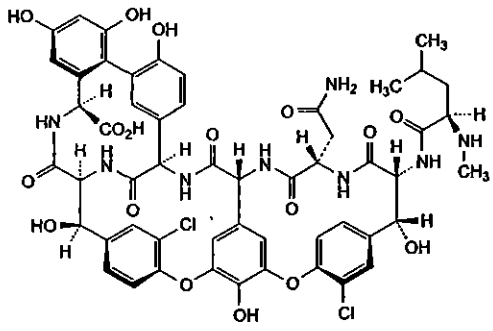
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *L.*



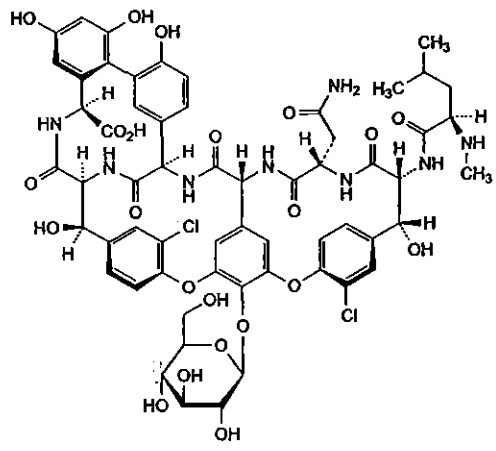
A. N^{2-1}-demethylvancomycin B,



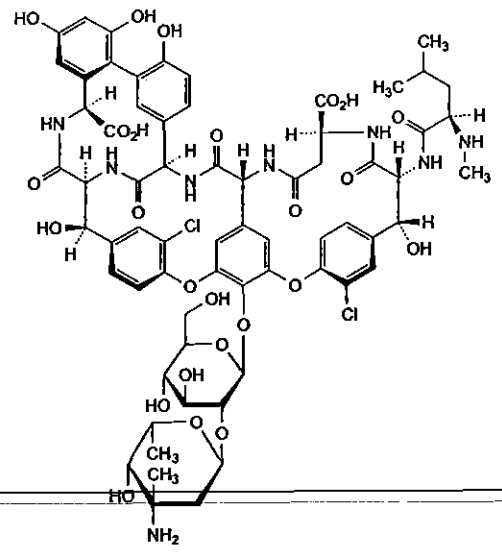
B. (1.2*M*)-[L-β-Asp^3]vancomycin B (3.2-*syn*-chloro[L-β-Asp^3] vancomycin B) (CDP-1 major),



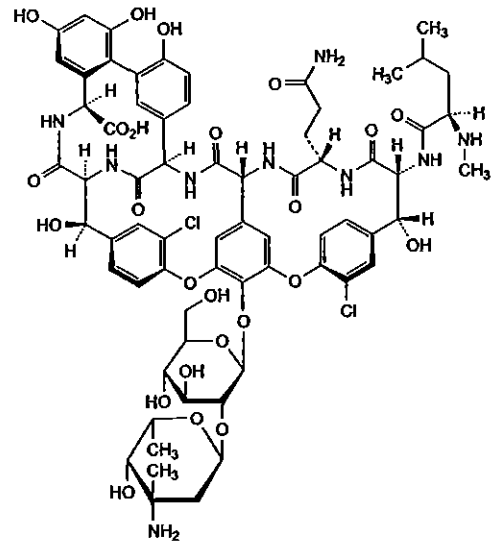
C. $O^{4.4}$-de-[2-*O*-(3-amino-2,3,6-trideoxy-3-*C*-methyl-α-L-*lyxo*-hexopyranosyl)-β-D-glucopyranosyl]vancomycin B (aglucovancomycin B),



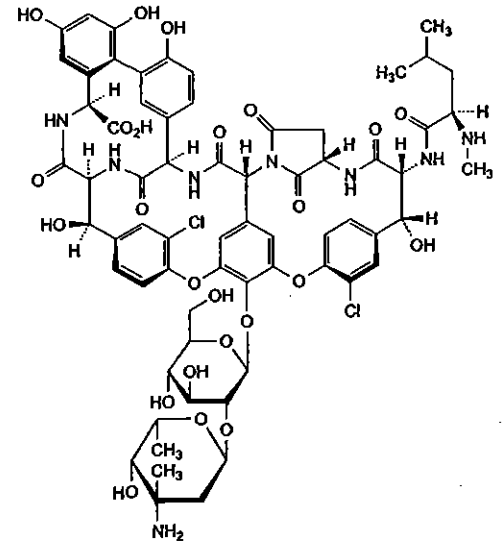
D. $O^{4.4}$-de-[2-*O*-(3-amino-2,3,6-trideoxy-3-*C*-methyl-α-L-*lyxo*-hexopyranosyl)-β-D-glucopyranosyl]-$O^{4.4}$-β-D-glucopyranosyl-vancomycin B (desvancosaminylvancomycin B),



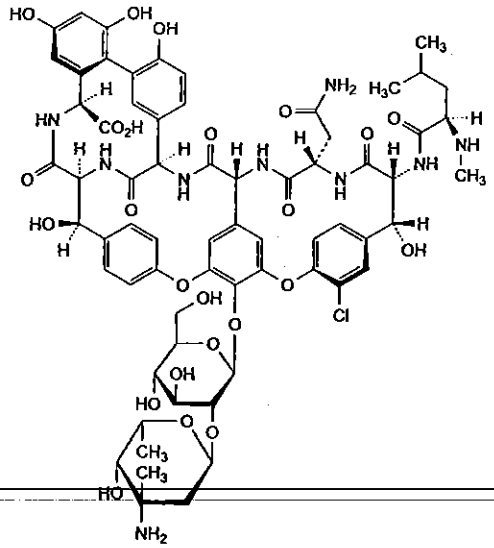
E. [L-β-Asp^3]vancomycin B (CDP-1 minor),



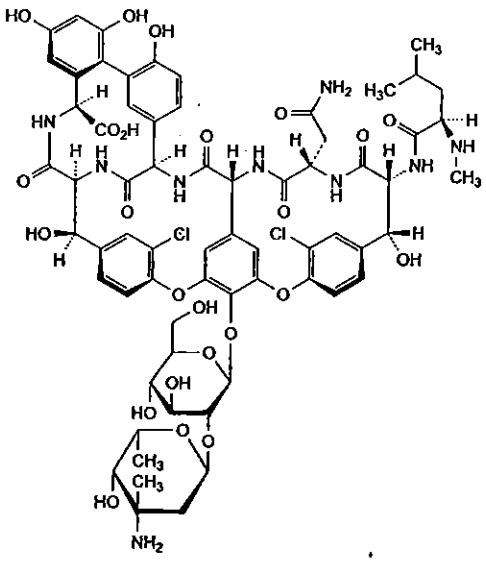
F. [L-α-Gln^3]vancomycin B,



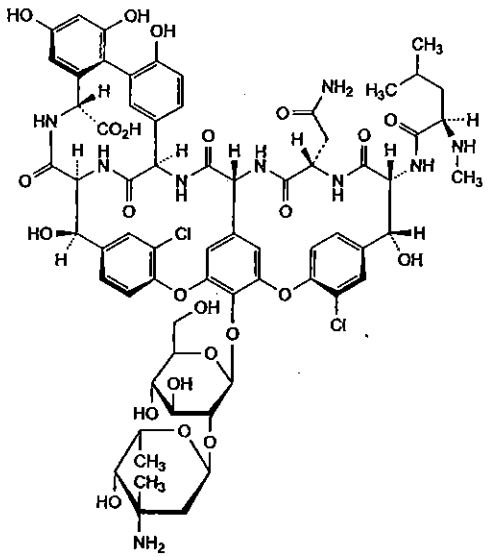
G. 4.3,$N^{\alpha.4}$-anhydro-[L-α-Asp^3]vancomycin B,



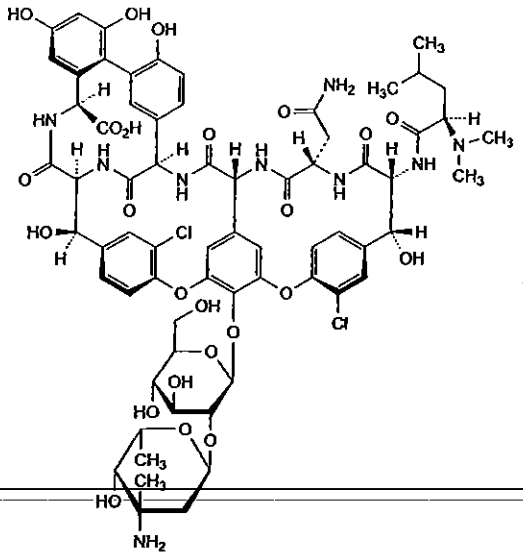
H. 3.6-dechlorovancomycin B (mono-dechloro-6-vancomycin B),



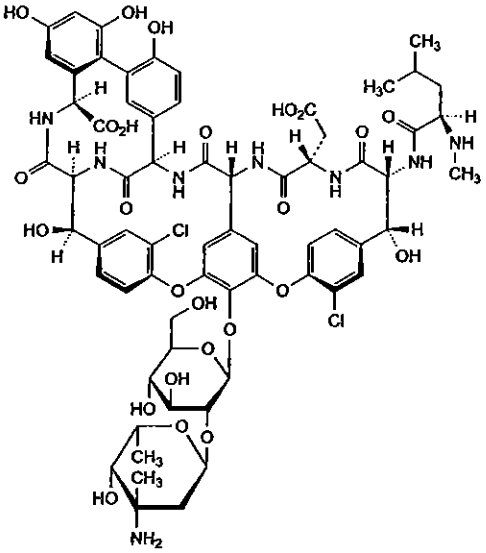
I. (1.2*M*)-vancomycin B (3.2-*syn*-chloro-vancomycin B),



J. ($C^{\alpha,7}R$)-vancomycin B (26-*epi*-vancomycin B),



K. [*N*,*N*-dimethyl-D-Leu^1]vancomycin B,



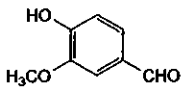
L. [L-α-Asp^3]vancomycin B),

M. unknown structure.

Ph Eur

Vanillin

(Ph. Eur. monograph 0747)



$C_8H_8O_3$ 152.1 *121-33-5*

Ph Eur

DEFINITION

Vanillin contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 4-hydroxy-3-methoxybenzaldehyde, calculated with reference to the dried substance.

CHARACTERS
White or slightly yellowish, crystalline powder or needles, slightly soluble in water, freely soluble in alcohol and in methanol. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION
First identification: B.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 81 °C to 84 °C.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *vanillin CRS*. Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for related substances in daylight after spraying. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To 5 mL of a saturated solution of the substance to be examined add 0.2 mL of *ferric chloride solution R1*. A blue colour is produced. Heat to 80 °C. The solution becomes brown. On cooling, a white precipitate is formed.

TESTS

Appearance of solution
Dissolve 1.0 g in *alcohol R* and dilute to 20 mL with the same solvent. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution B_6 (*2.2.2, Method II*).

Related substances
Examine by thin-layer chromatography (*2.2.27*), using *silica gel GF_{254} R* as the coating substance.

Test solution (a) Dissolve 0.1 g of the substance to be examined in *methanol R* and dilute to 5 mL with the same solvent.

Test solution (b) Dilute 1 mL of test solution (a) to 10 mL with *methanol R*.

Reference solution (a) Dissolve 10 mg of *vanillin CRS* in *methanol R* and dilute to 5 mL with the same solvent.

Reference solution (b) Dilute 0.5 mL of test solution (a) to 100 mL with *methanol R*.

Apply to the plate 5 µL of each solution. Develop in an unsaturated tank over a path of 10 cm using a mixture of 0.5 volumes of *anhydrous acetic acid R*, 1 volume of *methanol R* and 98.5 volumes of *methylene chloride R*. Dry the plate in a current of cold air. Examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). Spray with *dinitrophenylhydrazine-aceto-hydrochloric solution R* and examine in daylight. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Reaction with sulfuric acid
Dissolve 50 mg in 5 mL of *sulfuric acid R*. After 5 min, the solution is not more intensely coloured than a mixture of 4.9 mL of yellow primary solution and 0.1 mL of red primary solution or a mixture of 4.9 mL of yellow primary solution and 0.1 mL of blue primary solution (*2.2.2, Method I*).

Loss on drying (*2.2.32*)
Not more than 1.0 per cent, determined on 1.000 g by drying in a desiccator for 4 h.

Sulfated ash (*2.4.14*)
Not more than 0.05 per cent, determined on 2.0 g.

ASSAY
Dissolve 0.120 g in 20 mL of *alcohol R* and add 60 mL of *carbon dioxide-free water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

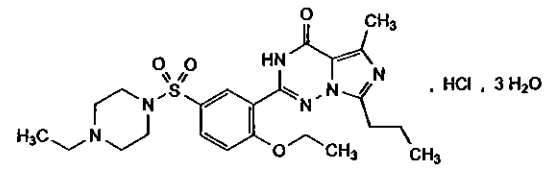
1 mL of *0.1 M sodium hydroxide* is equivalent to 15.21 mg of $C_8H_8O_3$.

STORAGE
Store protected from light.

Ph Eur

Vardenafil Hydrochloride Trihydrate

(Ph. Eur. monograph 2782)



$C_{23}H_{33}ClN_6O_4S,3H_2O$ 579.1 *330808-88-3*

Action and use
Selective inhibitor of cyclic GMP-specific phosphodiesterase type V with vasodilator action; treatment of erectile dysfunction.

Preparations
Vardenafil Orodispersible Tablets

Vardenafil Tablets

Ph Eur

DEFINITION
2-[2-Ethoxy-5-[(4-ethylpiperazin-1-yl)sulfonyl]phenyl]-5-methyl-7-propylimidazo[5,1-*f*][1,2,4]triazin-4(3*H*)-one hydrochloride trihydrate.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or slightly brown or yellow powder.

Solubility
Slightly soluble in water, freely soluble in anhydrous ethanol, practically insoluble in heptane.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison vardenafil hydrochloride CRS.

B. Water (see Tests).

C. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Related substances
Liquid chromatography (*2.2.29*). *Protect the solutions from light.*

Solvent mixture acetonitrile R, mobile phase A (20:80 *V/V*).

Test solution (a) Dissolve 50.0 mg of the substance to be examined in 20 mL of *acetonitrile R* and dilute to 100.0 mL with mobile phase A.

Test solution (b) Dilute 15.0 mL of test solution (a) to 50.0 mL with the solvent mixture.

Reference solution (a) Dissolve 50.0 mg of *vardenafil hydrochloride CRS* in 20 mL of *acetonitrile R* and dilute to 100.0 mL with mobile phase A. Dilute 15.0 mL of the solution to 50.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c) Dissolve 5 mg of *vardenafil for system suitability CRS* (containing impurity A) in 2 mL of *acetonitrile R* and dilute to 10 mL with mobile phase A.

Column:
- *size*: *l* = 0.25 m, Ø = 3.0 mm;
- *stationary phase*: *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer R* (5 µm);
- *temperature*: 45 °C.

Mobile phase:
- *mobile phase A*: solution containing 0.7 g/L of *disodium hydrogen phosphate dihydrate R* and 1.3 g/L of *potassium dihydrogen phosphate R*;
- *mobile phase B*: *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	80	20
2 - 22	80 → 25	20 → 75
22 - 27	25	75

Flow rate 0.5 mL/min.

Detection Spectrophotometer at 242 nm.

Injection 10 µL of test solution (a) and reference solutions (b) and (c).

Identification of impurities Use the chromatogram supplied with *vardenafil for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peak due to impurity A.

Relative retention With reference to vardenafil (retention time = about 16 min): impurity A = about 0.8.

System suitability Reference solution (c):
- *resolution*: minimum 5.0 between the peaks due to impurity A and vardenafil.

Calculation of percentage contents:
- for each impurity, use the concentration of vardenafil hydrochloride trihydrate in reference solution (b).

Limits:
- *impurity A*: maximum 0.15 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.3 per cent;
- *reporting threshold*: 0.05 per cent.

Sulfates (*2.4.13*)
Maximum 400 ppm.

Suspend 0.5 g in 20 mL of a 5.15 g/L solution of *hydrochloric acid R* and stir for 15 min. Filter if complete dissolution is not obtained.

Water (*2.5.12*)
8.8 per cent to 10.5 per cent, determined on 60.0 mg.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

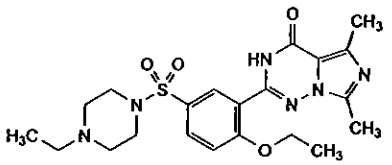
Injection 10 µL of test solution (b) and reference solution (a).

Calculate the percentage content of $C_{23}H_{33}ClN_6O_4S$ taking into account the assigned content of *vardenafil hydrochloride CRS*.

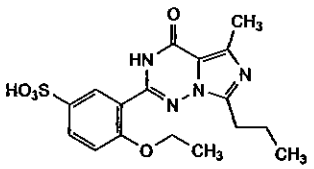
IMPURITIES

Specified impurities *A.*

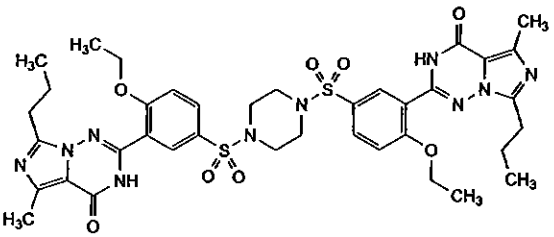
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C.*



A. 2-[2-ethoxy-5-[(4-ethylpiperazin-1-yl)sulfonyl]phenyl]-5,7-dimethylimidazo[5,1-*f*][1,2,4]triazin-4(3*H*)-one,



B. 4-ethoxy-3-(5-methyl-4-oxo-7-propyl-3,4-dihydroimidazo[5,1-*f*][1,2,4]triazin-2-yl)benzenesulfonic acid,

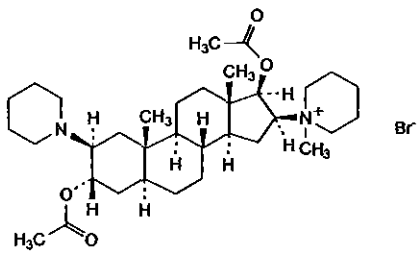


C. 2,2′-[piperazine-1,4-diylbis[(sulfonyl)(4-ethoxybenzene-1,3-diyl)]]bis[5-methyl-7-propylimidazo[5,1-*f*][1,2,4]triazin-4(3*H*)-one].

Ph Eur

Vecuronium Bromide

(Ph. Eur. monograph 1769)



$C_{34}H_{57}BrN_2O_4$ 638 *50700-72-6*

Action and use
Non-depolarizing neuromuscular blocker.

Preparation
Vecuronium Bromide for Injection

Ph Eur

DEFINITION
1-[3α,17β-Bis(acetyloxy)-2β-(piperidin-1-yl)-5α-androstan-16β-yl]-1-methylpiperidin-1-ium bromide.

Content
99.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white crystals or crystalline powder.

Solubility
Slightly soluble in water, freely soluble in methylene chloride, sparingly soluble in acetonitrile and in anhydrous ethanol.

IDENTIFICATION
A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *vecuronium bromide CRS.*

C. It gives reaction (a) of bromides (*2.3.1*).

TESTS
Solution S
Dissolve 0.500 g in a 5.15 g/L solution of *hydrochloric acid R* and dilute to 50.0 mL with the same solution.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_7 (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*)
+ 30.5 to + 35.0 (anhydrous substance), determined on solution S.

Impurity B
Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.10 g of the substance to be examined in *methylene chloride R* and dilute to 5.0 mL with the same solvent.

Reference solution (a) Dissolve 5 mg of the substance to be examined and 5 mg of *pancuronium bromide CRS* (impurity B) in *methylene chloride R* and dilute to 5.0 mL with the same solvent.

Reference solution (b) Dissolve 5.0 mg of *pancuronium bromide CRS* (impurity B) in *methylene chloride R* and dilute to 100.0 mL with the same solvent.

Plate *TLC silica gel plate R* (2-10 µm).

Mobile phase Dissolve 1 g of *sodium bromide R* in 5 mL of *water R*. Add 85 mL of *2-propanol R*, then 10 mL of *acetonitrile R*.

Application 1 µL.

Development In an unsaturated tank, over 2/3 of the plate.

Drying In air for 30 min.

Detection Spray with a 2.5 g/L solution of *iodine R* in a mixture of equal volumes of *methanol R* and *methylene chloride R*.

System suitability Reference solution (a):
— the chromatogram shows 2 clearly separated spots.

Limit:
— *impurity B*: any spot due to impurity B is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent).

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Solution A 0.2 g/L solution of *hydrochloric acid R* in *methanol R2.*

Test solution Dissolve 40 mg of the substance to be examined in solution A and dilute to 20.0 mL with solution A.

Reference solution (a) Dissolve 4 mg of *vecuronium for system suitability CRS* (containing impurities C and E) in solution A and dilute to 2.0 mL with the solution A.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with solution A. Dilute 1.0 mL of this solution to 10.0 mL with solution A.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 40 °C.

Mobile phase Mix 135 volumes of an 18.0 g/L solution of *tetramethylammonium hydroxide R* previously adjusted to pH 6.9 with *phosphoric acid R*, 250 volumes of *methanol R2* and 615 volumes of *acetonitrile R1.*

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 210 nm.

Autosampler Set at 4 °C.

Injection 20 µL.

Run time 5 times the retention time of vecuronium.

Identification of impurities Use the chromatogram supplied with *vecuronium for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities C and E.

Relative retention With reference to vecuronium (retention time = about 5 min): impurity C = about 0.8; impurity E = about 1.2.

System suitability:
— *signal-to-noise ratio*: minimum 28 for the principal peak in the chromatogram obtained with reference solution (b);
— *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the principal peak in the chromatogram obtained with reference solution (a).

Calculation of percentage contents:
— *correction factor*: multiply the peak area of impurity C by 1.4;

— for each impurity, use the concentration of vecuronium bromide in reference solution (b).

Limits:
— *impurity C*: maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.2 per cent;
— *reporting threshold*: 0.05 per cent.

Water (*2.5.12*)
Maximum 4.0 per cent, determined on 0.300 g.

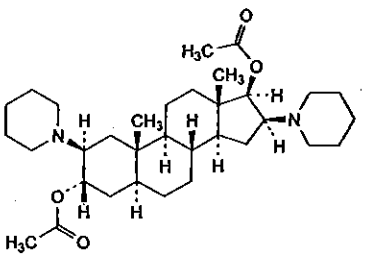
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.450 g in 50 mL of *glacial acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

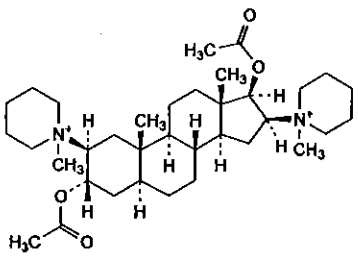
1 mL of *0.1 M perchloric acid* is equivalent to 63.8 mg of $C_{34}H_{57}BrN_2O_4$.

STORAGE
In an airtight container, protected from light and moisture.

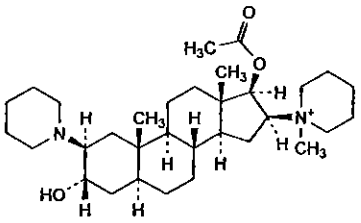
IMPURITIES
Specified impurities *B, C.*
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, D, E, F.*



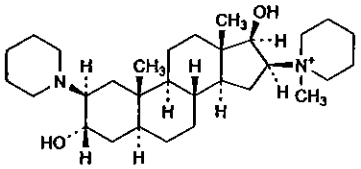
A. 2β,16β-di(piperidin-1-yl)-5α-androstane-3α,17β-diyl diacetate,



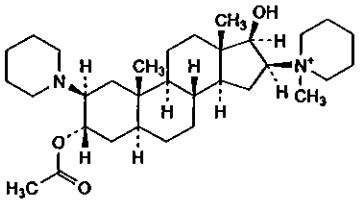
B. 1,1′-[3α,17β-bis(acetyloxy)-5α-androstane-2β,16β-diyl]bis(1-methylpiperidin-1-ium) (pancuronium),



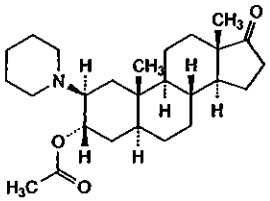
C. 1-[17β-(acetyloxy)-3α-hydroxy-2β-(piperidin-1-yl)-5α-androstan-16β-yl]-1-methylpiperidin-1-ium,



D. 1-[3α,17β-dihydroxy-2β-(piperidin-1-yl)-5α-androstan-16β-yl]-1-methylpiperidin-1-ium,



E. 1-[3α-(acetyloxy)-17β-hydroxy-2β-(piperidin-1-yl)-5α-androstan-16β-yl]-1-methylpiperidin-1-ium,



F. 2β-(piperidin-1-yl)-17-oxo-5α-androstan-3α-yl acetate.

Ph Eur

Vegetable Fatty Oils

(Ph. Eur. monograph 1579)

Ph Eur

DEFINITION
Vegetable fatty oils are mainly solid or liquid triglycerides of fatty acids. They may contain small amounts of other lipids such as waxes, free fatty acids, partial glycerides or unsaponifiable matters. Vegetable fatty oils are obtained from the seeds, the fruit or the pit/stone/kernel of various plants by expression and/or solvent extraction, then possibly refined and hydrogenated. A suitable antioxidant may be added if necessary.

Virgin oil An oil obtained from raw materials of special quality by mechanical procedures (e.g. by cold expression or centrifugation).

Refined oil An oil obtained by expression and/or solvent extraction, and subsequently either alkali refining (followed by bleaching and any deodorisation) or physical refining.

Hydrogenated oil An oil obtained by expression and/or solvent extraction, and subsequently either alkali refining or

physical refining, then possible bleaching, followed by drying, hydrogenation and subsequent bleaching and deodorisation.

Only alkali-refined oils are used in the manufacture of parenteral preparations.

PRODUCTION

Measures are taken to ensure that the oil complies with the limit for benzo[a]pyrene decided by the competent authority. A limit of 2.0 ppb is set in Commission Regulation (EC) No. 208/2005.

OBTENTION OF A CRUDE OIL

Where the plant has a high oil content, the oil is generally obtained by expression under heating followed by an extraction; where the plant has a low oil content, the oil is generally obtained by direct extraction.

Mechanical procedures

A. Expression

High-pressure screw-pressing It consists of some or all of the following steps: cleaning, drying, dehulling or decorticating, grinding, cooking and flaking.

During *cleaning* the foreign matter is eliminated. *Drying* may be necessary if the seed moisture content is higher than desirable for downstream processing. *Decorticating* is useful to obtain a high-protein meal by reduction of fibre and to reduce impurities in the oil. *Cooking* serves various purposes: completion of the breakdown of oil cells, lowering of the viscosity of the oil, coagulation of the protein in the meal, adjustment of the moisture level, sterilisation of the seed, detoxifying undesirable seed constituents (gossypol for cottonseed) and fixing certain phosphatides in the cake thus lowering subsequent refining losses. The efficacy of the expression process is such that only 3 per cent to 6 per cent of the oil is left in the cake.

Wet screw-pressing The bunches are loaded into cages (for palm fruit) and moved into a horizontal steriliser with application of live steam and heating. The purposes of this steriliser are inactivation of enzymes, loosening of the fruit on the bunch, coagulation of proteins, etc. After heating in a digester, the pulp is fed to a screw-press. The oil is centrifugally clarified and vacuum-dried.

Pre-pressing followed by solvent extraction The same sequence of steps is performed as above. The main function of pre-pressing is to obtain a cake of excellent permeability for the following solvent extraction stage. The extraction is performed either in a percolation-type or in an immersion-type apparatus. The efficacy of the solvent extraction process is such that residual oil levels in meal are generally below 1 per cent.

B. Centrifugation

Centrifugation separates the oily phase from the aqueous phase, which contains water-soluble components and residual solid particles. This operation can be carried out using:

- self-cleaning bowl or disc centrifuges;
- super-decanters, which are horizontal turbines equipped with a cylindrical bowl that tapers slightly at one end and which contains a continuously turning screw that scrapes the sides of the bowl; the screw and the bowl rotate at different speeds; the solid particles are discarded from the tapered end of the bowl and the oil flows out from the other end.

Solvent extraction

Prior to extraction, the following steps are carried out: the seeds are tempered for about a week at a temperature below 24 °C in order to loosen the hull from the seed and allow the seed moisture to attain equilibrium, then the seeds are cleaned, ground, dehulled and flaked. The most widely used solvent is a mixture of mainly *n*-hexane and methylpentanes (bp: 65-70 °C) commonly referred to as 'hexane'. Due to the major fire and explosive risks of this mixture, liquified gases and supercritical gases may also be used.

REFINING

The objective of refining is to remove impurities and contaminants of the oil with the least possible damage to the triglycerides and with minimal loss of oil. The contents of the following substances are reduced:

- free fatty acids, which may cause deterioration of the oil by oxidation, a smoked taste when heated and a sharp flavour (by alkali refining);
- water, which favours the enzymatic hydrolysis reactions (by alkali refining, drying);
- partial glycerides, which may cause foaming and a bitter taste (by neutralisation, washing);
- phosphatides and phosphorous compounds, which have emulsifying properties and may cause deposits, a darkening of the oil when heated, a cloudy appearance and bad organoleptic stability (by alkali refining);
- colouring matters such as chlorophyll (by alkali refining) and carotenoids (by bleaching);
- glycolipids, which may form colloidal solutions with water;
- free hydrocarbons, paraffin, waxes and resinous materials;
- metals (Fe, Cu, Pb, Sn, Pt, Pd, etc.), which are strong oxidation catalysts;
- pigments such as gossypol (in cottonseed oil) or mycotoxins such as aflatoxin (mainly in arachis seeds);
- pesticides;
- oxidation products (aldehydes, peroxides);
- proteins having possible allergic reactions;
- unsaponifiable matters (sterols, tocopherols and other vitamins);
- polycyclic aromatic hydrocarbons.

Alkali refining

It involves the following steps: degumming if necessary, neutralisation using alkali, washing and drying.

Degumming During this step of the refining, i.e. treatment with water and/or phosphoric acid and/or sodium chloride, the phosphatides, phosphorous compounds and metals are eliminated. The use of this step depends on the nature of the oil.

Neutralisation with alkali This step reduces the free-fatty-acid content below 0.1 per cent; the fatty acids are converted into oil-insoluble soaps, also called 'soapstocks'. Other substances may be removed by adsorption on these soaps: mucilaginous substances, phosphatides, oxidation products, colouring matters, etc. All substances that become insoluble in the oil on hydration are removed. Neutralisation with alkali has the disadvantage of saponifying a portion of neutral oil if the neutralisation is not well conducted.

Washing This operation consists in removing the excess of soaps and alkali as well as the remaining traces of metals, phosphatides and other impurities, using hot water.

Drying The remaining water is eliminated under vacuum before any further steps, such as bleaching.

Physical refining

It involves a steam treatment of the oil under high vacuum at a temperature greater than 235 °C. This technique can only be applied to oils naturally low in phosphatides and metals (palm and coconut) or from which phosphatides and metals have been removed by an acid treatment using concentrated phosphoric acid followed by an adsorptive treatment with

activated bleaching earth (for sunflower, rapeseed, soya-bean). Moreover, it cannot be used for heat-sensitive oils (cottonseed oil), which darken.

Bleaching
The common method of bleaching is by adsorption treatment of the oil, which is generally heated at 90 °C for 30 min under vacuum, with bleaching earth (natural or activated) or carbon (activated or not); synthetic silica adsorbents may also be added. Substances that have not been totally removed during refining are eliminated, for example carotenoids and chlorophyll.

Deodorisation
Deodorisation eliminates odours, volatile substances and any residual extraction solvents; it involves injecting dry vapour into the oil, which is kept under vacuum at a high temperature. Different temperatures are used according to the oil: 200-235 °C for 1.5-3 h or greater than 240 °C for 30 min.

One of the main side reactions is thermic decolourisation due to the destruction of carotenoids when the temperature is greater than 150 °C. This technique provokes a loss of substances that may be distilled (free fatty acids, sterols, tocopherols, part of the refined oil), and may cause *cis-trans* isomerisation of the unsaturated fatty-acid double bonds.

WINTERISATION
Elimination of solids and waxes by filtration at low temperature (also called dewaxing). These solids and waxes could affect the appearance of the oil and cause deposits.

HYDROGENATION
The hydrogenation of the dried and/or bleached oil is performed using a catalyst (e.g. Ni, Pt, Pd), at a temperature of about 100-200 °C under hydrogen pressure. The catalyst is then removed by filtration at 90 °C. The hydrogen must be pure: free of poisons for the catalyst, water-free, and low in carbon dioxide, methane and nitrogen contents. Small amounts of polymers may be obtained. *Trans*-fatty acids are formed during partial hydrogenation.

CHROMATOGRAPHIC PURIFICATION
In high-purity applications, mainly for parenteral uses, the oil may be further purified by passing the oil through a column containing an activated earth. A solvent may sometimes be used to improve the efficiency. High-polarity molecules, such as oxidised materials, acids, alcohols, partial glycerides and free sterols, are preferentially removed.

When the oil is used in the manufacture of parenteral preparations, the limits set in the monograph for the acid value, the peroxide value and the water content may be different.

LABELLING
The label states:
— where applicable, that the oil was obtained by expression or extraction;
— where applicable, that the oil is suitable for use in the manufacture of parenteral preparations.

Ph Eur

Hydrogenated Vegetable Oil

68334-00-9

Action and use
Excipient.

DEFINITION
Hydrogenated Vegetable Oil is a mixture of triglycerides of fatty acids of vegetable origin.

CHARACTERISTICS
An almost white, fine powder at room temperature and a pale yellow, oily liquid above its melting point.

Practically insoluble in *water*; soluble in *hexane* and in hot *propan-2-ol*.

IDENTIFICATION
Complies with the tests for Acid value, Iodine value and Saponification value.

TESTS
Melting point
57° to 70°, Appendix V A.

Acid value
Not more than 4.0, Appendix X B.

Iodine value
Not more than 5, Appendix X E, Method B.

Saponification value
175 to 205, Appendix X G, Method II.

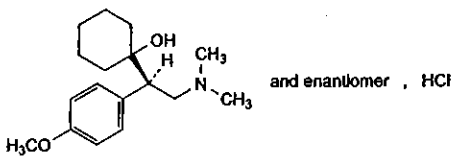
Unsaponifiable matter
Not more than 0.8% w/w, Appendix X H.

Loss on drying
When dried at 105° for 4 hours, loses not more than 0.1% of its weight. Use 5 g.

STORAGE
Hydrogenated Vegetable Oil should be stored at a temperature of 8° to 25°.

Venlafaxine Hydrochloride

(Ph. Eur. monograph 2119)



$C_{17}H_{28}ClNO_2$ 313.9 *99300-78-4*

Action and use
Inhibition of 5HT and noradrenaline reuptake; antidepressant.

Preparations
Venlafaxine Prolonged-release Capsules
Venlafaxine Prolonged-release Tablets
Venlafaxine Tablets

Ph Eur

DEFINITION
1-[(1*RS*)-2-(Dimethylamino)-1-(4-methoxyphenyl) ethyl]cyclohexanol hydrochloride.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Freely soluble in water and in methanol, soluble in anhydrous ethanol, slightly soluble or practically insoluble in acetone.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *venlafaxine hydrochloride CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *2-propanol R*, evaporate to dryness and record new spectra using the residues.

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Acidity or alkalinity
Dissolve 0.20 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent. Add 0.05 mL of *methyl red solution R* and 0.1 mL of *0.01 M hydrochloric acid.*
The solution is pink. Not more than 0.2 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to yellow.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 10.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve the contents of a vial of *venlafaxine for system suitability CRS* (containing impurities D and F) in 1.0 mL of the mobile phase.

Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: end-capped octylsilyl silica gel for chromatography R* (5 µm) with a pore size of 10 nm.

Mobile phase Mix 510 volumes of *acetonitrile R* and 1490 volumes of a solution prepared as follows: dissolve 17 g of *ammonium dihydrogen phosphate R* in 1490 mL of *water R* and adjust to pH 4.4 using *phosphoric acid R.*

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 225 nm.

Injection 20 µL.

Run time 10 times the retention time of venlafaxine.

Relative retention With reference to venlafaxine (retention time = about 9 min): impurity D = about 0.9; impurity F = about 3.4.

System suitability Reference solution (b):
— *resolution*: minimum 1.5 between the peaks due to impurity D and venlafaxine.

Limits:
— *impurity F*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 80 °C for 3 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

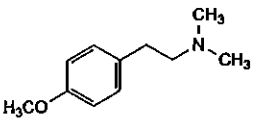
Dissolve 0.250 g in a mixture of 5.0 mL of *0.01 M hydrochloric acid* and 50 mL of *ethanol (96 per cent) R.* Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide.* Read the volume added between the 2 points of inflexion. Carry out a blank titration.

1 mL of *0.1 M sodium hydroxide* is equivalent to 31.39 mg of $C_{17}H_{28}ClNO_2$.

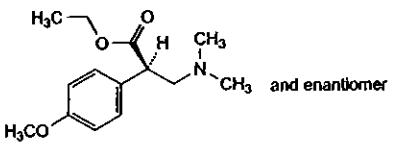
IMPURITIES

Specified impurities *F.*

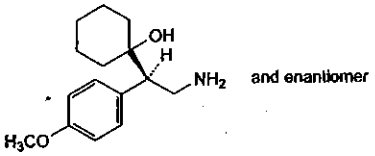
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, E, G, H.*



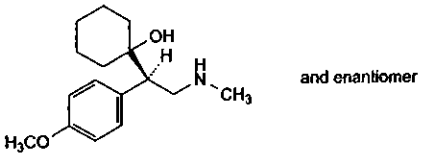
A. 2-(4-methoxyphenyl)-*N*,*N*-dimethylethanamine,



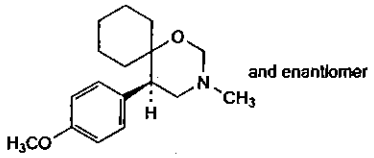
B. ethyl (2*RS*)-3-(dimethylamino)-2-(4-methoxyphenyl)propanoate,



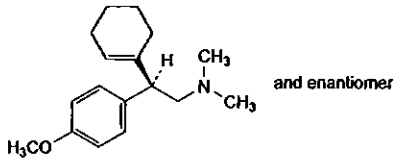
C. 1-[(1*RS*)-2-amino-1-(4-methoxyphenyl)ethyl]cyclohexanol,



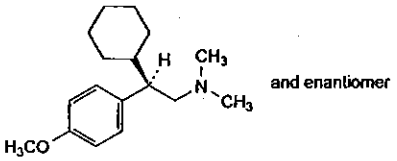
D. 1-[(1*RS*)-1-(4-methoxyphenyl)-2-(methylamino) ethyl]cyclohexanol,



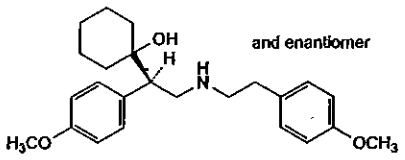
E. (5*RS*)-5-(4-methoxyphenyl)-3-methyl-1-oxa-3-azaspiro[5.5]undecane,



F. (2*RS*)-2-(cyclohex-1-enyl)-2-(4-methoxyphenyl)-*N*,*N*-dimethylethanamine,



G. (2*RS*)-2-cyclohexyl-2-(4-methoxyphenyl)-*N*,*N*-dimethylethanamine,

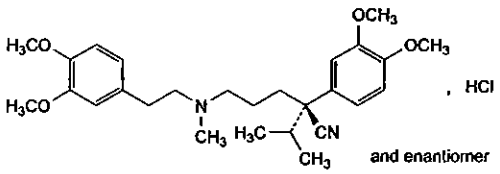


H. 1-[(1*RS*)-1-(4-methoxyphenyl)-2-[[2-(4-methoxyphenyl)ethyl]amino]ethyl]cyclohexanol.

Ph Eur

Verapamil Hydrochloride

(Ph. Eur. monograph 0573)



$C_{27}H_{39}ClN_2O_4$ 491.1 *152-11-4*

Action and use
Calcium channel blocker.

Preparations
Verapamil Prolonged-release Capsules
Verapamil Prolonged-release Tablets
Verapamil Injection
Verapamil Oral Solution
Verapamil Tablets

Ph Eur

DEFINITION
(2*RS*)-2-(3,4-Dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl)ethyl](methyl)amino]-2-(1-methylethyl) pentanenitrile hydrochloride.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Soluble in water, freely soluble in methanol, sparingly soluble in ethanol (96 per cent).

mp
About 144 °C.

IDENTIFICATION
First identification: B, D.

Second identification: A, C, D.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 20.0 mg in *0.01 M hydrochloric acid* and dilute to 100.0 mL with the same acid. Dilute 5.0 mL of this solution to 50.0 mL with *0.01 M hydrochloric acid.*

Spectral range 210-340 nm.

Absorption maxima At 229 nm and 278 nm.

Shoulder At 282 nm.

Absorbance ratio A_{278}/A_{229} = 0.35 to 0.39.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *verapamil hydrochloride CRS.*

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *methylene chloride R* and dilute to 5 mL with the same solvent.

Reference solution (a) Dissolve 20 mg of *verapamil hydrochloride CRS* in *methylene chloride R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 5 mg of *papaverine hydrochloride CRS* in reference solution (a) and dilute to 5 mL with the same solution.

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *diethylamine R, cyclohexane R* (15:85 *V/V*).

Application 5 µL.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution (b):
— the chromatogram shows 2 clearly separated principal spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. It gives reaction (b) of chlorides (*2.3.1*).

TESTS
Solution S
Dissolve 1.0 g in *carbon dioxide-free water R* while gently heating and dilute to 20.0 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)
4.5 to 6.0 for solution S.

Optical rotation (*2.2.7*)
−0.10° to + 0.10°, determined on solution S.

Related substances
Liquid chromatography (*2.2.29*).

Solvent mixture Mobile phase B, mobile phase A (37:63 *V/V*).

Test solution Dissolve 25.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (a) Dissolve 5 mg of *verapamil hydrochloride CRS*, 5 mg of *verapamil impurity I CRS* and 5 mg of *verapamil impurity M CRS* in the solvent mixture and dilute to 20 mL with the solvent mixture. Dilute 1 mL of this solution to 10 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer R* (5 µm).

Mobile phase:
— *mobile phase A*: 6.97 g/L solution of *dipotassium hydrogen phosphate R* adjusted to pH 7.20 with *phosphoric acid R*;
— *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 22	63	37
22 - 27	63 → 35	37 → 65
27 - 35	35	65

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 278 nm.

Injection 10 µL.

Relative retention With reference to verapamil (retention time = about 15 min): impurity I = about 1.3; impurity M = about 2.4.

System suitability Reference solution (a):
— *resolution*: minimum 5.0 between the peaks due to verapamil and impurity I;
— impurity M elutes from the column.

Limits:
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

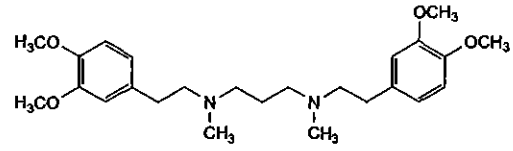
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.400 g in 50 mL of *anhydrous ethanol R* and add 5.0 mL of *0.01 M hydrochloric acid*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Measure the volume added between the 2 points of inflexion.

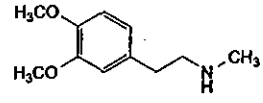
1 mL of *0.1 M sodium hydroxide* is equivalent to 49.11 mg of $C_{27}H_{39}ClN_2O_4$.

STORAGE
Protected from light.

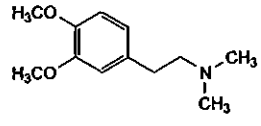
IMPURITIES
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P.*



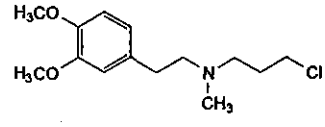
A. *N,N'*-bis[2-(3,4-dimethoxyphenyl)ethyl]-*N,N'*-dimethylpropane-1,3-diamine,



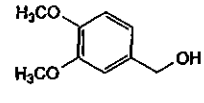
B. 2-(3,4-dimethoxyphenyl)-*N*-methylethanamine,



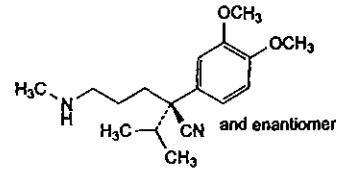
C. 2-(3,4-dimethoxyphenyl)-*N,N*-dimethylethanamine,



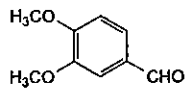
D. 3-chloro-*N*-[2-(3,4-dimethoxyphenyl)ethyl]-*N*-methylpropan-1-amine,



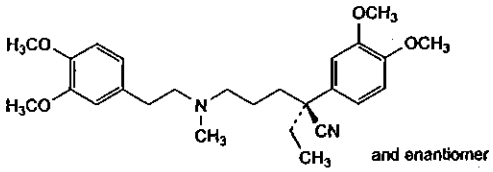
E. (3,4-dimethoxyphenyl)methanol,



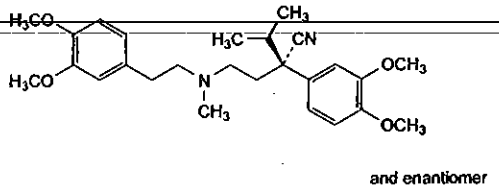
F. (2*RS*)-2-(3,4-dimethoxyphenyl)-5-(methylamino)-2-(1-methylethyl)pentanenitrile,



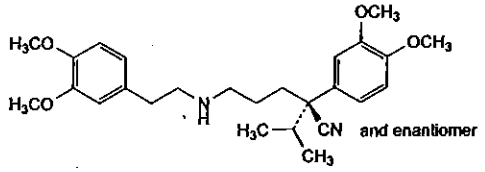
G. 3,4-dimethoxybenzaldehyde,



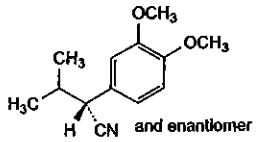
H. (2*RS*)-2-(3,4-dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl)ethyl](methyl)amino]-2-ethylpentanenitrile,



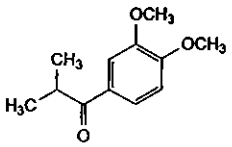
I. (2*RS*)-2-(3,4-dimethoxyphenyl)-2-[2-[[2-(3,4-dimethoxyphenyl)ethyl](methyl)amino]ethyl]-3-methylbutanenitrile,



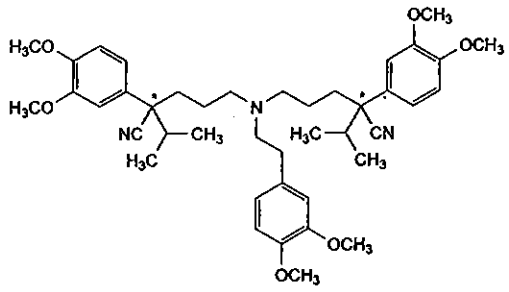
J. (2*RS*)-2-(3,4-dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl)ethyl]amino]-2-(1-methylethyl)pentanenitrile (*N*-norverapamil),



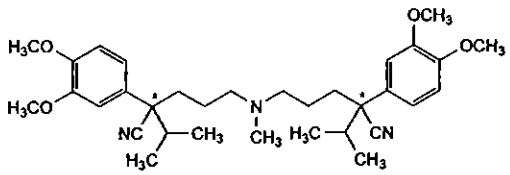
K. (2*RS*)-2-(3,4-dimethoxyphenyl)-3-methylbutanenitrile,



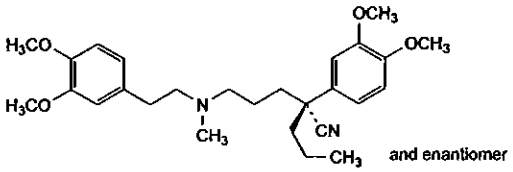
L. 1-(3,4-dimethoxyphenyl)-2-methylpropan-1-one,



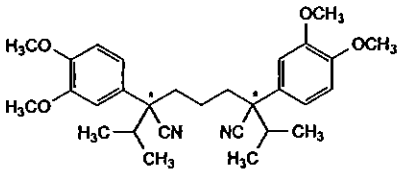
M. 5,5′-[[2-(3,4-dimethoxyphenyl)ethyl]imino]bis[2-(3,4-dimethoxyphenyl)-2-(1-methylethyl)pentanenitrile],



N. 5,5′-(methylimino)bis[2-(3,4-dimethoxyphenyl)-2-(1-methylethyl)pentanenitrile],



O. (2*RS*)-2-(3,4-dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl)ethyl](methyl)amino]-2-propylpentanenitrile,

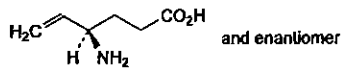


P. 2,6-bis(3,4-dimethoxyphenyl)-2,6-bis(1-methylethyl)-heptane-1,7-dinitrile.

Ph Eur

Vigabatrin

(Ph. Eur. monograph 2305)



$C_6H_{11}NO_2$ 129.2 *60643-86-9*

Action and use
Antiepileptic.

Preparations
Vigabatrin Oral Powder
Vigabatrin Tablets

Ph Eur

DEFINITION
(4*RS*)-4-Aminohex-5-enoic acid.

Content
98.5 per cent to 101.5 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white powder.

Solubility
Freely soluble in water, slightly soluble in methanol, practically insoluble in methylene chloride.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *vigabatrin CRS.*

TESTS

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in *water R*, using sonication if necessary, and dilute to 5.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *water R*.

Reference solution (b) Dissolve 7.5 mg of *vigabatrin impurity A CRS* and 7.5 mg of *vigabatrin impurity B CRS* in *water R* and dilute to 50.0 mL with the same solvent. Dilute 2.0 mL of the solution to 20.0 mL with *water R*.

Reference solution (c) Dissolve 5.0 mg of *vigabatrin impurity E CRS* and 10.0 mg of *vigabatrin impurity D CRS* in *water R* and dilute to 50.0 mL with the same solvent. To 2.0 mL of the solution add 1.0 mL of reference solution (a) and dilute to 20.0 mL with *water R*.

Reference solution (d) Dilute 2 mL of reference solution (b) to 10 mL with *water R*.

Column:
- *size*: *l* = 0.10 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped solid core phenylhexylsilyl silica gel for chromatography R* (2.7 µm);
- *temperature*: 45 °C.

Mobile phase Dissolve 2.1 g of *perfluoroheptanoic acid R* in a mixture of 195 mL of *methanol R2* and 805 mL of *water for chromatography R*.

Flow rate 1.0 mL/min.

Post-column solution *methanol R2*.

Post-column flow rate 0.8 mL/min.

Detection Charged aerosol detector (gas source: nitrogen at 35 psi) and, for impurities A and B, spectrophotometer at 210 nm.

Autosampler Set at 15 °C.

Injection 30 µL of the test solution and reference solutions (b), (c) and (d).

Run time 3 times the retention time of vigabatrin.

Identification of impurities Use the chromatogram obtained with the spectrophotometer and with reference solution (b) to identify the peaks due to impurities A and B; use the chromatogram obtained with the charged aerosol detector and with reference solution (c) to identify the peaks due to impurities D and E.

Relative retention With reference to vigabatrin (retention time = about 11 min): impurity A = about 0.3; impurity E = about 0.5; impurity D = about 0.6; impurity B = about 2.3.

System suitability:
- *resolution*: minimum 1.5 between the peaks due to impurities E and D in the chromatogram obtained with reference solution (c);
- *signal-to-noise ratio*: minimum 15 for the peak due to vigabatrin in the chromatogram obtained with reference solution (c); minimum 10 for the peak due to impurity B in the chromatogram obtained with reference solution (d);
- *symmetry factor*: minimum 0.6 for the peaks due to impurity D and vigabatrin in the chromatogram obtained with reference solution (c); minimum 0.6 for the peaks due to impurities A and B in the chromatogram obtained with reference solution (b).

Calculation of percentage contents:
- for impurities A and B, use the concentration of each impurity in reference solution (b);
- for impurity D, use the concentration of impurity D in reference solution (c);
- for impurities other than A, B and D, use the concentration of vigabatrin in reference solution (c).

Limits:
- *impurity D*: maximum 0.2 per cent;
- *impurities A, B (spectrophotometer at 210 nm)*: for each impurity, maximum 0.15 per cent;
- *unspecified impurities*: for each impurity, maximum 0.05 per cent;
- *total*: maximum 0.5 per cent;
- *reporting threshold*: 0.03 per cent.

Water (*2.5.12*)
Maximum 0.5 per cent, determined on 0.300 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

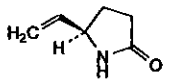
Dissolve 90 mg in 50 mL of *glacial acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 12.92 mg of $C_6H_{11}NO_2$.

IMPURITIES

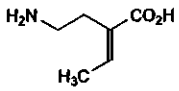
Specified impurities *A, B, D.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *E.*

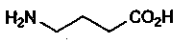


and enantiomer

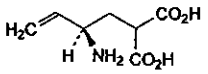
A. (5*RS*)-5-ethenylpyrrolidin-2-one,



B. (2*E*)-2-(2-aminoethyl)but-2-enoic acid,



D. 4-aminobutanoic acid (GABA),



and enantiomer

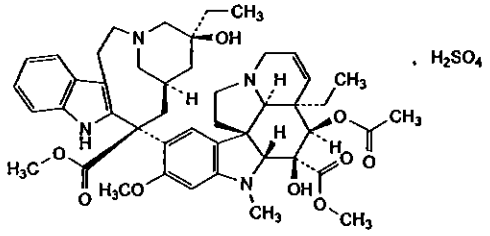
E. 2-[(2*RS*)-2-aminobut-3-en-1-yl]propanedioic acid.

Ph Eur

Vinblastine Sulfate

Vinblastine Sulphate

(Ph. Eur. monograph 0748)



$C_{46}H_{60}N_4O_{13}S$ 909 *143-67-9*

Action and use
Vinca alkaloid cytotoxic.

Preparation
Vinblastine Injection

Ph Eur

DEFINITION

Vinblastine sulfate contains not less than 95.0 per cent and not more than the equivalent of 104.0 per cent of methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-4-(acetyloxy)-3a-ethyl-9-[(5*S*,7*R*,9*S*)5-ethyl-5-hydroxy-9-(methoxycarbonyl)-1,4,5,6,7,8,9,10-octahydro-2*H*-3,7-methanoazacycloundecino[5,4-*b*]indol-9-yl]-5-hydroxy-8-methoxy-6-methyl-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate sulfate, calculated with reference to the dried substance.

CHARACTERS

A white or slightly yellowish, crystalline powder, very hygroscopic, freely soluble in water, practically insoluble in alcohol.

IDENTIFICATION

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the *Ph. Eur. reference spectrum of vinblastine sulfate*.

B. Examine the chromatograms obtained in the assay. The principal peak in the chromatogram obtained with the test solution is similar in position and approximate size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Solution S

Dissolve 50.0 mg in *carbon dioxide-free water R* and dilute to 10.0 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method I*).

pH (*2.2.3*)

Dilute 3 mL of solution S to 10 mL with *carbon dioxide-free water R*. The pH of this solution is 3.5 to 5.0.

Related substances

Examine the chromatograms obtained in the assay. In the chromatogram obtained with the test solution, the area of any peak apart from the principal peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (c) (2.0 per cent) and the sum of the areas of any such peaks is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (5.0 per cent). Disregard any peak with an area less than that of the peak in the chromatogram obtained with reference solution (d).

Loss on drying

Not more than 15.0 per cent, determined on 3 mg by thermogravimetry (*2.2.34*). Heat to 200 °C at a rate of 5 °C/min, under a stream of *nitrogen for chromatography R*, at a flow rate of 40 mL/min.

ASSAY

Examine by liquid chromatography (*2.2.29*).

Keep the solutions in iced water before use.

Test solution Dilute 1.0 mL of solution S (see Tests) to 5.0 mL with *water R*.

Reference solution (a) Dissolve the contents of a vial of *vinblastine sulfate CRS* in 5.0 mL of *water R* to obtain a concentration of 1.0 mg/mL.

Reference solution (b) Dissolve 1.0 mg of *vincristine sulfate CRS* in 1.0 mL of reference solution (a).

Reference solution (c) Dilute 1.0 mL of reference solution (a) to 50.0 mL with *water R*.

Reference solution (d) Dilute 1.0 mL of reference solution (c) to 20.0 mL with *water R*.

The chromatographic procedure may be carried out using:
- a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with *octylsilyl silica gel for chromatography R* (5 µm). Place between the injector and the column a precolumn packed with suitable silica gel,
- as mobile phase at a flow rate of 1.0 mL/min a mixture of 38 volumes of a 1.5 per cent *V/V* solution of *diethylamine R* adjusted to pH 7.5 with *phosphoric acid R*, 12 volumes of *acetonitrile R* and 50 volumes of *methanol R*,
- as detector a spectrophotometer set at 262 nm,
- a loop injector.

Inject 10 µL of each solution and record the chromatograms for 3 times the retention time of the peak due to vinblastine. The assay is not valid unless: in the chromatogram obtained with reference solution (b) the resolution between the peaks due to vincristine and vinblastine is not less than 4; the peak in the chromatogram obtained with reference solution (d) has a signal-to-noise ratio not less than 5. Calculate the percentage content of $C_{46}H_{60}N_4O_{13}S$ from the area of the principal peak in each of the chromatograms obtained with the test solution and reference solution (a) and from the declared content of *vinblastine sulfate CRS*.

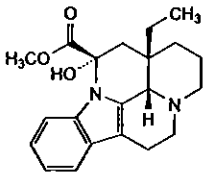
STORAGE

Store in an airtight, glass container, protected from light, at a temperature not exceeding −20 °C. If the substance is sterile, store in a sterile, airtight, tamper-evident glass container.

Ph Eur

Vincamine

(Ph. Eur. monograph 1800)



$C_{21}H_{26}N_2O_3$ 354.5 *1617-90-9*

Action and use
Vasodilator.

Ph Eur

DEFINITION
Methyl 14-hydroxyvincane-14β-carboxylate.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, soluble in methylene chloride, slightly soluble in anhydrous ethanol.

IDENTIFICATION
A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *vincamine CRS.*

TESTS
Specific optical rotation (*2.2.7*)
+ 44.3 to + 49.0 (dried substance).
Dissolve 0.1 g in *dimethylformamide R* and dilute to 20.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use. Dissolve the samples using sonication, while avoiding any overheating.*

Test solution Dissolve 50.0 mg of the substance to be examined in 10 mL of *tetrahydrofuran R* and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 200.0 mL with the mobile phase.

Reference solution (b) Dissolve 5 mg of *vincamine for system suitability CRS* (containing impurities A, B and C) in 1 mL of *tetrahydrofuran R* and dilute to 10 mL with the mobile phase.

Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: end-capped octadecylsilyl silica gel for chromatography with embedded polar groups R* (5 µm).

Mobile phase *tetrahydrofuran R, acetonitrile R,* 15.4 g/L solution of *ammonium acetate R* (17:18:65 *V/V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 272 nm.

Injection 20 µL.

Run time 3.5 times the retention time of vincamine.

Identification of impurities Use the chromatogram supplied with *vincamine for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B and C.

Relative retention With reference to vincamine (retention time = about 10 min): impurity A = about 0.8; impurity B = about 0.9; impurity C = about 1.35.

System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the peaks due to impurity B and vincamine.

Calculation of percentage contents:
— for each impurity, use the concentration of vincamine in reference solution (a).

Limits:
— *impurities A, C*: for each impurity, maximum 0.5 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 1.0 per cent;
— *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

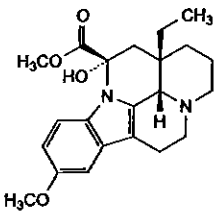
Sulfated ash (*2.4.14*)
Maximum 0.2 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.300 g in 30 mL of a mixture of 1 volume of *acetic anhydride R* and 5 volumes of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

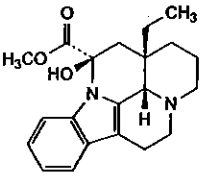
1 mL of *0.1 M perchloric acid* is equivalent to 35.45 mg of $C_{21}H_{26}N_2O_3$.

IMPURITIES
Specified impurities *A, C.*

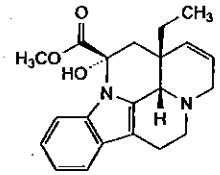
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, D.*



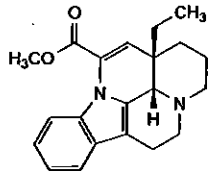
A. methyl 14-hydroxy-10-methoxyvincane-14β-carboxylate (10-methoxyvincamine),



B. methyl 14-hydroxyvincane-14α-carboxylate (14-*epi*-vincamine),



C. methyl 14-hydroxy-17,18-didehydrovincane-14β-carboxylate (17,18-didehydrovincamine),



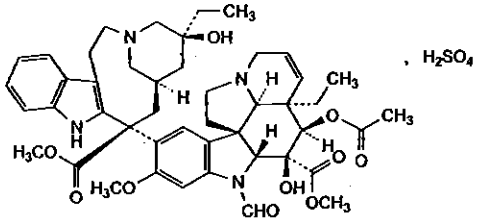
D. methyl 14,15-didehydrovincane-14-carboxylate (apovincamine).

Ph Eur

Vincristine Sulfate

Vincristine Sulphate

(Ph. Eur. monograph 0749)



$C_{46}H_{58}N_4O_{14}S$ 923 *2068-78-2*

Action and use
Vinca alkaloid cytotoxic.

Preparation
Vincristine Injection

Ph Eur

DEFINITION
Methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-4-(acetyloxy)-3a-ethyl-9-[(5*S*,7*R*,9*S*)-5-ethyl-5-hydroxy-9-(methoxycarbonyl)-1,4,5,6,7,8,9,10-octahydro-2*H*-3,7-methanoazacycloundecino[5,4-*b*]indol-9-yl]-6-formyl-5-hydroxy-8-methoxy-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate sulfate.

Content
95.0 per cent to 104.0 per cent (dried substance).

CHARACTERS

Appearance
White or slightly yellowish, crystalline powder, very hygroscopic.

Solubility
Freely soluble in water, slightly soluble in alcohol.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *Ph. Eur. reference spectrum of vincristine sulfate.*

TESTS

Solution S
Dissolve 50.0 mg in *carbon dioxide-free water R* and dilute to 10.0 mL with the same solvent. *Keep the solution in iced water to carry out the test for related substances.*

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method I*).

pH (*2.2.3*)
3.5 to 4.5.
Dilute 2 mL of solution S to 10 mL with *carbon dioxide-free water R*.

Related substances
Liquid chromatography (*2.2.29*). *Keep the solutions in iced water before use.*

Test solution Dilute 1.0 mL of solution S to 5.0 mL with *water R*.

Reference solution (a) Dissolve the contents of a vial of *vincristine sulfate CRS* in 5.0 mL of *water R* to obtain a concentration of 1.0 mg/mL.

Reference solution (b) Dissolve 1.0 mg of *vinblastine sulfate CRS* in 1.0 mL of reference solution (a).

Reference solution (c) Dilute 1.0 mL of the test solution to 50.0 mL with *water R*.

Reference solution (d) Dilute 1.0 mL of reference solution (c) to 20.0 mL with *water R*.

Precolumn:
— *stationary phase*: *octylsilyl silica gel for chromatography R*.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm,
— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: 1.5 per cent *V/V* solution of *diethylamine R* adjusted to pH 7.5 with *phosphoric acid R*,
— *mobile phase B*: *methanol R*,

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 12	38	62
12 - 27	38 → 8	62 → 92

Flow rate 2 mL/min.

Detection Spectrophotometer at 297 nm.

Injection 20 µL.

System suitability Reference solution (b):
— *resolution*: minimum 4 between the peaks due to vincristine and vinblastine.

Limits:
— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (2.0 per cent),
— *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (5.0 per cent),
— *disregard limit*: area of the peak in the chromatogram obtained with reference solution (d) (0.1 per cent).

Loss on drying
Maximum 12.0 per cent, determined on 3 mg by thermogravimetry (*2.2.34*). Heat the substance to be examined to 200 °C increasing the temperature by 5 °C/min,

under a current of *nitrogen for chromatography R*, at a flow rate of 40 mL/min.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances, with the following modifications.

Mobile phase Mix 30 volumes of a 1.5 per cent *V/V* solution of *diethylamine R* adjusted to pH 7.5 with *phosphoric acid R* and 70 volumes of *methanol R*.

Flow rate 1.0 mL/min.

Calculate the percentage content of $C_{46}H_{58}N_4O_{14}S$ using the chromatogram obtained with reference solution (a) and the declared content of *vincristine sulfate CRS*.

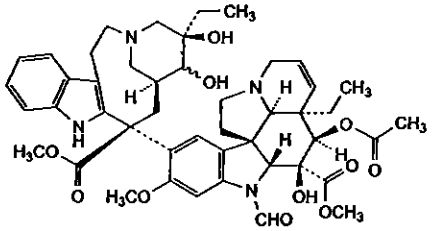
STORAGE

In an airtight, glass container, protected from light, at a temperature not exceeding −20 °C. If the substance is sterile, store in a sterile, airtight, tamper-evident glass container.

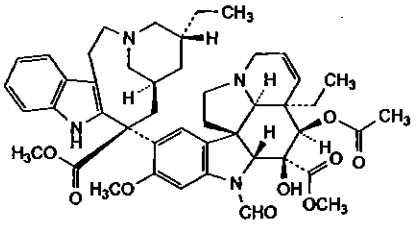
IMPURITIES

Specified impurities *A, B, C, D, H.*

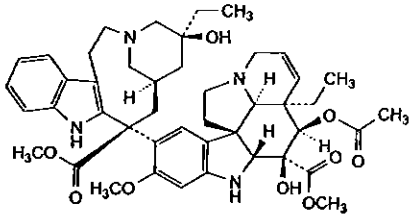
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *E, F, G.*



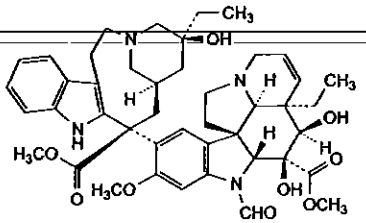
A. methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-4-(acetyloxy)-3a-ethyl-9-[(5*R*,7*S*,9*S*)-5-ethyl-5,6-dihydroxy-9-(methoxycarbonyl)-1,4,5,6,7,8,9,10-octahydro-2*H*-3,7-methanoazacycloundecino[5,4-b]indol-9-yl]-6-formyl-5-hydroxy-8-methoxy-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate (3′-hydroxy-VCR),



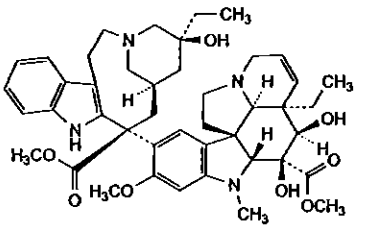
B. methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-4-(acetyloxy)-3a-ethyl-9-[(5*R*,7*S*,9*S*)-5-ethyl-9-(methoxycarbonyl)-1,4,5,6,7,8,9,10-octahydro-2*H*-3,7-methanoazacycloundecino[5,4-*b*]indol-9-yl]-6-formyl-5-hydroxy-8-methoxy-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate (4′-deoxyvincristine),



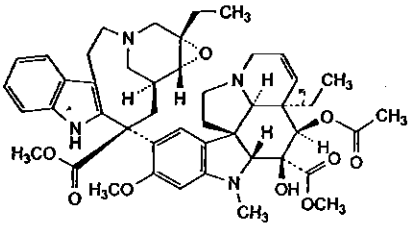
C. methyl (3a*R*,4*R*,5*S*,5a*R*,10b*S*,13a*R*)-4-(acetyloxy)-3a-ethyl-9-[(5*S*,7*R*,9*S*)-5-ethyl-5-hydroxy-9-(methoxycarbonyl)-1,4,5,6,7,8,9,10-octahydro-2*H*-3,7-methanoazacycloundecino[5,4-*b*]indol-9-yl]-5-hydroxy-8-methoxy-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate (*N*-desmethylvinblastine),



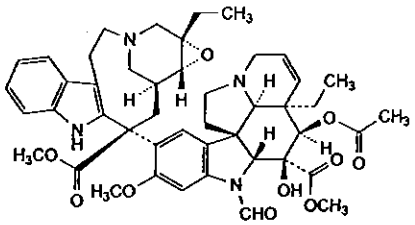
D. methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-3a-ethyl-9-[(5*S*,7*R*,9*S*)-5-ethyl-5-hydroxy-9-(methoxycarbonyl)-1,4,5,6,7,8,9,10-octahydro-2*H*-3,7-methanoazacycloundecino[5,4-*b*]indol-9-yl]-6-formyl-4,5-dihydroxy-8-methoxy-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate (deacetylvincristine),



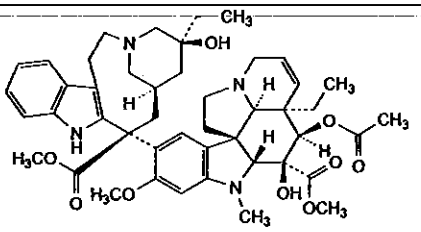
E. methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-3a-ethyl-9-[(5*S*,7*R*,9*S*)-5-ethyl-5-hydroxy-9-(methoxycarbonyl)-1,4,5,6,7,8,9,10-octahydro-2*H*-3,7-methanoazacycloundecino[5,4-*b*]indol-9-yl]-4,5-dihydroxy-8-methoxy-6-methyl-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate (deacetylvinblastine),



F. methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-4-(acetyloxy)-3a-ethyl-9-[(1a*S*,11*S*,13*S*,13a*R*)-1a-ethyl-11-(methoxycarbonyl)-1a,4,5,10,11,12,13,13a-octahydro-2*H*-3,13-methano-oxireno[9,10]azacycloundecino[5,4-*b*]indol-11-yl]-5-hydroxy-8-methoxy-6-methyl-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate (leurosine),



G. methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-4-(acetyloxy)-3a-ethyl-9-[(1a*S*,11*S*,13*S*,13a*R*)-1a-ethyl-11-(methoxycarbonyl)-1a,4,5,10,11,12,13,13a-octahydro-2*H*-3,13-methano-oxireno[9,10]azacycloundecino[5,4-*b*]indol-11-yl]-6-formyl-5-hydroxy-8-methoxy-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate (formylleurosine),



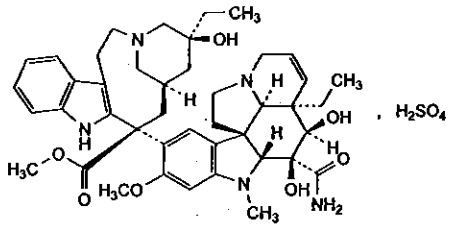
H. vinblastine.

Ph Eur

Vindesine Sulfate

Vindesine Sulphate

(Ph. Eur. monograph 1276)



$C_{43}H_{57}N_5O_{11}S$ 852 *59917-39-4*

Action and use
Vinca alkaloid cytotoxic.

Preparation
Vindesine Injection

Ph Eur

DEFINITION
3-(Carbamoyl)-O^4-deacetyl-3-de(methoxycarbonyl) vincaleukoblastine sulfate.

Content
96.0 per cent to 103.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white, amorphous, hygroscopic substance.

Solubility
Freely soluble in water and in methanol, practically insoluble in cyclohexane.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of vindesine sulfate.*

TESTS
Solution S
Dissolve 50 mg in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method I*).

pH (*2.2.3*)
3.5 to 5.5 for solution S.

Related substances
Liquid chromatography (*2.2.29*). *Keep the solutions in iced water before use.*

Test solution Dissolve 10.0 mg of the substance to be examined in *water R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 50.0 mL with *water R*. Dilute 1.0 mL of this solution to 10.0 mL with *water R*.

Reference solution (b) Dissolve 1.0 mg of *desacetylvinblastine CRS* in *water R*, add 1.0 mL of the test solution and dilute to 50.0 mL with *water R*.

Reference solution (c) In order to prepare impurity A *in situ*, dissolve 0.2 g of the substance to be examined in *dilute hydrogen peroxide solution R* and dilute to 20.0 mL with the same solvent. Dilute 2.0 mL of the solution to 10.0 mL with *water R*. Inject the solution within 1 h of preparation.

Column:
— *size*: *l* = 0.15 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
— *mobile phase A*: 1.5 per cent *V/V* solution of *diethylamine R* adjusted to pH 7.4 with *phosphoric acid R*;
— *mobile phase B*: *methanol R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 40	49	51
40 - 49	49 → 30	51 → 70
49 - 60	30	70

Flow rate 2 mL/min.

Detection Spectrophotometer at 270 nm.

Injection 200 µL.

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peak due to impurity A.

Relative retention With reference to vindesine (retention time = about 25 min): impurity A = about 0.2.

System suitability Reference solution (b):
— the retention time of vindesine is less than 40 min;
— *resolution*: minimum 2.0 between the peaks due to vindesine and desacetylvinblastine;
— *symmetry factor*: maximum 2.0 for the peak due to vindesine.

Limits:
— *impurity A*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

— *unspecified impurities:* for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total:* not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.8 per cent);
— *disregard limit:* 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.32*)
Maximum 5.0 per cent, determined on 50.0 mg using the evaporation technique at 150 °C; weigh the sample in an inert atmosphere and carry out a blank test.

ASSAY
Liquid chromatography (*2.2.29*). *Keep the solutions in iced water before use.*

Test solution Dissolve 5.0 mg of the substance to be examined in *water R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dissolve and dilute the entire contents of a vial of *vindesine sulfate CRS* with *water R* to yield a concentration of approximately 0.50 mg/mL.

Reference solution (b) Add 1.0 mg of *desacetylvinblastine CRS* to 2.0 mL of reference solution (a).

Column:
— *size:* l = 0.15 m, Ø = 4.6 mm;
— *stationary phase: octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 38 volumes of a 1.5 per cent *V/V* solution of *diethylamine R*, previously adjusted to pH 7.4 with *phosphoric acid R*, and 62 volumes of *methanol R*.

Flow rate 1 mL/min.

Detection Spectrophotometer at 270 nm.

Injection 20 µL.

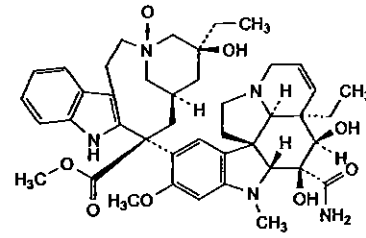
System suitability Reference solution (b):
— *resolution:* minimum 1.5 between the peaks due to vindesine and desacetylvinblastine;
— *symmetry factor:* maximum 2.0 for the peak due to vindesine;
— *repeatability:* maximum relative standard deviation of 1.5 per cent for the peak due to vindesine after 5 injections.

Calculate the percentage content of $C_{43}H_{57}N_5O_{11}S$ taking into account the assigned content of *vindesine sulfate CRS*.

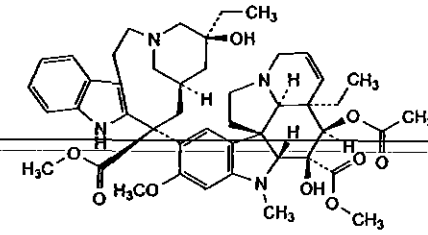
STORAGE
In an airtight, high-density polyethylene container with a high-density polyethylene cap, at a temperature of −50 °C or below. If the substance is sterile, store in a sterile, airtight, tamper-evident container.

IMPURITIES
Specified impurities *A.*

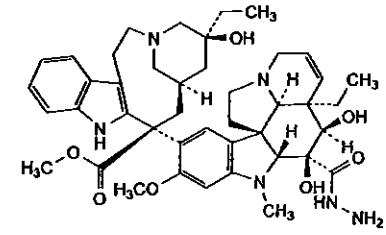
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C.*



A. 3-(carbamoyl)-O^4-deacetyl-3-de(methoxycarbonyl) vincaleukoblastine $N^{6\prime}$-oxide (vindesine N^3-oxide),



B. vincaleukoblastine (vinblastine),

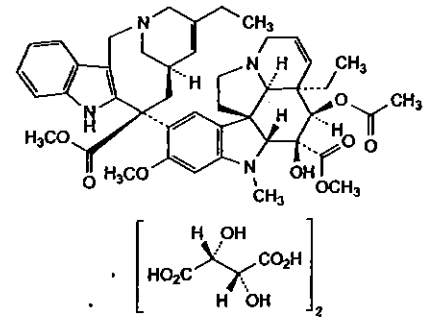


C. O^4-deacetyl-23-demethoxy-23-hydrazinylvincaleukoblastine (deacetylvinblastine hydrazide).

Ph Eur

Vinorelbine Tartrate

(Ph. Eur. monograph 2107)



$C_{53}H_{66}N_4O_{20}$ 1079 *125317-39-7*

Action and use
Vinca alkaloid cytotoxic.

Ph Eur

DEFINITION
4′-Deoxy-3′,4′-didehydro-8′-norvincaleukoblastine dihydrogen bis[(2*R*,3*R*)-2,3-dihydroxybutanedioate].

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white powder, hygroscopic.

Solubility

Freely soluble in water and in methanol, practically insoluble in hexane.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Dissolve 10 mg in 5 mL of *water R*. Add 0.5 mL of *sodium hydroxide solution R*. Extract with 5 mL of *methylene chloride R*. Dry the organic layer over *anhydrous sodium sulfate R*, filter and reduce its volume to about 0.5 mL by evaporation and apply to a disc of *potassium bromide R*. Evaporate and record the spectrum.

Comparison *vinorelbine tartrate CRS*, treated as described above.

B. It gives reaction (b) of tartrates (*2.3.1*).

TESTS

Solution S

Dissolve a quantity equivalent to 0.140 g of the anhydrous substance in *carbon dioxide-free water R* and dilute to 10.0 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and its absorbance (*2.2.25*) at 420 nm is not greater than 0.030.

pH (*2.2.3*)

3.3 to 3.8 for solution S.

Related substances

Liquid chromatography (*2.2.29*): use the normalisation procedure.

Solvent mixture Mobile phase A, mobile phase B (45:55 *V/V*).

Test solution Dissolve 35.0 mg of the substance to be examined in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

Reference solution (a) Dissolve 14 mg of *vinorelbine tartrate CRS* in *water R* and dilute to 10 mL with the same solvent. Expose this solution for 1 h to a xenon lamp apparatus at a wavelength of 310-880 nm, supplying a dose of 1600 kJ/m^2 at a fluence rate of 500 W/m^2 in order to generate impurity A.

Reference solution (b) Dilute 1.0 mL of the test solution to 20.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 100.0 mL with the solvent mixture.

Reference solution (c) Dissolve 7 mg of *vinorelbine for peak identification A CRS* (containing impurity K) in the solvent mixture and dilute to 5 mL with the solvent mixture.

Reference solution (d) Dissolve 7 mg of *vinorelbine for peak identification B CRS* (containing impurities C and I) in the solvent mixture and dilute to 5 mL with the solvent mixture.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 30 °C.

Mobile phase:
- *mobile phase A*: mix 14 mL of *diethylamine R* and 986 mL of *water for chromatography R* and adjust to pH 7.5 with *dilute phosphoric acid R*;
- *mobile phase B*: *acetonitrile R*, *methanol R* (20:80 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	45	55
5 - 45	45 → 20	55 → 80
45 - 50	20	80

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 267 nm.

Autosampler Set at 5 °C.

Injection 20 µL.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity A; use the chromatogram supplied with *vinorelbine for peak identification B CRS* and the chromatogram obtained with reference solution (d) to identify the peaks due to impurities C and I; use the chromatogram supplied with *vinorelbine for peak identification A CRS* and the chromatogram obtained with reference solution (c) to identify the peak due to impurity K.

Relative retention With reference to vinorelbine (retention time = about 20 min): impurity C = about 0.65; impurity K = about 0.8; impurity A = about 0.9; impurity I = about 1.2.

System suitability Reference solution (a):
- *resolution*: minimum 1.5 between the peaks due to impurity A and vinorelbine.

Limits:
- *impurity A*: maximum 0.3 per cent;
- *impurities C, I, K*: for each impurity, maximum 0.2 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.5 per cent;
- *reporting threshold*: 0.05 per cent (principal peak in the chromatogram obtained with reference solution (b)).

Boron

Maximum 50 ppm.

Test solution Dissolve 0.10 g of the substance to be examined in 2 mL of *water R*. Slowly add 10.0 mL of *sulfuric acid R* while cooling in iced water. Stir and allow to warm to room temperature. Add 10.0 mL of a 0.5 g/L solution of *carminic acid R* in *sulfuric acid R*.

Reference solution Dilute 2.5 mL of a 0.572 g/L solution of *boric acid R* to 100.0 mL with *water R*. To 2.0 mL of this solution slowly add 10.0 mL of *sulfuric acid R* while cooling in iced water. Stir and allow to warm to room temperature. Add 10.0 mL of a 0.5 g/L solution of *carminic acid R* in *sulfuric acid R*.

Blank solution To 2.0 mL of *water R* slowly add 10.0 mL of *sulfuric acid R* while cooling in iced water. Stir and allow to warm to room temperature. Add 10.0 mL of a 0.5 g/L solution of *carminic acid R* in *sulfuric acid R*.

After 45 min, measure the absorbance (*2.2.25*) of the test solution and the reference solution, between 560 nm and 650 nm, using the blank solution as compensation liquid. The maximum absorbance value of the test solution is not greater than that of the reference solution.

Fluorides

Maximum 50 ppm.

Potentiometry (*2.2.36, Method I*) using a fluoride-selective indicator electrode and a silver-silver chloride reference electrode.

Test solution Dissolve 0.19 g of the substance to be examined in 20 mL of *water R*. Add 5.0 mL of *total-ionic-strength-adjustment buffer R* and dilute to 50 mL with *water R*.

Reference solutions To 0.6 mL, 0.8 mL, 1.0 mL, 1.2 mL and 1.4 mL of *fluoride standard solution (10 ppm F) R*, add 5.0 mL of *total-ionic-strength-adjustment buffer R* and dilute to 50 mL with *water R*.

Introduce the electrodes into the reference solutions and allow to stand for 5 min. Determine the potential difference between the electrodes after 1 min of stabilisation. Using semi-logarithmic paper plot the potential difference obtained for each reference solution as a function of concentration of fluoride. Using exactly the same conditions, determine the potential difference obtained with the test solution and calculate the content of fluoride.

Water (*2.5.12*)

Maximum 4.0 per cent, determined on 0.250 g.

ASSAY

Dissolve 0.350 g in 40 mL of *glacial acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 53.96 mg of $C_{53}H_{66}N_4O_{20}$.

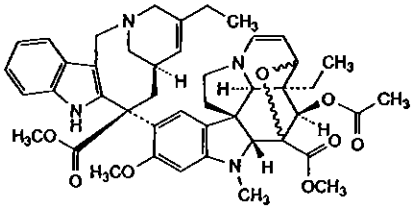
STORAGE

Under an inert gas, protected from light, at a temperature not exceeding −15 °C.

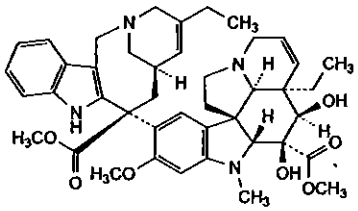
IMPURITIES

Specified impurities *A, C, I, K.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, D, E, F, G, H, J.*

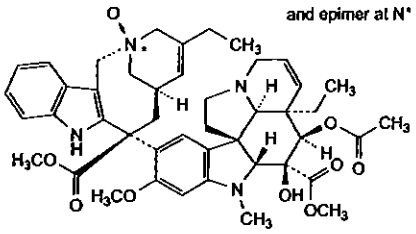


A. 3,4′-dideoxy-3,6ξ-epoxy-3′,4′,7,8-tetradehydro-6,7-dihydro-8′-nor-3ξ-vincaleukoblastine,

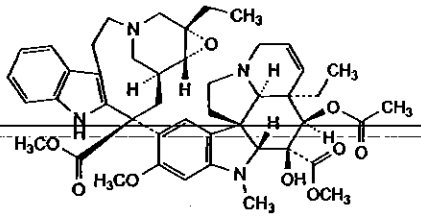


B. O^4-deacetyl-4′-deoxy-3′,4′-didehydro-8′-norvincaleukoblastine,

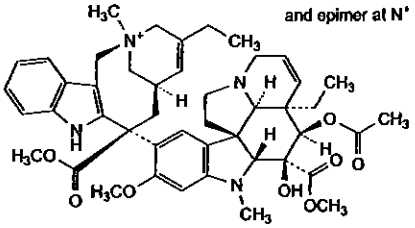
C. unknown structure,



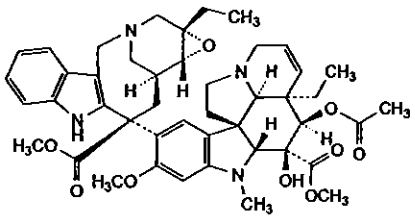
D. (6′*RS*)-4′-deoxy-3′,4′-didehydro-8′-norvincaleukoblastine 6′-oxide,



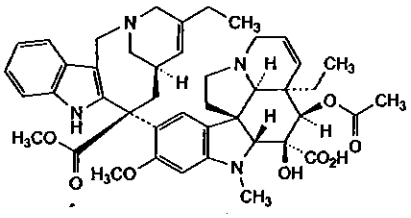
E. 4′-deoxy-3′α,4′α-epoxyvincaleukoblastine (leurosine),



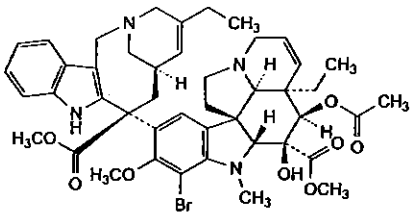
F. (6′*RS*)-4′-deoxy-6′-methyl-3′,4′-didehydro-6′-azonia-8′-norvincaleukoblastine,



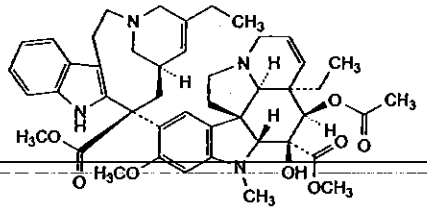
G. 4′-deoxy-3′α,4′α-epoxy-8′-norvincaleukoblastine,



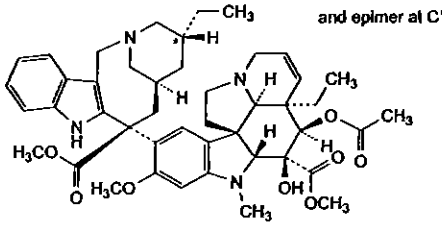
H. 4′-deoxy-3′,4′-didehydro-8′,24-dinorvincaleukoblastine,



I. 17-bromo-4′-deoxy-3′,4′-didehydro-8′-norvincaleukoblastine,



J. 4′-deoxy-3′,4′-didehydrovincaleukoblastine,

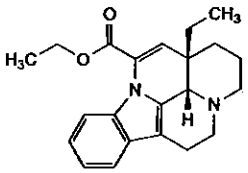


K. 4′-deoxy-8′-nor-4′αβ-vincaleukoblastine.

Ph Eur

Vinpocetine

(Ph. Eur. monograph 2139)



$C_{22}H_{26}N_2O_2$ 350.5 *42971-09-5*

Action and use
Vasodilator.

Ph Eur

DEFINITION

Ethyl (13a*S*,13b*S*)-13a-ethyl-2,3,5,6,13a,13b-hexahydro-1*H*-indolo[3,2,1-*de*]pyrido[3,2,1-*ij*][1,5]naphthyridine-12-carboxylate.

Content
98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance
White or slightly yellow, crystalline powder.

Solubility
Practically insoluble in water, soluble in methylene chloride, slightly soluble in anhydrous ethanol.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *vinpocetine CRS*.

TESTS

Specific optical rotation (*2.2.7*)
+ 127 to + 134 (dried substance).

Dissolve 0.25 g in *dimethylformamide R* and dilute to 25.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 50.0 mL with the mobile phase.

Reference solution (b) Dissolve 5.0 mg of *vinpocetine impurity B CRS*, 6.0 mg of *vinpocetine impurity A CRS*, 5.0 mg of *vinpocetine impurity C CRS* and 5.0 mg of *vinpocetine impurity D CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (c) Dilute 1.0 mL of reference solution (a) and 1.0 mL of reference solution (b) to 20.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase 15.4 g/L solution of *ammonium acetate R*, *acetonitrile R* (45:55 *V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 15 µL.

Run time 3 times the retention time of vinpocetine.

Relative retention With reference to vinpocetine (retention time = about 16 min): impurity A = about 0.4; impurity D = about 0.68; impurity B = about 0.75; impurity C = about 0.83.

System suitability Reference solution (c):
— *resolution*: minimum 2.0 between the peaks due to impurities D and B.

Limits:
— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.6 per cent);
— *impurities B, D*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.5 per cent);
— *impurity C*: not more than 0.6 times the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.3 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the peak due to vinpocetine in the chromatogram obtained with reference solution (c) (0.10 per cent);
— *total*: not more than 10 times the area of the peak due to vinpocetine in the chromatogram obtained with reference solution (c) (1.0 per cent);
— *disregard limit*: 0.5 times the area of the peak due to vinpocetine in the chromatogram obtained with reference solution (c) (0.05 per cent).

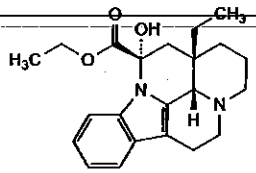
Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* in an oven at 100 °C for 3 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

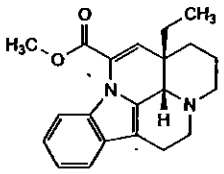
ASSAY
Dissolve 0.300 g in 50 mL of a mixture of equal volumes of *acetic anhydride R* and *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 35.05 mg of $C_{22}H_{26}N_2O_2$.

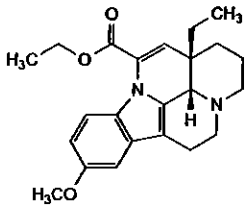
IMPURITIES
Specified impurities *A, B, C, D.*



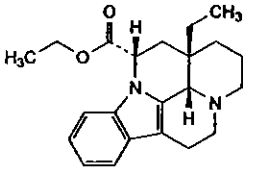
A. ethyl (12*S*,13a*S*,13b*S*)-13a-ethyl-12-hydroxy-2,3,5,6,12,13,13a,13b-octahydro-1*H*-indolo[3,2,1-*de*]pyrido[3,2,1-*ij*][1,5]naphthyridine-12-carboxylate (ethyl vincaminate),



B. methyl (13a*S*,13b*S*)-13a-ethyl-2,3,5,6,13a,13b-hexahydro-1*H*-indolo[3,2,1-*de*]pyrido[3,2,1-*ij*][1,5]naphthyridine-12-carboxylate (apovincamine),



C. ethyl (13a*S*,13b*S*)-13a-ethyl-10-methoxy-2,3,5,6,13a,13b-hexahydro-1*H*-indolo[3,2,1-*de*]pyrido[3,2,1-*ij*][1,5]naphthyridine-12-carboxylate (methoxyvinpocetine),



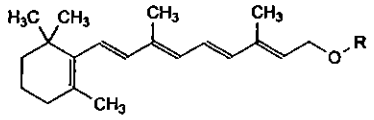
D. ethyl (12*RS*,13a*RS*,13b*RS*)-13a-ethyl-2,3,5,6,12,13,13a,13b-octahydro-1*H*-indolo[3,2,1-*de*]pyrido[3,2,1-*ij*][1,5]naphthyridine-12-carboxylate (dihydrovinpocetine).

Ph Eur

Vitamin A

(Ph. Eur. monograph 0217)

In the British Pharmacopoeia, the term 'Retinol' is used within titles for preparations containing synthetic ester(s) and the term 'Vitamin A' within the title for the preparation containing material of natural origin.



Substance	R	Molecular formula	M_r
all-(*E*)-retinol	H	$C_{20}H_{30}O$	286.5
all-(*E*)-retinol acetate	CO-CH_3	$C_{22}H_{32}O_2$	328.5
all-(*E*)-retinol propionate	CO-C_2H_5	$C_{23}H_{34}O_2$	342.5
all-(*E*)-retinol palmitate	CO-$C_{15}H_{31}$	$C_{36}H_{60}O_2$	524.9

Preparation
Paediatric Vitamins A, C and D Oral Drops

Ph Eur

DEFINITION
Vitamin A Refers to a number of substances of very similar structure (including (*Z*)-isomers) found in animal tissues and possessing similar activity. The principal and biologically most active substance is all-(*E*)-retinol (all-(*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraen-1-ol; $C_{20}H_{30}O$). Vitamin A is generally used in the form of esters such as the acetate, propionate and palmitate.

Synthetic retinol ester Refers to an ester (acetate, propionate or palmitate) or a mixture of synthetic retinol esters.

The activity of vitamin A is expressed in retinol equivalents (R.E.). 1 mg R.E. corresponds to the activity of 1 mg of all-(*E*)-retinol. The activity of the other retinol esters is calculated stoichiometrically, so that 1 mg R.E. of vitamin A corresponds to the activity of:
— 1.147 mg of all-(*E*)-retinol acetate,
— 1.195 mg of all-(*E*)-retinol propionate,
— 1.832 mg of all-(*E*)-retinol palmitate.

International Units (IU) are also used to express the activity of vitamin A. 1 IU of vitamin A is equivalent to the activity of 0.300 µg of all-(*E*)-retinol. The activity of the other retinol esters is calculated stoichiometrically, so that 1 IU of vitamin A is equivalent to the activity of:
— 0.344 µg of all-(*E*)-retinol acetate,
— 0.359 µg of all-(*E*)-retinol propionate,
— 0.550 µg of all-(*E*)-retinol palmitate,

1 mg of retinol equivalent is equivalent to 3333 IU.

CHARACTERS
Appearance
Retinol acetate: pale-yellow crystals (mp: about 60 °C). Once melted retinol acetate tends to yield a supercooled melt.

Retinol propionate: reddish-brown oily liquid.

Retinol palmitate: a fat-like, light yellow solid or a yellow oily liquid, if melted (mp: about 26 °C).

Solubility
All retinol esters are practically insoluble in water, soluble or partly soluble in anhydrous ethanol and miscible with organic solvents.

Vitamin A and its esters are very sensitive to the action of air, oxidising agents, acids, light and heat.

Carry out the assay and all tests as rapidly as possible, avoiding exposure to actinic light and air, oxidising agents, oxidation catalysts (e.g. copper, iron), acids and heat; use freshly prepared solutions.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Prepare a solution containing about 3.3 IU of vitamin A per microlitre in *cyclohexane R* containing 1 g/L of *butylhydroxytoluene R*.

Reference solution Prepare a 10 mg/mL solution of *retinol esters CRS* (i.e. 3.3 IU of each ester per microlitre) in *cyclohexane R* containing 1 g/L of *butylhydroxytoluene R*.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *ether R, cyclohexane R (20:80 V/V)*.

Application 3 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution:

— the chromatogram shows the individual spots of the corresponding esters. The elution order from bottom to top is: retinol acetate, retinol propionate and retinol palmitate.

Results The composition of esters is confirmed by the correspondence of the principal spot or spots of the test solution with those obtained with the reference solution.

B. Related substances (see Tests).

TESTS

Retinol

Thin-layer chromatography (*2.2.27*).

Test solution Prepare a solution in *cyclohexane R*, stabilised with a solution containing 1 g/L of *butylhydroxytoluene R*, containing about 330 IU of vitamin A per microlitre.

Reference solution Shake 1 mL of the test solution with 20 mL of *0.1 M tetrabutylammonium hydroxide in 2-propanol* for 2 min and dilute to 100 mL with *cyclohexane R*, stabilised with a solution containg 1 g/L of *butylhydroxytoluene R*.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *ether R, cyclohexane R* (20:80 *V/V*).

Application 3 µL.

Development Over a path of 15 cm.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability Reference solution:

— in the chromatogram obtained no or only traces of the retinol esters are seen.

Limit Any spot corresponding to retinol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (1.0 per cent).

Related substances

Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution The solution described under Activity.

Absorption maximum At 325 nm to 327 nm.

Absorbance ratios:

— A_{300}/A_{326} = maximum 0.60;

— A_{350}/A_{326} = maximum 0.54;

— A_{370}/A_{326} = maximum 0.14.

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

ACTIVITY

The activity of the substance is determined in order to be taken into account for the production of concentrates.

Dissolve 25-100 mg, weighed with an accuracy of 0.1 per cent, in 5 mL of *pentane R* and dilute with *2-propanol R1* to a presumed concentration of 10 IU/mL to 15 IU/mL. Measure the absorbance (*2.2.25*) at the absorption maximum at 326 nm. Calculate the activity of vitamin A in International Units per gram from the expression:

$$\frac{A_{326} \times V \times 1900}{100 \times m}$$

A_{326} = absorbance at 326 nm,

m = mass of the substance to be examined, in grams,

V = total volume to which the substance to be examined is diluted to give 10 IU/mL to 15 IU/mL,

1900 = factor to convert the specific absorbance of esters of retinol into International Units per gram.

STORAGE

In an airtight container, protected from light.

Once the container has been opened, its contents are to be used as soon as possible; any part of the contents not used at once should be protected by an atmosphere of inert gas.

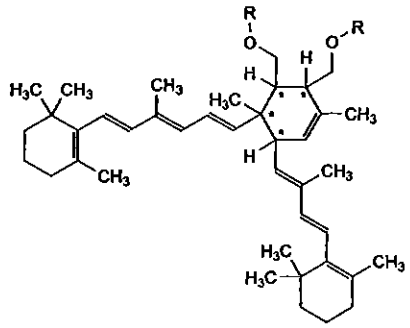
LABELLING

The label states:

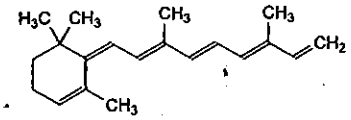
— the number of International Units per gram,

— the name of the ester or esters.

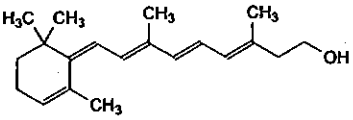
IMPURITIES



A. R = H, CO-CH_3: kitols (Diels-Alder dimers of vitamin A),



B. (3*E*,5*E*,7*E*)-3,7-dimethyl-9-[(1*Z*)-2,6,6-trimethylcyclohex-2-enylidene]nona-1,3,5,7-tetraene (anhydro-vitamin A),



C. (3*E*,5*E*,7*E*)-3,7-dimethyl-9-[(1*Z*)-2,6,6-trimethylcyclohex-2-enylidene]nona-3,5,7-trien-1-ol (*retro*-vitamin A),

D. oxidation products of vitamin A.

Natural Vitamin A Ester Concentrate

DEFINITION

Natural Vitamin A Ester Concentrate consists of a natural ester or a mixture of natural esters of retinol or of a solution of the ester or mixture of esters in Arachis Oil or other suitable vegetable oil. It contains in 1 g not less than 485,000 IU of vitamin A and not less than 97.0% of the number of IU of vitamin A stated on the label. It may contain a suitable antioxidant or mixture of antioxidants.

CHARACTERISTICS

A yellow oil or a mixture of oil and crystalline material which yields a homogeneous yellow oil on warming.

Solubility

Practically insoluble in *water*; soluble or partly soluble in *ethanol (96%)*; miscible with *ether* and with petroleum spirit.

IDENTIFICATION

A. Dissolve a quantity containing 10 IU in a mixture of 100 parts of *absolute ethanol* and 1 part of *hydrochloric acid*. The *light absorption*, Appendix II B, of the solution immediately after preparation exhibits a single maximum at 326 nm. Heat the solution in a water bath for 30 seconds and cool rapidly. The *light absorption* in the range 300 to 400 nm exhibits a low maximum or inflection at 332 nm and sharp maxima at 348, 367 and 389 nm.

B. Dissolve a quantity containing 30 IU in 1 mL of *chloroform* and add 10 mL of *antimony trichloride solution*. A transient bright blue colour is produced immediately.

TESTS

Acid value

Not more than 2.0, Appendix X B.

Peroxide value

Place 1 g in a boiling tube (20 cm × 2.5 cm) and dissolve in 20 mL of a mixture of 2 volumes of *glacial acetic acid* and 1 volume of *ethanol-free chloroform*. Add 1 g of finely powdered *potassium iodide* and pass a rapid current of *oxygen-free nitrogen* through the mixture for 1 minute. Stopper the tube loosely, partly immerse in boiling water for 30 seconds and then in water at 80° for 2 minutes, tighten the stopper and cool rapidly. Transfer the contents to a flask containing 25 mL of a freshly prepared 1% w/v solution of *potassium iodide*, rinse the tube with a further 25 mL of the potassium iodide solution, shake the combined solution and rinsings and titrate with 0.01M *sodium thiosulfate VS*. Repeat the procedure without the concentrate. The difference between the titrations does not exceed 1.4 mL.

Retinol

Carry out the method for *descending paper chromatography*, Appendix III E, using a mixture of 70 volumes of *1,4-dioxan*, 15 volumes of *methanol* and 15 volumes of *water* containing 1% w/v of *butylated hydroxyanisole* in the bottom of the tank and as the mobile phase. Saturate the paper with a 10% w/v solution of *liquid paraffin* in *petroleum spirit (boiling range, 40° to 60°)* and dry without the aid of heat. Apply separately to the impregnated paper 5 µL and 10 µL of each of the following freshly prepared solutions. For solution (1) dissolve sufficient of the concentrate in *petroleum spirit (boiling range, 40° to 60°)* to yield a solution containing 16,150 to 17,850 IU per mL. For solution (2) mix a quantity of the concentrate containing not less than 500 IU of vitamin A and not more than 1 g of fat with 30 mL of *absolute ethanol* and 3 mL of a 50% w/w solution of *potassium hydroxide*. Boil gently under a reflux condenser in a current of *oxygen-free nitrogen* for 30 minutes, cool rapidly and add 30 mL of water. Transfer the hydrolysate to a separating funnel using three 50 mL quantities of *ether* and extract the vitamin A by shaking for 1 minute. After complete separation discard the aqueous layer and wash the extract with four 50 mL quantities of *water*, mixing very cautiously during the first two washes to avoid the formation of emulsions. Evaporate the separated extract to about 5 mL and remove the remaining solvent in a current of *oxygen-free nitrogen* without the application of heat. Dissolve the residue in sufficient *petroleum spirit (boiling range, 40° to 60°)* to produce a solution of retinol containing about 340 IU per mL.

Develop until the solvent front approaches the bottom of the paper. Examine the dried paper under *ultraviolet light (365 nm)*. The fluorescence of any spot corresponding to retinol in the chromatograms obtained with solution (1) is not more intense than that of the spot in the corresponding chromatogram obtained with solution (2).

ASSAY

Carry out the test as rapidly as possible, avoiding exposure to actinic light and air, oxidising agents, oxidation catalysts (e.g. copper and iron) and acids.

Examine by *ultraviolet absorption spectrophotometry*, Appendix II B (Method A). If method A is found not to be valid, examine by *liquid chromatography*, Appendix III D (Method B).

Method A

Test solution To a quantity of the substance being examined containing 50,000 IU in a round-bottomed flask, add 3 mL of a freshly prepared 50% w/w solution of *potassium hydroxide* and 30 mL of *absolute ethanol*. Boil under a reflux condenser in a current of *nitrogen* for 30 minutes. Cool rapidly and add 30 mL of *water*. Extract with four 50 mL quantities of *ether* discarding the lower layer after complete separation. Wash the combined upper layers with four 50 mL quantities of *water* and evaporate to dryness under a gentle current of *nitrogen* at a temperature not exceeding 30° or in a rotary evaporator at a temperature not exceeding 30° under reduced pressure (water ejector). Dissolve the residue in sufficient *propan-2-ol* to give an expected concentration of vitamin A equivalent to 10 to 15 IU per mL.

Measure the *absorbances*, Appendix II B, of the solution at 300 nm, 310 nm, 325 nm and 334 nm and at the wavelength of maximum absorption with a suitable spectrophotometer in 1-cm specially matched cells, using *propan-2-ol* as the compensation liquid.

Calculate the content of vitamin A, as all-*trans*-retinol, in IU per gram from the expression:

$$A_{325} \times \frac{1830}{100m} \times V$$

where A_{325} = absorbance at 325 nm,
m = mass of the substance to be examined in grams,
V = total volume of solution containing 10 IU to 15 IU of vitamin A per mL,
1830 = conversion factor for the specific absorbance of all-*trans*-retinol in IU.

The above expression can be used only if A_{325} has a value of not greater than $A_{325,\,corr}$ / 0.970 where $A_{325,\,corr}$ is the corrected *absorbance* at 325 nm and is given by the equation:

$$A_{325,\,corr} = 6.815A_{325} - 2.555A_{310} - 4.260A_{334}$$

where A designates the absorbance at the wavelength indicated by the subscript.

If A_{325} has a value greater than $A_{325,\,corr}$ / 0.970, calculate the content of vitamin A from the expression:

$$A_{325,corr} \times \frac{1830}{100m} \times V$$

The assay is not valid unless:

(a) the wavelength of maximum absorption lies between 323 nm and 327 nm and

(b) the *absorbance* at 300 nm relative to that at 325 nm is at most 0.73.

Method B

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Prepare solution (1) as follows. To a quantity of the substance being examined containing 50,000 IU in a round-bottomed flask, add 5 mL of a freshly prepared 10% w/v solution of *ascorbic acid* and 10 mL of a freshly prepared 80% w/v solution of *potassium hydroxide* and 100 mL of *ethanol (96%)*. Boil under a reflux condenser on a water bath for 15 minutes. Add 100 mL of a 1% w/v solution of *sodium chloride* and cool. Transfer the solution to a 500 mL separating funnel rinsing the round-bottomed flask with about 75 mL of a 1% w/v solution of *sodium chloride* and then with 150 mL of a mixture of equal volumes of *petroleum spirit (boiling range, 40° to 60°)* and *ether*. Shake for 1 minute. When the layers have separated completely, discard the lower layer and wash the upper layer, first with 50 mL of a 3% w/v solution of *potassium hydroxide* in a 10% v/v solution of *ethanol (96%)* and then with three 50 mL quantities of a 1% w/v solution of *sodium chloride*. Filter the upper layer through 5 g of *anhydrous sodium sulfate* on a fast filter paper into a 250 mL flask suitable for a rotary evaporator. Wash the funnel with 10 mL of fresh extraction mixture, filter and combine the upper layers. Distil them at a temperature not exceeding 30° under reduced pressure (water ejector) and fill with *nitrogen* when evaporation is completed. Alternatively evaporate the solvent under a gentle current of *nitrogen* at a temperature not exceeding 30°. Dissolve the residue in *propan-2-ol*, transfer to a 25 mL volumetric flask and dilute to 25 mL with *propan-2-ol*. Gentle heating in an ultrasonic bath may be required. For solution (2) prepare a solution of *retinyl acetate EPCRS* in *propan-2-ol R1* so that 1 mL contains about 1000 IU of all-*trans*-retinol.

The exact concentration of solution (2) is assessed by ultraviolet absorption spectrophotometry, Appendix II B. Dilute solution (2) with *propan-2-ol R1* to a presumed concentration of 10 IU per mL to 15 IU per mL and measure the *absorbance* at 326 nm in matched 1-cm cells using *propan-2-ol R1* as the compensation liquid.

Calculate the content of vitamin A in IU per mL of solution (2) from the following expression, taking into account the assigned content of *retinyl acetate EPCRS*:

$$A_{326} \times \frac{1900 \times V_2}{100 \times V_1}$$

where A_{326} = *absorbance* at 326 nm,
V_2 = volume of the diluted solution,
V_1 = volume of solution (2) used,
1900 = conversion factor for the specific absorbance of *retinyl acetate EPCRS* in IU.

For solution (3) proceed as described for solution (1) but use 2 mL of solution (2) in place of the substance being examined.

The exact concentration of solution (3) is assessed by *ultraviolet absorption spectrophotometry*, Appendix II B. Dilute solution (3) with *propan-2-ol R1* to a presumed concentration of 10 IU per mL to 15 IU per mL of all-*trans*-retinol and measure the absorbance at 325 nm in matched 1 cm cells using *propan-2-ol R1* as the compensation liquid.

Calculate the content of all-*trans*-retinol in IU per millilitre of solution (3) from the expression:

$$A_{325} \times \frac{1830 \times V_4}{100 \times V_3}$$

where A_{325} = *absorbance* at 325 nm,
V_3 = volume of the diluted solution,
V_4 = volume of solution (3) used,
1830 = conversion factor for the specific absorbance of all-*trans*-retinol in IU.

The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm to 10 µm), (b) as mobile phase at a flow rate of 1 mL per minute a mixture of 3 volumes of *water* and 97 volumes of *methanol*, (c) a detection wavelength of 325 nm, (d) a 10 µL loop injector and (e) an electronic integrator.

Inject in triplicate solution (1) and solution (3). The retention time of all-*trans*-retinol is 5 minute ± 1 minute.

The assay is not valid unless (a) the chromatogram obtained with solution (1) shows a peak corresponding to that of all-*trans*-retinol in the chromatogram obtained with solution (3), (b) when using the method of standard additions to solution (1) there is greater than 95% recovery of the added *retinyl acetate EPCRS*, and (c) the recovery of all-*trans*-retinol in solution (3) as assessed by direct absorption spectrophotometry is greater than 95%.

Calculate the content of vitamin A using the following expression:

$$A_1 \times \frac{C \times V}{A_2} \times \frac{1}{m}$$

where A_1 = area of the peak corresponding to all-*trans*-retinol in the chromatogram obtained with solution (1),
A_2 = area of the peak corresponding to all-*trans*-retinol in the chromatogram obtained with solution (3),
C = concentration of *retinyl acetate EPCRS* in solution (2) as assessed prior to the saponification in International Units per mL (1000 IU per mL),
V = volume of solution (2) treated,
m = mass of the substance being examined in solution (1).

STORAGE

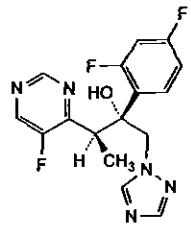
Vitamin A Ester Concentrate (Natural) should be kept in an airtight container, protected from light and stored at a temperature of 8° to 15°. Once the container has been opened its contents should be used as soon as possible; any part of the contents not used at once should be protected by an atmosphere of an inert gas.

LABELLING

The label states (1) the number of IU (Units) of vitamin A per g; (2) the name of the ester or esters; (3) the name and proportion of the principal excipients; (4) the method of restoring the solution if partial crystallisation has occurred.

Voriconazole

(Ph. Eur. monograph 2576)



$C_{16}H_{14}F_3N_5O$ 349.3 *137234-62-9*

Action and use
Antifungal.

Ph Eur

DEFINITION
(2*R*,3*S*)-2-(2,4-Difluorophenyl)-3-(5-fluoropyrimidin-4-yl)-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol.

Content
97.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white powder.

Solubility
Very slightly soluble in water, freely soluble in acetone and in methylene chloride.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *voriconazole CRS.*

B. Enantiomeric purity (see Tests).

TESTS
Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 0.5 g in a 103 g/L solution of *hydrochloric acid R* and dilute to 20 mL with the same solution.

Enantiomeric purity
Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in 2 mL of *acetonitrile R* and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 5.0 mg of *voriconazole impurity D CRS* in 2 mL of *acetonitrile R* and dilute to 50.0 mL with the mobile phase.

Reference solution (b) Dissolve 25 mg of the substance to be examined in 2 mL of *acetonitrile R*, add 1 mL of reference solution (a) and dilute to 50.0 mL with the mobile phase.

Reference solution (c) Dilute 1.0 mL of reference solution (a) to 100.0 mL with the mobile phase.

Column:
— *size:* l = 0.25 m, Ø = 4.6 mm;
— *stationary phase: silica gel BC for chiral chromatography R* (5 µm);
— *temperature:* 30 °C.

Mobile phase Mix 18 volumes of *acetonitrile R* and 82 volumes of a 0.77 g/L solution of *ammonium acetate R* previously adjusted to pH 5.0 with *glacial acetic acid R.*

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 256 nm.

Injection 20 µL of the test solution and reference solutions (b) and (c).

Run time 2.5 times the retention time of voriconazole.

Relative retention With reference to voriconazole (retention time = about 7 min): impurity D = about 1.5.

System suitability Reference solution (b):
— *resolution:* minimum 4.0 between the peaks due to voriconazole and impurity D.

Limit:
— *impurity D:* not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent).

Impurity E
Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in 5.0 mL of *methanol R* and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dissolve 25.0 mg of *voriconazole impurity E CRS* in 50 mL of *methanol R* and dilute to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve 17 mg of *sodium chloride R* in *water R* and dilute to 200.0 mL with the same solvent. Mix 1 mL of the solution, 1 mL of reference solution (a) and 25 mL of *methanol R* and dilute to 50.0 mL with the mobile phase.

Reference solution (c) To 1.0 mL of reference solution (a) add 25 mL of *methanol R* and dilute to 50.0 mL with the mobile phase.

Column:
— *size:* l = 0.25 m, Ø = 4.0 mm;
— *stationary phase: strongly basic anion-exchange resin for chromatography R* (8.5 µm);
— *temperature:* 40 °C.

Mobile phase To 1500 mL of *water R* add 500 mL of *methanol R*, mix and degas; add about 175 µL of a 470 g/L solution of *sodium hydroxide R* and mix.

Flow rate 1.0 mL/min.

Detection Conductivity detector; use a self-regenerating anion suppressor.

Injection 20 µL of the test solution and reference solutions (b) and (c).

Run time Twice the retention time of impurity E.

Relative retention With reference to impurity E (retention time = about 4 min): chloride = about 1.5.

System suitability:
— *resolution:* minimum 3.5 between the peaks due to impurity E and chloride in the chromatogram obtained with reference solution (b);
— *symmetry factor:* maximum 1.7 for the peak due to impurity E in the chromatogram obtained with reference solution (c).

Limit:
— *impurity E:* not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent).

Related substances
Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the mobile phase, sonicating if necessary, and dilute to 100.0 mL with the mobile phase. Mix well to ensure complete dissolution.

Test solution (b) Dilute 5.0 mL of test solution (a) to 100.0 mL with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *voriconazole CRS* in the mobile phase, sonicating if necessary, and dilute to 100.0 mL with the mobile phase. Mix well to ensure complete dissolution. Dilute 5.0 mL of the solution to 100.0 mL with the mobile phase.

Reference solution (b) Suspend 0.100 g of the substance to be examined in 10 mL of a 40 g/L solution of *sodium hydroxide R* and dilute to 20 mL with the mobile phase; sonicate if necessary. Allow to stand for 30 min. Dilute 1.0 mL of the solution to 100.0 mL with the mobile phase (*in situ* degradation to obtain impurities A and C).

Reference solution (c) Dilute 1.0 mL of test solution (a) to 100.0 mL with the mobile phase and mix. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase and mix well.

Reference solution (d) Dissolve 2 mg of *voriconazole impurity B CRS* in the mobile phase and dilute to 200 mL with the mobile phase. Dilute 1.0 mL of the solution to 10.0 mL with the mobile phase.

Column:
- *size*: *l* = 0.15 m, Ø = 3.9 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (4 µm);
- *temperature*: 35 °C.

Mobile phase Mix 15 volumes of *acetonitrile R*, 30 volumes of *methanol R* and 55 volumes of a 1.90 g/L solution of *ammonium formate R* previously adjusted to pH 4.0 with *anhydrous formic acid R* while stirring continuously.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 256 nm.

Injection 20 µL of test solution (a) and reference solutions (b), (c) and (d).

Run time 3 times the retention time of voriconazole.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and C; use the chromatogram obtained with reference solution (d) to identify the peak due to impurity B.

Relative retention With reference to voriconazole (retention time = about 8 min): impurity A = about 0.25; impurity C = about 0.3; impurity B = about 0.6.

System suitability Reference solution (b):
- *resolution*: minimum 1.8 between the peaks due to impurities A and C.

Limits:
- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.7; impurity B = 2.1; impurity C = 0.7;
- *impurities A, B, C*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);
- *sum of impurities A, B, C, D, E and unspecified impurities*: maximum 0.5 per cent;
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Water (*2.5.12*)
Maximum 0.4 per cent, determined on 1.00 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g in a platinum crucible.

Bacterial endotoxins (*2.6.14*)
Less than 0.2 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (a).

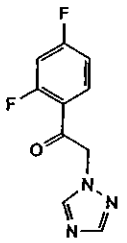
Calculate the percentage content of $C_{16}H_{14}F_3N_5O$ from the declared content of *voriconazole CRS*.

STORAGE

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

IMPURITIES

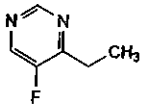
Specified impurities *A, B, C, D, E.*



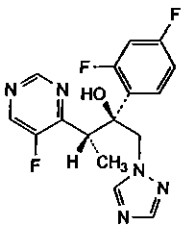
A. 1-(2,4-difluorophenyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanone,

and enantiomer

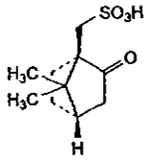
B. (2*RS*,3*SR*)-2-(2,4-difluorophenyl)-3-pyrimidin-4-yl-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol,



C. 4-ethyl-5-fluoropyrimidine,



D. (2*S*,3*R*)-2-(2,4-difluorophenyl)-3-(5-fluoropyrimidin-4-yl)-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol (voriconazole enantiomer),



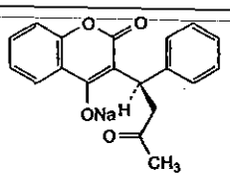
and enantiomer

E. [(1*R*,4*S*)-7,7-dimethyl-2-oxobicyclo[2.2.1]hept-1-yl] methanesulfonic acid ((±)-10-camphorsulfonic acid).

Ph Eur

Warfarin Sodium

(Ph. Eur. monograph 0698)



and enantiomer

$C_{19}H_{15}NaO_4$ 330.3 *129-06-6*

Action and use
Vitamin K epoxide reductase inhibitor; oral anticoagulant (coumarin).

Preparations
Warfarin Oral Suspension
Warfarin Tablets

Ph Eur

DEFINITION
Sodium 2-oxo-3-[(1*RS*)-3-oxo-1-phenylbutyl]-2*H*-1-benzopyran-4-olate.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white, hygroscopic, amorphous powder.

Solubility
Very soluble in water and in ethanol (96 per cent), soluble in acetone, very slightly soluble in methylene chloride.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *warfarin sodium CRS.*

B. Propan-2-ol (see Tests).

C. It gives reaction (b) of sodium (*2.3.1*).

TESTS
Appearance of solution
The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 1.0 g in *water R* and dilute to 20 mL with the same solvent.

pH (*2.2.3*)
7.6 to 8.6.

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 100 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).
Solvent mixture *methanol R, water R* (25:75 *V/V*).

Test solution Dissolve 40.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dissolve 2 mg of *4-hydroxycoumarin R* (impurity B) and 2 mg of benzalacetone R (impurity C) in 25 mL of *methanol R* and dilute to 100 mL with *water R.*

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Column:
— *size:* *l* = 0.25 m, Ø = 4.0 mm;
— *stationary phase: cyanosilyl silica gel for chromatography R* (5 µm);
— *temperature:* 30 °C.

Mobile phase *glacial acetic acid R, acetonitrile R, water R* (1:25:75 *V/V/V*).

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 260 nm.

Injection 20 µL.

Run time Twice the retention time of warfarin.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peaks due to impurities B and C.

Relative retention With reference to warfarin (retention time = about 9 min): impurity B = about 0.4; impurity C = about 0.6.

System suitability Reference solution (a):
— *resolution:* minimum 2.0 between the peaks due to impurities B and C.

Limits:
— *correction factors:* for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.5; impurity C = 0.4;
— *impurities B, C:* for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
— *unspecified impurities:* for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
— *total:* not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
— *disregard limit:* 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Phenolic ketones
Dissolve 1.25 g in a 20 g/L solution of *sodium hydroxide R* and dilute to 10.0 mL with the same solvent.
The absorbance (*2.2.25*) is maximum 0.20 measured at 385 nm within 15 min of preparing the solution.

Propan-2-ol (*2.4.24, System A*)
Maximum 0.5 per cent.

Water (*2.5.12*)
Maximum 4.0 per cent, determined on 0.750 g.

ASSAY
Dissolve 0.100 g in *0.01 M sodium hydroxide* and dilute to 100.0 mL with the same solvent. Dilute 10.0 mL of the solution to 100.0 mL with *0.01 M sodium hydroxide.* Dilute 10.0 mL of this solution to 100.0 mL with *0.01 M sodium hydroxide.* Measure the absorbance (*2.2.25*) at the absorption maximum at 308 nm.

Calculate the percentage content of $C_{19}H_{15}NaO_4$ taking the specific absorbance to be 431.

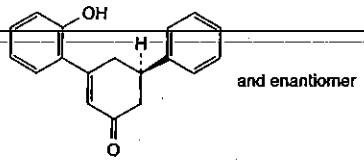
STORAGE

In an airtight container, protected from light.

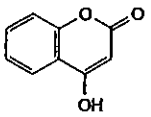
IMPURITIES

Specified impurities *B, C.*

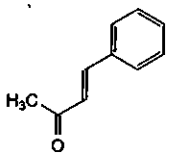
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A.*



A. (5*RS*)-3-(2-hydroxyphenyl)-5-phenylcyclohex-2-enone,



B. 4-hydroxy-2*H*-1-benzopyran-2-one (4-hydroxycoumarin),

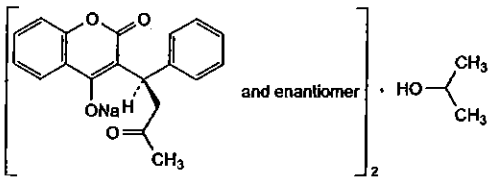


C. (3*E*)-4-phenylbut-3-en-2-one (benzalacetone).

Ph Eur

Warfarin Sodium Clathrate

(Ph. Eur. monograph 0699)



Action and use

Vitamin K epoxide reductase inhibitor; oral anticoagulant (coumarin).

Preparation

Warfarin Tablets

Ph Eur

DEFINITION

Mixture, in the form of a clathrate, of warfarin sodium (sodium 2-oxo-3-[(1*RS*)-3-oxo-1-phenylbutyl]-2*H*-1-benzopyran-4-olate) and propan-2-ol in molecular proportions 2:1 (equivalent to about 92 per cent of warfarin sodium).

Content

— *warfarin sodium*: 98.0 per cent to 102.0 per cent (anhydrous and propan-2-ol-free substance);
— *propan-2-ol*: 8.0 per cent to 8.5 per cent.

CHARACTERS

Appearance

White or almost white, hygroscopic, crystalline powder.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent), soluble in acetone, very slightly soluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *warfarin sodium clathrate CRS.*

B. Propan-2-ol (see Tests).

C. It gives reaction (b) of sodium (*2.3.1*).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 1.0 g in *water R* and dilute to 20 mL with the same solvent.

pH (*2.2.3*)

7.6 to 8.6.

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 100 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture *methanol R, water R* (25:75 *V/V*).

Test solution Dissolve 40.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dissolve 2 mg of *4-hydroxycoumarin R* (impurity B) and 2 mg of benzalacetone R (impurity C) in 25 mL of *methanol R* and dilute to 100 mL with *water R*.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.25 m, Ø = 4.0 mm;
— *stationary phase*: *cyanosilyl silica gel for chromatography R* (5 µm);
— *temperature*: 30 °C.

Mobile phase *glacial acetic acid R, acetonitrile R, water R* (1:25:75 *V/V/V*).

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 260 nm.

Injection 20 µL.

Run time Twice the retention time of warfarin.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peaks due to impurities B and C.

Relative retention With reference to warfarin (retention time = about 9 min): impurity B = about 0.4; impurity C = about 0.6.

System suitability Reference solution (a):
— *resolution*: minimum 2.0 between the peaks due to impurities B and C.

Limits:
- — *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.5; impurity C = 0.4;
- — *impurities B, C*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
- — *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- — *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
- — *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Phenolic ketones
Dissolve 1.25 g in a 20 g/L solution of *sodium hydroxide R* and dilute to 10.0 mL with the same solvent.
The absorbance (*2.2.25*) is maximum 0.20 measured at 385 nm within 15 min of preparing the solution.

Propan-2-ol (*2.4.24, System A*)
8.0 per cent to 8.5 per cent.

Water (*2.5.12*)
Maximum 0.3 per cent, determined on 2.500 g.

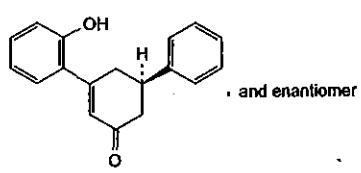
ASSAY
Dissolve 0.100 g in *0.01 M sodium hydroxide* and dilute to 100.0 mL with the same solvent. Dilute 10.0 mL of the solution to 100.0 mL with *0.01 M sodium hydroxide*. Dilute 10.0 mL of this solution to 100.0 mL with *0.01 M sodium hydroxide*. Measure the absorbance (*2.2.25*) at the absorption maximum at 308 nm.

Calculate the percentage content of warfarin sodium ($C_{19}H_{15}NaO_4$) taking the specific absorbance to be 431.

STORAGE
In an airtight container, protected from light.

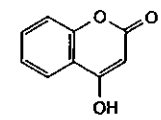
IMPURITIES
Specified impurities *B, C.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A.*

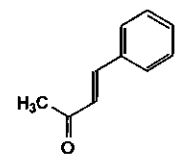


and enantiomer

A. (5*RS*)-3-(2-hydroxyphenyl)-5-phenylcyclohex-2-enone,



B. 4-hydroxy-2*H*-1-benzopyran-2-one (4-hydroxycoumarin),



C. (3*E*)-4-phenylbut-3-en-2-one (benzalacetone).

Ph Eur

Purified Water

(Ph. Eur. monograph 0008)

H_2O 18.02

Ph Eur

DEFINITION
Water for the preparation of medicines other than those that are required to be both sterile and apyrogenic, unless otherwise justified and authorised.

PURIFIED WATER IN BULK

PRODUCTION
Purified water in bulk is prepared by distillation, by ion exchange, by reverse osmosis or by any other suitable method from water that complies with the regulations on water intended for human consumption laid down by the competent authority.

Purified water in bulk is stored and distributed in conditions designed to prevent growth of micro-organisms and to avoid any other contamination.

Microbiological monitoring
During production and subsequent storage, appropriate measures are taken to ensure that the microbial count is adequately controlled and monitored. Appropriate alert and action levels are set so as to detect adverse trends. Under normal conditions, an appropriate action level is a microbial count of 100 CFU/mL, determined by filtration through a membrane with a nominal pore size not greater than 0.45 µm, using R2A agar and incubating at 30-35 °C for not less than 5 days. The size of the sample is to be chosen in relation to the expected result.

R2A agar

Yeast extract	0.5 g
Proteose peptone	0.5 g
Casein hydrolysate	0.5 g
Glucose	0.5 g
Starch	0.5 g
Dipotassium hydrogen phosphate	0.3 g
Magnesium sulfate, anhydrous	0.024 g
Sodium pyruvate	0.3 g
Agar	15.0 g
Purified water	to 1000 mL

Adjust the pH so that after sterilisation it is 7.2 ± 0.2.
Sterilise by heating in an autoclave at 121 °C for 15 min.

Growth promotion of R2A agar

— *Preparation of test strains.* Use standardised stable suspensions of test strains or prepare them as stated in Table 0008.-1. Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable micro-organisms used for inoculation are not more than 5 passages removed from the original master seed-lot. Grow each of the bacterial strains separately as described in Table 0008.-1. Use buffered sodium chloride-peptone solution pH 7.0 or phosphate buffer solution pH 7.2 to make test suspensions. Use the suspensions within 2 h, or within 24 h if stored at 2-8 °C. As an alternative to preparing and then diluting a fresh suspension of vegetative cells of *Bacillus subtilis*, a stable spore suspension is prepared and then an appropriate volume of the spore suspension is used for test inoculation. The stable spore suspension may be maintained at 2-8 °C for a validated period of time.

— *Growth promotion.* Test each batch of ready-prepared medium and each batch of medium, prepared either from dehydrated medium or from the ingredients described. Inoculate plates of R2A agar separately with a small number (not more than 100 CFU) of the micro-organisms indicated in Table 0008.-1. Incubate under the conditions described in the table. Growth obtained must not differ by a factor greater than 2 from the calculated value for a standardised inoculum. For a freshly prepared inoculum, growth of the micro-organisms must be comparable to that obtained with a previously tested and approved batch of medium.

Table 0008.-1. – *Growth promotion of R2A agar*

Micro-organism	Preparation of the test strain	Growth promotion
Pseudomonas aeruginosa such as: ATCC 9027 NCIMB 8626 CIP 82.118 NBRC 13275	Casein soyabean digest agar or casein soyabean digest broth 30-35 °C 18-24 h	R2A agar ≤ 100 CFU 30-35 °C ≤ 3 days
Bacillus subtilis such as: ATCC 6633 NCIMB 8054 CIP 52.62 NBRC 3134	Casein soyabean digest agar or casein soyabean digest broth 30-35 °C 18-24 h	R2A agar ≤ 100 CFU 30-35 °C ≤ 3 days

Total organic carbon or oxidisable substances

Carry out the test for total organic carbon (*2.2.44*) with a limit of 0.5 mg/L or alternatively the following test for oxidisable substances: to 100 mL add 10 mL of *dilute sulfuric acid R* and 0.1 mL of *0.02 M potassium permanganate* and boil for 5 min; the solution remains faintly pink.

Conductivity

Determine the conductivity off-line or in-line under the following conditions.

EQUIPMENT

Conductivity cell:

— electrodes of a suitable material such as stainless steel;

— cell constant: the cell constant is generally certified by the supplier and is subsequently verified at suitable intervals using a certified reference solution with a conductivity less than 1500 $\mu S \cdot cm^{-1}$ or by comparison with a cell having a certified cell constant; the cell constant is confirmed if the value found is within 2 per cent of the certified value, otherwise re-calibration must be performed.

Conductometer Accuracy of 0.1 $\mu S \cdot cm^{-1}$ or better at the lowest range.

System calibration (conductivity cell and conductometer):

— against one or more suitable certified reference solutions;

— accuracy: within 3 per cent of the measured conductivity plus 0.1 $\mu S \cdot cm^{-1}$.

Conductometer calibration Calibration is carried out for each range of measurement to be used, after disconnection of the conductivity cell, using certified precision resistors or equivalent devices with an uncertainty not greater than 0.1 per cent of the certified value.

If in-line conductivity cells cannot be dismantled, system calibration may be performed against a calibrated conductivity-measuring instrument with a conductivity cell placed close to the cell to be calibrated in the water flow.

Temperature measurement Tolerance ± 2 °C.

PROCEDURE

Measure the conductivity without temperature compensation, recording simultaneously the temperature. Temperature-compensated measurement may be performed after suitable validation.

The water to be examined meets the requirements if the measured conductivity at the recorded temperature is not greater than the value in Table 0008.-2.

Table 0008.-2. – *Temperature and conductivity requirements*

Temperature (°C)	Conductivity ($\mu S \cdot cm^{-1}$)
0	2.4
10	3.6
20	4.3
25	5.1
30	5.4
40	6.5
50	7.1
60	8.1
70	9.1
75	9.7
80	9.7
90	9.7
100	10.2

For temperatures not listed in Table 0008.-2, calculate the maximal permitted conductivity by interpolation between the next lower and next higher data points in the table.

Elemental impurities

If purified water in bulk does not meet the requirement for conductivity prescribed for *Water for injections (0169)* in bulk, a risk assessment according to general chapter *5.20. Elemental impurities* is carried out. The risk assessment should consider the role of water in the manufacturing process, in particular when water is used in a process but is no longer present in the final product.

CHARACTERS

Appearance

Clear and colourless liquid.

TESTS

Nitrates

Maximum 0.2 ppm.

Place 5 mL in a test-tube immersed in iced water, add 0.4 mL of a 100 g/L solution of *potassium chloride R*, 0.1 mL

of *diphenylamine solution R* and, dropwise with shaking, 5 mL of *nitrogen-free sulfuric acid R*. Transfer the tube to a water-bath at 50 °C. After 15 min, any blue colour in the solution is not more intense than that in a reference solution prepared at the same time in the same manner using a mixture of 4.5 mL of *nitrate-free water R* and 0.5 mL of *nitrate standard solution (2 ppm NO_3) R*.

Aluminium (*2.4.17*)
Maximum 10 ppb, if intended for use in the manufacture of dialysis solutions.

Prescribed solution To 400 mL of the water to be examined add 10 mL of *acetate buffer solution pH 6.0 R* and 100 mL of *distilled water R*.

Reference solution Mix 2 mL of *aluminium standard solution (2 ppm Al) R*, 10 mL of *acetate buffer solution pH 6.0 R* and 98 mL of *distilled water R*.

Blank solution Mix 10 mL of *acetate buffer solution pH 6.0 R* and 100 mL of *distilled water R*.

Bacterial endotoxins (*2.6.14*)
Less than 0.25 IU/mL, if intended for use in the manufacture of dialysis solutions without a further appropriate procedure for removal of bacterial endotoxins.

LABELLING
The label states, where applicable, that the substance is suitable for use in the manufacture of dialysis solutions.

PURIFIED WATER IN CONTAINERS

DEFINITION
Purified water in bulk that has been filled and stored in conditions designed to assure the required microbiological quality. It is free from any added substances.

CHARACTERS
Appearance
Clear and colourless liquid.

TESTS
It complies with the tests prescribed in the section on Purified water in bulk and with the following additional tests.

Acidity or alkalinity
To 10 mL, freshly boiled and cooled in a borosilicate glass flask, add 0.05 mL of *methyl red solution R*. The solution is not coloured red.

To 10 mL add 0.1 mL of *bromothymol blue solution R1*. The solution is not coloured blue.

Oxidisable substances
To 100 mL add 10 mL of *dilute sulfuric acid R* and 0.1 mL of *0.02 M potassium permanganate* and boil for 5 min. The solution remains faintly pink.

Chlorides
To 10 mL add 1 mL of *dilute nitric acid R* and 0.2 mL of *silver nitrate solution R2*. The solution shows no change in appearance for at least 15 min.

Sulfates
To 10 mL add 0.1 mL of *dilute hydrochloric acid R* and 0.1 mL of *barium chloride solution R1*. The solution shows no change in appearance for at least 1 h.

Ammonium
Maximum 0.2 ppm.

To 20 mL add 1 mL of *alkaline potassium tetraiodomercurate solution R*. After 5 min, examine the solution down the vertical axis of the tube. The solution is not more intensely coloured than a standard prepared at the same time by adding 1 mL of *alkaline potassium tetraiodomercurate solution R* to a mixture of 4 mL of *ammonium standard solution (1 ppm NH_4) R* and 16 mL of *ammonium-free water R*.

Calcium and magnesium
To 100 mL add 2 mL of *ammonium chloride buffer solution pH 10.0 R*, 50 mg of *mordant black 11 triturate R* and 0.5 mL of *0.01 M sodium edetate*. A pure blue colour is produced.

Residue on evaporation
Maximum 0.001 per cent.

Evaporate 100 mL to dryness on a water-bath and dry in an oven at 100-105 °C. The residue weighs a maximum of 1 mg.

Microbial contamination
TAMC: acceptance criterion 10^2 CFU/mL (*2.6.12*).
Use casein soya bean digest agar.

LABELLING
The label states, where applicable, that the substance is suitable for use in the manufacture of dialysis solutions.

Ph Eur

Water for Injections

(Ph. Eur. monograph 0169)

H_2O 18.02

Ph Eur

DEFINITION
Water for the preparation of medicines for parenteral administration when water is used as vehicle (water for injections in bulk) and for dissolving or diluting substances or preparations for parenteral administration (sterilised water for injections).

WATER FOR INJECTIONS IN BULK

PRODUCTION
Water for injections in bulk is obtained from water that complies with the regulations on water intended for human consumption laid down by the competent authority or from purified water. It is produced either:
- by distillation in an apparatus of which the parts in contact with the water are of neutral glass, quartz or a suitable metal and which is fitted with an effective device to prevent the entrainment of droplets; or
- by a purification process that is equivalent to distillation. Reverse osmosis, which may be single-pass or double-pass, coupled with other appropriate techniques such as electro-deionisation, ultrafiltration or nanofiltration, is suitable. Notice is given to the supervisory authority of the manufacturer before implementation.

For all methods of production, correct operation monitoring and maintenance of the system are essential. In order to ensure the appropriate quality of the water, validated procedures, in-process monitoring of the electrical conductivity, and regular monitoring of total organic carbon and microbial contamination are applied.

The first portion of water obtained when the system begins to function is discarded.

Water for injections in bulk is stored and distributed in conditions designed to prevent growth of micro-organisms and to avoid any other contamination.

Microbiological monitoring
During production and subsequent storage, appropriate measures are taken to ensure that the microbial count is adequately controlled and monitored. Appropriate alert and action levels are set so as to detect adverse trends. Under normal conditions, an appropriate action level is a microbial count of 10 CFU per 100 mL when determined by filtration through a membrane with a nominal pore size not greater than 0.45 µm, using R2A agar, using at least 200 mL of water for injections in bulk and incubating at 30-35 °C for not less than 5 days. For aseptic processing, stricter alert levels may need to be applied.

R2A agar

Yeast extract	0.5 g
Proteose peptone	0.5 g
Casein hydrolysate	0.5 g
Glucose	0.5 g
Starch	0.5 g
Dipotassium hydrogen phosphate	0.3 g
Magnesium sulfate, anhydrous	0.024 g
Sodium pyruvate	0.3 g
Agar	15.0 g
Purified water	to 1000 mL

Adjust the pH so that after sterilisation it is 7.2 ± 0.2. Sterilise by heating in an autoclave at 121 °C for 15 min.

Growth promotion of R2A agar

— *Preparation of test strains.* Use standardised stable suspensions of test strains or prepare them as stated in Table 0169.-1. Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable micro-organisms used for inoculation are not more than 5 passages removed from the original master seed-lot. Grow each of the bacterial strains separately as described in Table 0169.-1. Use buffered sodium chloride-peptone solution pH 7.0 or phosphate buffer solution pH 7.2 to make test suspensions. Use the suspensions within 2 h, or within 24 h if stored at 2-8 °C. As an alternative to preparing and then diluting a fresh suspension of vegetative cells of *Bacillus subtilis*, a stable spore suspension is prepared and then an appropriate volume of the spore suspension is used for test inoculation. The stable spore suspension may be maintained at 2-8 °C for a validated period of time.

— *Growth promotion.* Test each batch of ready-prepared medium and each batch of medium, prepared either from dehydrated medium or from the ingredients described. Inoculate plates of R2A agar separately with a small number (not more than 100 CFU) of the micro-organisms indicated in Table 0169.-1. Incubate under the conditions described in the table. Growth obtained must not differ by a factor greater than 2 from the calculated value for a standardised inoculum. For a freshly prepared inoculum, growth of the micro-organisms must be comparable to that obtained with a previously tested and approved batch of medium.

Total organic carbon (*2.2.44*)
Maximum 0.5 mg/L.

Conductivity
Determine the conductivity off-line or in-line under the following conditions.

Table 0169.-1. – *Growth promotion of R2A agar*

Micro-organism	Preparation of the test strain	Growth promotion
Pseudomonas aeruginosa such as: ATCC 9027 NCIMB 8626 CIP 82.118 NBRC 13275	Casein soyabean digest agar or casein soyabean digest broth 30-35 °C 18-24 h	R2A agar ≤ 100 CFU 30-35 °C ≤ 3 days
Bacillus subtilis such as: ATCC 6633 NCIMB 8054 CIP 52.62 NBRC 3134	Casein soyabean digest agar or casein soyabean digest broth 30-35 °C 18-24 h	R2A agar ≤ 100 CFU 30-35 °C ≤ 3 days

EQUIPMENT

Conductivity cell:

— electrodes of a suitable material such as stainless steel;

— cell constant: the cell constant is generally certified by the supplier and is subsequently verified at suitable intervals using a certified reference solution with a conductivity less than 1500 $\mu S \cdot cm^{-1}$ or by comparison with a cell having a certified cell constant. The cell constant is confirmed if the value found is within 2 per cent of the certified value, otherwise re-calibration must be performed.

Conductometer Accuracy of 0.1 $\mu S \cdot cm^{-1}$ or better at the lowest range.

System calibration (conductivity cell and conductometer):

— against one or more suitable certified reference solutions;

— accuracy: within 3 per cent of the measured conductivity plus 0.1 $\mu S \cdot cm^{-1}$.

Conductometer calibration Calibration is carried out for each range of measurement to be used, after disconnection of the conductivity cell, using certified precision resistors or equivalent devices with an uncertainty not greater than 0.1 per cent of the certified value.

If in-line conductivity cells cannot be dismantled, system calibration may be performed against a calibrated conductivity-measuring instrument with a conductivity cell placed close to the cell to be calibrated in the water flow.

Temperature measurement Tolerance ± 2 °C.

PROCEDURE

Stage 1

1. Measure the conductivity without temperature compensation, recording simultaneously the temperature. Temperature-compensated measurement may be performed after suitable validation.

2. Using Table 0169.-2, find the closest temperature value that is not greater than the measured temperature. The corresponding conductivity value is the limit at that temperature.

3. If the measured conductivity is not greater than the value in Table 0169.-2, the water to be examined meets the requirements of the test for conductivity. If the conductivity is higher than the value in Table 0169.-2, proceed with stage 2.

Table 0169.-2. – *Stage 1 Temperature and conductivity requirements (for non-temperature-compensated conductivity measurements)*

Temperature (°C)	Conductivity ($\mu S \cdot cm^{-1}$)
0	0.6
5	0.8
10	0.9
15	1.0
20	1.1
25	1.3
30	1.4
35	1.5
40	1.7
45	1.8
50	1.9
55	2.1
60	2.2
65	2.4
70	2.5
75	2.7
80	2.7
85	2.7
90	2.7
95	2.9
100	3.1

Stage 2

4. Transfer a sufficient amount of the water to be examined (100 mL or more) to a suitable container, and stir the test sample. Adjust the temperature, if necessary, and while maintaining it at 25 ± 1 °C, begin vigorously agitating the test sample while periodically observing the conductivity. When the change in conductivity (due to uptake of atmospheric carbon dioxide) is less than 0.1 $\mu S.cm^{-1}$ per 5 min, note the conductivity.

5. If the conductivity is not greater than 2.1 $\mu S.cm^{-1}$, the water to be examined meets the requirements of the test for conductivity. If the conductivity is greater than 2.1 $\mu S.cm^{-1}$, proceed with stage 3.

Stage 3

6. Perform this test within approximately 5 min of the conductivity determination in step 5 under stage 2, while maintaining the sample temperature at 25 ± 1 °C. Add a recently prepared saturated solution of *potassium chloride R* to the test sample (0.3 mL per 100 mL of the test sample), and determine the pH (*2.2.3*) to the nearest 0.1 pH unit.

7. Using Table 0169.-3, determine the conductivity limit at the measured pH value in step 6. If the measured conductivity in step 4 under stage 2 is not greater than the conductivity requirements for the pH determined, the water to be examined meets the requirements of the test for conductivity. If either the measured conductivity is greater than this value or the pH is outside the range of 5.0-7.0, the water to be examined does not meet the requirements of the test for conductivity.

Table 0169.-3. – *Stage 3 pH and conductivity requirements (for atmosphere- and temperature-equilibrated samples)*

pH	Conductivity ($\mu S \cdot cm^{-1}$)
5.0	4.7
5.1	4.1
5.2	3.6
5.3	3.3
5.4	3.0
5.5	2.8
5.6	2.6
5.7	2.5
5.8	2.4
5.9	2.4
6.0	2.4
6.1	2.4
6.2	2.5
6.3	2.4
6.4	2.3
6.5	2.2
6.6	2.1
6.7	2.6
6.8	3.1
6.9	3.8
7.0	4.6

CHARACTERS

Appearance

Clear and colourless liquid.

TESTS

Nitrates

Maximum 0.2 ppm.

Place 5 mL in a test-tube immersed in iced water, add 0.4 mL of a 100 g/L solution of *potassium chloride R*, 0.1 mL of *diphenylamine solution R* and, dropwise with shaking, 5 mL of *nitrogen-free sulfuric acid R*. Transfer the tube to a water-bath at 50 °C. After 15 min, any blue colour in the solution is not more intense than that in a reference solution prepared at the same time in the same manner using a mixture of 4.5 mL of *nitrate-free water R* and 0.5 mL of *nitrate standard solution (2 ppm NO_3) R*.

Aluminium (*2.4.17*)

Maximum 10 ppb, if intended for use in the manufacture of dialysis solutions.

Prescribed solution To 400 mL of the water to be examined add 10 mL of *acetate buffer solution pH 6.0 R* and 100 mL of *distilled water R*.

Reference solution Mix 2 mL of *aluminium standard solution (2 ppm Al) R*, 10 mL of *acetate buffer solution pH 6.0 R* and 98 mL of *distilled water R*.

Blank solution Mix 10 mL of *acetate buffer solution pH 6.0 R* and 100 mL of *distilled water R*.

Bacterial endotoxins (*2.6.14*)

Less than 0.25 IU/mL.

STERILISED WATER FOR INJECTIONS

DEFINITION

Water for injections in bulk that has been distributed into suitable containers, closed and sterilised by heat in conditions which ensure that the product still complies with the test for bacterial endotoxins. Sterilised water for injections is free from any added substances.

Examined in suitable conditions of visibility, it is clear and colourless.

Each container contains a sufficient quantity of water for injections to permit the nominal volume to be withdrawn.

TESTS

Acidity or alkalinity

To 20 mL add 0.05 mL of *phenol red solution R*. If the solution is yellow, it becomes red on the addition of 0.1 mL of *0.01 M sodium hydroxide*; if red, it becomes yellow on the addition of 0.15 mL of *0.01 M hydrochloric acid*.

Conductivity

Maximum 25 $\mu S \cdot cm^{-1}$ for containers with a nominal volume of 10 mL or less; maximum 5 $\mu S \cdot cm^{-1}$ for containers with a nominal volume greater than 10 mL.

Use equipment and the calibration procedure as defined under Water for injections in bulk, maintaining the sample temperature at 25 ± 1 °C.

Oxidisable substances

For containers with a nominal volume less than 50 mL: heat 100 mL to boiling with 10 mL of *dilute sulfuric acid R*, add 0.4 mL of *0.02 M potassium permanganate* and boil for 5 min; the solution remains faintly pink.

For containers with a nominal volume equal to or greater than 50 mL: heat 100 mL to boiling with 10 mL of *dilute sulfuric acid R*, add 0.2 mL of *0.02 M potassium permanganate* and boil for 5 min; the solution remains faintly pink.

Chlorides (*2.4.4*)

Maximum 0.5 ppm for containers with a nominal volume of 100 mL or less.

15 mL complies with the limit test for chlorides. Prepare the standard using a mixture of 1.5 mL of *chloride standard solution (5 ppm Cl) R* and 13.5 mL of *water R*. Examine the solutions down the vertical axes of the tubes.

For containers with a nominal volume greater than 100 mL, use the following test: to 10 mL add 1 mL of *dilute nitric acid R* and 0.2 mL of *silver nitrate solution R2*. The solution shows no change in appearance for at least 15 min.

Nitrates

Maximum 0.2 ppm.

Place 5 mL in a test-tube immersed in iced water, add 0.4 mL of a 100 g/L solution of *potassium chloride R*, 0.1 mL of *diphenylamine solution R* and, dropwise with shaking, 5 mL of *nitrogen-free sulfuric acid R*. Transfer the tube to a water-bath at 50 °C. After 15 min, any blue colour in the solution is not more intense than that in a reference solution prepared at the same time in the same manner using a mixture of 4.5 mL of *nitrate-free water R* and 0.5 mL of *nitrate standard solution (2 ppm NO_3) R*.

Sulfates

To 10 mL add 0.1 mL of *dilute hydrochloric acid R* and 0.1 mL of *barium chloride solution R1*. The solution shows no change in appearance for at least 1 h.

Aluminium (*2.4.17*)

Maximum 10 ppb, if intended for use in the manufacture of dialysis solutions.

Prescribed solution To 400 mL of the water to be examined add 10 mL of *acetate buffer solution pH 6.0 R* and 100 mL of *distilled water R*.

Reference solution Mix 2 mL of *aluminium standard solution (2 ppm Al) R*, 10 mL of *acetate buffer solution pH 6.0 R* and 98 mL of *distilled water R*.

Blank solution Mix 10 mL of *acetate buffer solution pH 6.0 R* and 100 mL of *distilled water R*.

Ammonium

For containers with a nominal volume less than 50 mL: maximum 0.6 ppm; for containers with a nominal volume equal to or greater than 50 mL: maximum 0.2 ppm.

Containers with a nominal volume less than 50 mL: to 20 mL add 1 mL of *alkaline potassium tetraiodomercurate solution R*; after 5 min, examine the solution down the vertical axis of the tube; the solution is not more intensely coloured than a standard prepared at the same time by adding 1 mL of *alkaline potassium tetraiodomercurate solution R* to a mixture of 4 mL of *ammonium standard solution (3 ppm NH_4) R* and 16 mL of *ammonium-free water R*.

Containers with a nominal volume equal to or greater than 50 mL: to 20 mL add 1 mL of *alkaline potassium tetraiodomercurate solution R*; after 5 min, examine the solution down the vertical axis of the tube; the solution is not more intensely coloured than a standard prepared at the same time by adding 1 mL of *alkaline potassium tetraiodomercurate solution R* to a mixture of 4 mL of *ammonium standard solution (1 ppm NH_4) R* and 16 mL of *ammonium-free water R*.

Calcium and magnesium

To 100 mL add 2 mL of *ammonium chloride buffer solution pH 10.0 R*, 50 mg of *mordant black 11 triturate R* and 0.5 mL of *0.01 M sodium edetate*. A pure blue colour is produced.

Residue on evaporation

Maximum 4 mg (0.004 per cent) for containers with a nominal volume of 10 mL or less; maximum 3 mg (0.003 per cent) for containers with a nominal volume greater than 10 mL.

Evaporate 100 mL to dryness on a water-bath and dry in an oven at 100-105 °C.

Particulate contamination: sub-visible particles (*2.9.19*)

It complies with test A or test B, as appropriate.

Sterility (*2.6.1*)

It complies with the test for sterility.

Bacterial endotoxins (*2.6.14*)

Less than 0.25 IU/mL.

Ph Eur

Water for Preparation of Extracts

(Ph. Eur. monograph 2249)

Ph Eur

DEFINITION

Water intended for the preparation of *Herbal drug extracts (0765)* complies with the sections Purified water in bulk or Purified water in containers in the monograph *Purified water (0008)*, or is water intended for human consumption of a quality equivalent to that defined in Directive 98/83/EC which is monitored according to the Production section described below.

PRODUCTION

When water intended for human consumption is used as water for preparation of extracts it is a clear, colourless liquid. It is stored (where necessary) and distributed under conditions designed to prevent growth of micro-organisms and to avoid other contamination.

For monitoring purposes, the following tests are carried out at regular intervals to demonstrate consistency in the quality of the water used for the preparation of extracts.

Conductivity (*2.2.38*)

Maximum 2500 $\mu S \cdot cm^{-1}$, measured at 20 °C.

Nitrate

Liquid chromatography (*2.2.29*).

Test solution The substance to be examined.

Reference solution Dissolve 0.163 g of *potassium nitrate R* and 0.149 g of *potassium bromide R* in *water R* and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of the solution to 100.0 mL with *water R*.

Column:
— *size*: l = 0.25 m, Ø = 4 mm;
— *stationary phase*: anion-exchange resin R3.

Mobile phase Dissolve 0.265 g of *anhydrous sodium carbonate R* and 0.210 g of *sodium hydrogen carbonate R* in *water R* and dilute to 1000.0 mL with the same solvent.

Flow rate 1.2 mL/min.

Detection Conductivity detector, using a self-regenerating anion suppressor.

Injection 25 µL.

Run time Twice the retention time of nitrate.

Relative retention With reference to nitrate (retention time = about 7 min): bromide = about 0.9.

System suitability Reference solution:
— *resolution*: minimum 2.0 between the peaks due to bromide and nitrate.

Limit:
— *nitrate*: maximum 50 ppm.

Microbiological monitoring

Appropriate measures are taken to ensure that the microbial count is adequately controlled and monitored. Appropriate alert and action levels are set so as to detect adverse trends.

Under normal conditions, an appropriate action level is a microbial count of 100 CFU/mL, determined by filtration through a membrane with a nominal pore size not greater than 0.45 µm, using casein soya bean digest agar and incubating at 30-35 °C for not less than 5 days.

The size of the sample is to be chosen in relation to the expected result.

Casein soya bean digest agar

Pancreatic digest of casein	15.0 g
Papaic digest of soya bean	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Purified water	to 1000 mL

Adjust the pH so that after sterilisation it is 7.3 ± 0.2. Sterilise in an autoclave using a validated cycle.

Growth promotion of casein soya bean digest agar

— *Preparation of test strains*. Use standardised stable suspensions of test strains or prepare them as stated in Table 2249.-1. Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable micro-organisms used for inoculation are not more than 5 passages removed from the original master seed-lot. Grow each of the bacterial strains separately as described in Table 2249.-1. Use buffered sodium chloride-peptone solution pH 7.0 or phosphate buffer solution pH 7.2 to make test suspensions. Use the suspensions within 2 h, or within 24 h if stored at 2-8 °C. As an alternative to preparing and then diluting a fresh suspension of vegetative cells of *Bacillus subtilis*, a stable spore suspension is prepared and then an appropriate volume of the spore suspension is used for test inoculation. The stable spore suspension may be maintained at 2-8 °C for a validated period of time.

— *Growth promotion*. Test each batch of ready-prepared medium and each batch of medium, prepared either from dehydrated medium or from the ingredients described. Inoculate plates of casein soya bean digest agar separately with a small number (not more than 100 CFU) of the micro-organisms indicated in Table 2249.-1. Incubate under the conditions described in this table. Growth obtained must not differ by a factor greater than 2 from the calculated value for a standardised inoculum. For a freshly prepared inoculum, growth of the micro-organisms must be comparable to that obtained with a previously tested and approved batch of medium.

Table 2249.-1. – *Growth promotion of casein soya bean digest agar*

Micro-organism	Preparation of the test strain	Growth promotion
Pseudomonas aeruginosa such as: ATCC 9027 NCIMB 8626 CIP 82.118 NBRC 13275	Casein soya bean digest agar or casein soya bean digest broth 30-35 °C 18-24 h	Casein soya bean digest agar ≤ 100 CFU 30-35 °C ≤ 3 days
Bacillus subtilis such as: ATCC 6633 NCIMB 8054 CIP 52.62 NBRC 3134	Casein soya bean digest agar or casein soya bean digest broth 30-35 °C 18-24 h	Casein soya bean digest agar ≤ 100 CFU 30-35 °C ≤ 3 days

Ph Eur

Virgin Wheat-germ Oil

(Ph. Eur. monograph 1480)

Ph Eur

DEFINITION

Fatty oil obtained from the germ of the grain of *Triticum aestivum* L. by cold expression or other suitable mechanical means.

PRODUCTION

The oil is prepared using materials and methods designed to ensure that the content of brassicasterol (*2.4.23*) in the sterol fraction of the oil is not greater than 0.3 per cent.

CHARACTERS

Appearance

Clear, light yellow or golden-yellow liquid.

Solubility

Practically insoluble in water and in ethanol (96 per cent), miscible with light petroleum (bp: 40-60 °C).

Relative density

About 0.925.

Refractive index

About 1.475.

IDENTIFICATION

First identification: B.

Second identification: A.

A. Identification of fatty oils by thin-layer chromatography (*2.3.2*).

Results The chromatogram obtained is similar to the corresponding chromatogram shown in Figure 2.3.2.-1.

B. Composition of fatty acids (see Tests).

TESTS

Acid value (*2.5.1*)
Maximum 20.0.

Peroxide value (*2.5.5, Method A*)
Maximum 15.0.

Unsaponifiable matter (*2.5.7*)
Maximum 5.0 per cent, determined on 5.0 g.

Composition of fatty acids (*2.4.22, Method C*)
Composition of the fatty-acid fraction of the oil:
— *palmitic acid*: 14.0 per cent to 19.0 per cent;
— *stearic acid*: maximum 2.0 per cent;
— *oleic acid*: 12.0 per cent to 23.0 per cent;
— *linoleic acid*: 52.0 per cent to 59.0 per cent;
— *linolenic acid*: 3.0 per cent to 10.0 per cent;
— *eicosenoic acid*: maximum 2.0 per cent.

Water (*2.5.32*)
Maximum 0.1 per cent, determined on 1.00 g.

STORAGE
In an airtight, well-filled container, protected from light.

Ph Eur

Refined Wheat-germ Oil

(Ph. Eur. monograph 1379)

Ph Eur

DEFINITION
Fatty oil obtained from the germ of the grain of *Triticum aestivum* L. by cold expression or by other suitable mechanical means and/or by extraction. It is then refined. A suitable antioxidant may be added.

PRODUCTION
The oil is prepared using materials and methods designed to ensure that the content of brassicasterol (*2.4.23*) in the sterol fraction of the oil is not greater than 0.3 per cent.

CHARACTERS

Appearance
Clear, light yellow liquid.

Solubility
Practically insoluble in water and in ethanol (96 per cent), miscible with light petroleum (bp: 40-60 °C).

Relative density
About 0.925.

Refractive index
About 1.475.

IDENTIFICATION
First identification: B.

Second identification: A.

A. Identification of fatty oils by thin-layer chromatography (*2.3.2*).

Results The chromatogram obtained is similar to the corresponding chromatogram shown in Figure 2.3.2.-1.

B. Composition of fatty acids (see Tests).

TESTS

Acid value (*2.5.1*)
Maximum 0.9, or maximum 0.3 if intended for use in the manufacture of parenteral preparations.

Peroxide value (*2.5.5, Method A*)
Maximum 10.0, or maximum 5.0 if intended for use in the manufacture of parenteral preparations.

Unsaponifiable matter (*2.5.7*)
Maximum 5.0 per cent, determined on 5.0 g.

Alkaline impurities (*2.4.19*)
It complies with the test.

Composition of fatty acids (*2.4.22, Method C*)
Use the mixture of calibrating substances in Table 2.4.22.-3.
Composition of the fatty-acid fraction of the oil:
— *palmitic acid*: 14.0 per cent to 19.0 per cent;
— *stearic acid*: maximum 2.0 per cent;
— *oleic acid*: 12.0 per cent to 23.0 per cent;
— *linoleic acid*: 52.0 per cent to 59.0 per cent;
— *linolenic acid*: 3.0 per cent to 10.0 per cent;
— *eicosenoic acid*: maximum 2.0 per cent.

Water (*2.5.32*)
Maximum 0.1 per cent, determined on 1.00 g.

STORAGE
In an airtight, well-filled container, protected from light.

LABELLING
The label states:
— where applicable, that the substance is suitable for use in the manufacture of parenteral preparations;
— whether the oil is obtained by mechanical means, by extraction or by a combination of the 2.

Ph Eur

Wool Alcohols

Wool Wax Alcohols

(Ph. Eur. monograph 0593)

Preparation
Wool Alcohols Ointment

Ph Eur

DEFINITION
Mixture of sterols and higher aliphatic alcohols from wool fat. A suitable antioxidant may be added.

Content
Minimum 30.0 per cent of cholesterol.

CHARACTERS

Appearance
Pale-yellow or brownish-yellow, brittle mass becoming plastic on heating.

Solubility
Practically insoluble in water, soluble in boiling anhydrous ethanol and in methylene chloride, slightly soluble in ethanol (90 per cent *V/V*).

IDENTIFICATION
Dissolve 50 mg in 5 mL of *methylene chloride R* and add 1 mL of *acetic anhydride R* and 0.1 mL of *sulfuric acid R*. Within a few seconds, a green colour develops.

TESTS

Appearance of solution
To 1.0 g add 10 mL of *light petroleum R1* and shake while warming in a water-bath. The substance dissolves completely. After cooling, the solution is clear (*2.2.1*).

Alkalinity
Dissolve 2.0 g in 25 mL of hot *ethanol (90 per cent V/V) R* and add 0.5 mL of *phenolphthalein solution R1*. No red colour develops.

Melting point (*2.2.15*)
Minimum 56 °C.

Melt the substance to be examined by heating in a water-bath at a temperature which exceeds the expected melting point by not more than 10 °C; introduce the substance to be examined into the capillary tubes and allow to stand on ice for at least 2 h.

Water-absorption capacity
Place 0.6 g of the substance to be examined and 9.4 g of *white soft paraffin R* in a mortar and melt on a water-bath. Allow to cool and incorporate 20 mL of *water R*, added in portions. Within 24 h no water is released from the almost white, ointment-like emulsion.

Acid value (*2.5.1*)
Maximum 2.0.

If necessary, heat in a water-bath under a reflux condenser to dissolve the substance to be examined.

Hydroxyl value (*2.5.3, Method A*)
120 to 180.

Peroxide value (*2.5.5, Method A*)
Maximum 15.

Take from the substance to be examined wedge-shaped pieces whose base consists of part of the surface. Melt the pieces before carrying out the determination. Before adding 0.5 mL of *saturated potassium iodide solution R*, cool the solution obtained to room temperature.

Saponification value (*2.5.6*)
Maximum 12.0, determined on 10.00 g. Heat under reflux for 4 h.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 2.000 g by drying in an oven at 105 °C.

Total ash (*2.4.16*)
Maximum 0.1 per cent.

ASSAY
Gas chromatography (*2.2.28*). *Homogenise the sample before use.*

Internal standard solution Dissolve 0.125 g of *pregnenolone isobutyrate CRS* in *heptane R* and dilute to 50.0 mL with the same solvent.

Test solution Dissolve 75.0 mg of the substance to be examined in 10.0 mL of the internal standard solution and dilute to 25.0 mL with *heptane R*.

Reference solution Dissolve 25.0 mg of *cholesterol CRS* in 10.0 mL of the internal standard solution and dilute to 25.0 mL with *heptane R*.

Injection liner:
— *packing material*: quartz wool;
— *size*: *l* = 78.5 mm, Ø = 4.0 mm.

Column:
— *material*: fused silica;
— *size*: *l* = 30 m, Ø = 0.25 mm;
— *stationary phase*: *methylpolysiloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R*.

Flow rate 1 mL/min.

Split ratio 1:50.

Temperature:
— *column*: 275 °C;
— *injection port*: 285 °C;
— *detector*: 300 °C.

Detection Flame ionisation.

Injection 1 µL.

Relative retention With reference to pregnenolone isobutyrate (retention time = about 8 min): cholesterol = about 1.2.

System suitability Reference solution:
— *resolution*: minimum 5.0 between the peaks due to pregnenolone isobutyrate and cholesterol.

Calculate the percentage content of cholesterol in the substance to be examined taking into account the assigned content of *cholesterol CRS*.

STORAGE
In a well-filled container, protected from light.

FUNCTIONALITY-RELATED CHARACTERISTICS
This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for wool alcohols used in water-emulsifying ointments and lipophilic creams.

Melting point
(see Tests).

Water-absorption capacity
(see Tests).

Ph Eur

Wool Fat

Anhydrous Lanolin

(Ph. Eur. monograph 0134)

Ph Eur

DEFINITION
Purified, anhydrous, waxy substance obtained from the wool of sheep (*Ovis aries*). A suitable antioxidant may be added.

CHARACTERS
Appearance
Yellow, unctuous substance. When melted, it is a clear or almost clear, yellow liquid. A solution in light petroleum is opalescent.

Solubility
Practically insoluble in water, slightly soluble in boiling anhydrous ethanol.

Characteristic odour.

IDENTIFICATION
A. In a test-tube, dissolve 0.5 g in 5 mL of *methylene chloride R* and add 1 mL of *acetic anhydride R* and 0.1 mL of *sulfuric acid R*. A green colour develops.

B. Dissolve 50 mg in 5 mL of *methylene chloride R*, add 5 mL of *sulfuric acid R* and shake. A red colour develops and an intense green fluorescence appears in the lower layer when examined in daylight, with illumination from behind the observer.

TESTS
Water-soluble acid or alkaline substances
Melt 5.0 g on a water-bath and shake vigorously for 2 min with 75 mL of *water R* previously heated to 90-95 °C. Allow

to cool and filter through filter paper previously rinsed with *water R*. To 60 mL of the filtrate (which may not be clear) add 0.25 mL of *bromothymol blue solution R1*. Not more than 0.2 mL of *0.02 M hydrochloric acid* or 0.15 mL of *0.02 M sodium hydroxide* is required to change the colour of the indicator.

Water-absorption capacity

Place 10 g of molten wool fat in a mortar and allow to cool to room temperature. Weigh the mortar. Add *water R* in portions of 0.2-0.5 mL from a burette, stirring vigorously after each addition to incorporate the *water R*. Instead of a pestle, use a high-density polypropylene cylindrical rod (120 mm long and 10 mm in diameter, for example). The end-point is reached when visible droplets remain which cannot be incorporated. Weigh the mortar again and determine the amount of water absorbed by weight difference. Not less than 20 mL of *water R* is absorbed.

Acid value (*2.5.1*)

Maximum 1.0, determined on 5.0 g dissolved in 25 mL of the prescribed mixture of solvents.

Peroxide value (*2.5.5, Method A*)

Maximum 20.

Before adding 0.5 mL of *saturated potassium iodide solution R*, cool the solution obtained to room temperature.

Saponification value (*2.5.6*)

90 to 105, determined on 2.00 g while heating under reflux for 4 h.

Water-soluble oxidisable substances

To 10 mL of the filtrate obtained in the test for water-soluble acid or alkaline substances add 1 mL of *dilute sulfuric acid R* and 0.1 mL of *0.02 M potassium permanganate*. After 10 min, the solution is not completely decolourised.

Paraffins

Maximum 1.0 per cent.

The tap and cotton plugs used must be free from grease Prepare a column of anhydrous aluminium oxide 0.23 m long and 20 mm in diameter by adding a slurry of *anhydrous aluminium oxide R* and *light petroleum R1* to a glass tube fitted with a tap and containing *light petroleum R1* (before use, dehydrate the anhydrous aluminium oxide by heating it in an oven at 600 °C for 3 h). Allow to settle and reduce the depth of the layer of solvent above the column to about 40 mm. Dissolve 3.0 g of the substance to be examined in 50 mL of warm *light petroleum R1*, cool, pass the solution through the column at a flow rate of 3 mL/min and wash with 250 mL of *light petroleum R1*. Concentrate the combined eluate and washings to low bulk by distillation, evaporate to dryness on a water-bath and heat the residue at 105 °C for periods of 10 min until 2 successive weighings do not differ by more than 1 mg. The residue weighs a maximum of 30 mg.

Pesticide residues

Maximum 0.05 ppm for each organochlorine pesticide, 0.5 ppm for each other pesticide and 1 ppm for the sum of all the pesticides.

All glassware used is thoroughly washed using phosphate-free detergent as follows. The glassware is immersed in a bath of detergent solution (5 per cent in deionised water) and allowed to soak for 24 h. The detergent is washed off with copious amounts of acetone and hexane for pesticide analysis. It is important to keep glassware specifically for pesticide analyses, it must not be mixed up with glassware used for other applications. The glassware used must be free of chlorinated solvents, plastics and rubber materials, in particular phthalate plasticisers, oxygenated compounds and nitrogenated solvents such as acetonitrile. Use hexane, toluene and acetone for pesticide analysis. Use HPLC grade reagents for ethyl acetate, cyclohexane and water.

The test consists of the isolation of pesticide residues by size-exclusion chromatography (*2.2.30*) followed by solid phase extraction and identification by gas chromatography coupled with an electron capture detector or a thermionic detector.

ISOLATION OF THE PESTICIDE RESIDUES

As detector, use a UV-visible spectrophotometer set at a wavelength of 254 nm to calibrate the chromatographic column for gel permeation.

Calibration is extremely important in gel permeation chromatography (GPC) to check that the pressure, solvent flow rate, solvent ratio, temperature and column conditions remain constant. The gel permeation column is to be calibrated at regular intervals using a standard mixture prepared as follows: into a 1000 mL volumetric flask, introduce 50.00 g of *maize oil R*, 0.20 g of *methoxychlor R* and 50.0 mg of *perylene R*. Dilute to 1000.0 mL with a mixture of equal volumes of *cyclohexane R* and *ethyl acetate R*.

To calibrate the column, set the mobile phase at a flow rate of 5 mL/min with a mixture of equal volumes of *cyclohexane R* and *ethyl acetate R*. Inject 5 mL of the standard mixture and record the resulting chromatogram.

The retention times for the analytes must not vary by more than ± 5 per cent between calibrations. If the retention time shift is greater than ± 5 per cent, take corrective action. Excessive retention time shifts may be caused by:

— poor laboratory temperature control;
— the pump containing air; this can be verified by measuring the flow rate: collect 25 mL of column eluate in a volumetric flask and record the time (300 ± 5 s);
— a leak in the system.

Changes in pressure, in mobile phase flow rate or in column temperature conditions, as well as column contamination, can affect pesticide retention times and are to be monitored. If the flow rate or column pressure are outside desired bands the precolumn or column is to be replaced.

Test solution In a volumetric flask, dissolve 1 g of the substance to be examined, accurately weighed, in a mixture of 1 volume of *ethyl acetate R* and 7 volumes of *cyclohexane R*. Add 1 mL of an internal standard (2 ppm, either *isodrin R* or *ditalimphos R*) and dilute to 20 mL. *The internal standard solutions are used to establish that recoveries of the pesticides from the GPC purification stage, evaporation and solid phase extraction stage are at acceptable levels. Recovery levels of the internal standard solutions from the wool fat are determined by comparing the peak areas of the wool fat extracts with peak areas of solutions of the internal standards.*

Precolumn:

— *size*: *l* = 0.075 m, Ø = 21.2 mm;
— *stationary phase*: *styrene-divinylbenzene copolymer R* (5 µm).

Gel permeation column:

— *size*: *l* = 0.3 m, Ø = 21.2 mm;
— *stationary phase*: *styrene-divinylbenzene copolymer R* (5 µm).

Mobile phase *ethyl acetate R, cyclohexane R* (10:70 *V/V*).

Flow rate 5 mL/min.

Detection Spectrophotometer at 254 nm.

Inject 5 mL of the test solution. Discard the first 95 mL (19 min) of eluate containing the substance to be examined. Collect the next 155 mL of eluate (31 min) containing any pesticide residues in an evaporating vessel.

Place the 155 mL of the eluate collected from the gel permeation chromatography column into an evaporating vessel. Place this vessel in an autoevaporator setting the

water-bath temperature at 45 °C and the nitrogen pressure at 55 kPa. Evaporate the eluate down to 0.5 mL.

To prepare the solid phase extraction cartridges take some *magnesium silicate for pesticide residue analysis R* and heat it in a muffle furnace at 700 °C for 4 h to remove moisture and polychlorinated biphenyls. Subsequently allow the magnesium silicate to cool for 2 h and transfer it directly to an oven at 100-105 °C, and allow to stand for 30 min. Transfer the magnesium silicate to a stoppered glass jar and allow to equilibrate for 48 h. This material may be used for up to 2 weeks. After that period the magnesium silicate is to be reactivated, by heating at 600 °C for 2 h in a muffle furnace. Remove the magnesium silicate from the furnace, cool and store in a stoppered glass jar. The magnesium silicate is deactivated by adding 1 per cent of *water R*. After the water has been added, shake the magnesium silicate intermittently over 15 min just prior to use. The deactivated magnesium silicate is suitable for use for up to 1 week. It is essential that only deactivated magnesium silicate is used.

Take a 6 mL empty solid phase extraction cartridge and weigh into the cartridge 1 g of the deactivated magnesium silicate.

At this stage the GPC fraction still contains about 10 per cent of the substance to be examined, so further clean-up is necessary. A separate isolation procedure is carried out a) for organochlorine and synthetic pyrethroid pesticides and b) for organophosphorus pesticides. Place a preconditioned solid phase extraction cartridge containing 1 g of deactivated *magnesium silicate for pesticide residue analysis R* onto a vacuum manifold.

Condition the cartridge by adding 10 mL of *toluene R* and allowing the solvent to elute through the cartridge. Place the 0.5 mL of the solvent fraction from the evaporating vessel on the preconditioned cartridge. Elute the pesticide fractions from the cartridges using 20 mL of either of the 2 different solvent systems shown below:

a) for determination of the organochlorine and synthetic pyrethroid pesticides: *toluene R*; a very small amount of the substance to be examined is co-eluted;

b) for determination of the organophosphorus pesticides: a mixture of 2 volumes of *acetone R* and 98 volumes of *toluene R*; this solvent system is used to elute all the pesticides including the more polar organophosphorus pesticides; unfortunately, some of the substance to be examined is co-eluted with this solvent system, which can interfere with the electron capture detector.

Collect the eluate from the extraction cartridges in 25 mL glass vials. Quantitatively transfer the eluate to an evaporating vessel, washing the vial with 3 quantities, each of 10 mL, of *hexane R*.

Place the evaporating vessel on the autoevaporator and evaporate the solid phase extraction fractions down to 0.5 mL. The water-bath temperature is set at 45 °C and the nitrogen pressure is 55 kPa.

Examine the residues by gas chromatography (*2.2.28*) using electron capture and thermionic detectors as described below.

Recovery Calculate the recovery correction factor (R_{cf}) of the internal standards (*ditalimphos R* or *isodrin R*) added to the test solution using the following expression:

$$\frac{A_2}{A_1} \times 100$$

A_1 = peak area of an internal standard 1 ppm in solution;
A_2 = peak area of internal standard extracted from the test solution.

5 mL of the 20 mL test solution containing 1 mL of 2 ppm internal standard concentrated to 0.5 mL is equivalent to 1 ppm of the internal standard in the solution.

If the recovery of the internal standards falls outside the range of 70 per cent to 110 per cent the test is not valid.

Reference solutions Prepare reference solutions of pesticides using the pesticides standards at a concentration of 0.5 ppm (see composition of reference solutions A to D in Table 0134.-1). Commercially available pesticides may be purchased. The individual standards have a concentration of 10 ppm.

Table 0134.-1. – *Composition of the reference solutions*

Reference solution A (0.5 ppm or 0.5 mg/L) (organochlorine and synthetic pyrethroid pesticides)	Reference solution B (0.5 ppm or 0.5 mg/L) (organochlorine and synthetic pyrethroid pesticides)
Cyhalothrin R	*Aldrin R*
Cypermethrin R	*o,p'-DDT R*
o,p'-DDE R	*o,p'-DDD R*
p,p'-DDE R	*p,p'-DDD R*
p,p'-DDT R	*Dieldrin R*
Deltamethrin R	*α-Endosulfan R*
Endrin R	*β-Endosulfan R*
Heptachlor R	*Fenvalerate R*
Heptachlor epoxide R	*α-Hexachlorocyclohexane R*
Hexachlorobenzene R	*β-Hexachlorocyclohexane R*
Lindane R	*δ-Hexachlorocyclohexane R*
Tecnazene R	*Methoxychlor R*
	Permethrin R
Reference solution C (0.5 ppm or 0.5 mg/L) (organophosphorus pesticides)	**Reference solution D (0.5 ppm or 0.5 mg/L) (organophosphorus pesticides)**
Bromophos-ethyl R	*Bromophos R*
Carbophenothion R	*Chlorpyriphos R*
Chlorfenvinphos R	*Chlorpyriphos-methyl R*
Diazinon R	*Coumaphos R*
Dichlofenthion R	*Phosalone R*
Ethion R	*Pirimiphos-ethyl R*
Fenchlorphos R	*Tetrachlorvinphos R*
Malathion R	
Propetamphos R	
Reference solution E (electron capture detector calibration mixture)	**Reference solution F (thermionic detector calibration mixture)**
Aldrin R (0.01 mg/L)	*Chlorfenvinphos R* (0.05 mg/L)
Cypermethrin R (0.1 mg/L)	*Diazinon R* (0.05 mg/L)
o,p'-DDD R (0.01 mg/L)	*Ethion R* (0.05 mg/L)
Deltamethrin R (0.1 mg/L)	*Fenchlorphos R* (0.05 mg/L)
Endrin R (0.01 mg/L)	*Propetamphos R* (0.05 mg/L)
β-Hexachlorocyclohexane R (0.01 mg/L)	
Reference solution G (internal standard organophosphorus pesticide)	**Reference solution H (internal standard organochlorine pesticide)**
Ditalimphos R (2 ppm or 2.0 mg/L)	*Isodrin R* (2 ppm or 2.0 mg/L)
Ditalimphos R (1 ppm or 1.0 mg/L)	*Isodrin R* (1 ppm or 1.0 mg/L)

At the same time prepare solutions of pesticides equivalent to the limit of detection of the method (see recommended compositions in Table 0134.-1). These reference solutions are used to optimise the electron capture detector and thermionic detector to achieve the detection limits of the method (reference solutions E and F).

To prepare the reference solutions at the different concentrations use a calibrated pipette and volumetric flasks. To prepare the internal standard solutions G and H use a four-place analytical balance, pipette and volumetric flasks.

IDENTIFICATION AND QUANTIFICATION OF THE PESTICIDE RESIDUES

To identify any pesticide residues compare the chromatograms obtained with chromatograms obtained with reference solutions A to D.

The identity of the pesticides can be confirmed by spiking samples or overlaying chromatograms using an integration package on a computer. The interpretation of pesticides in trace residue analyses is extremely complex. The detectors, particularly the electron capture detector, are prone to interference, both from the substance to be examined itself, and from solvents, reagents and apparatus used in the extraction. These peaks can easily be misinterpreted or quoted as a false positive. Confirmation of pesticides can be achieved by running samples and standards on different capillary columns (see chromatographic systems A or B described below). The peaks can be identified by using the information in Table 0134.-2.

A knowledge of the different responses the pesticides have with the 2 detectors is useful in identification of unknown peaks.

Table 0134.-2. – *Elution order of the pesticides on chromatographic systems A and B*

Chromatographic system A	Chromatographic system B
Tecnazene	Tecnazene
α-Hexachlorocyclohexane	Hexachlorobenzene
Hexachlorobenzene	α-Hexachlorocyclohexane
β-Hexachlorocyclohexane	Diazinon
Lindane	Lindane
Propetamphos	Propetamphos
δ-Hexachlorocyclohexane	Heptachlor
Diazinon	Dichlofenthion
Dichlofenthion	Aldrin
Chlorpyriphos-methyl	Chlorpyriphos-methyl
Heptachlor	Fenchlorphos
Fenchlorphos	β-Hexachlorocyclohexane
Aldrin	δ-Hexachlorocyclohexane
Malathion	Pirimiphos-ethyl
Chlorpyriphos	Chlorpyriphos
Bromophos	Bromophos
Pirimiphos-ethyl	Malathion
Heptachlor epoxide	Heptachlor epoxide
Chlorfenvinphos (*E*)	*o,p'*-DDE
Chlorfenvinphos (*Z*)	Chlorfenvinphos (*E*)
Bromophos-ethyl	α-Endosulfan
o,p'-DDE	Chlorfenvinphos (*Z*)
α-Endosulfan	Bromophos-ethyl
Tetrachlorvinphos	*p,p'*-DDE
Dieldrin	Dieldrin
p,p'-DDE	Tetrachlorvinphos
o,p'-DDT	*o,p'*-DDT
Endrin	Endrin
β-Endosulfan	*o,p'*-DDD
o,p'-DDD	*p,p'*-DDD
p,p'-DDD	β-Endosulfan
Ethion	Ethion
Carbophenothion	*p,p'*-DDT
p,p'-DDT	Carbophenothion
Methoxychlor	Methoxychlor
Phosalone	Cyhalothrin
Cyhalothrin (2 isomers)	*cis*-Permethrin
cis-Permethrin	Phosalone
trans-Permethrin	*trans*-Permethrin
Coumaphos	Cypermethrin (4 isomers)
Cypermethrin (4 isomers)	Coumaphos
Fenvalerate (2 isomers)	Fenvalerate (2 isomers)
Deltamethrin	Deltamethrin

Once the pesticides have been identified, calculate the content of each pesticide using the following expression:

$$C_P = \frac{P_P \times D \times C_e}{P_e} \times \frac{100}{R_{cf}}$$

C_P = concentration of identified pesticide (ppm);
P_P = peak area of the individual pesticide in the test sample obtained;
C_e = concentration of the individual pesticide in the external standard (ppm);
P_e = peak area of the individual pesticide in the external standard;
D = dilution factor;
R_{cf} = recovery correction factor.

The dilution factor (D) can be defined as follows:

$$\frac{V_1}{m \times \frac{V_2}{V_3}}$$

V_1 = volume of sample obtained after the 2nd evaporation stage;
m = sample weight;
V_2 = GPC injection volume;
V_3 = sample volumetric flask volume.

Chromatographic system A:

Precolumn:
— *material*: deactivated silica;
— *size*: *l* = 4.5 m, Ø = 0.53 mm.

Column:
— *material*: fused silica;
— *size*: *l* = 60 m, Ø = 0.25 mm;
— *stationary phase*: *phenyl(5)methyl(95)polysiloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R.*

Linear velocity 25 cm/s.

Pressure 180 kPa.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 1	75
	1 - 5	75 → 175
	5 - 30	175 → 275
	30 - 40	275 → 285
	40 - 55	285
Injection port		300
Detector		350

Detection Electron capture or thermionic specific detector.

Injection 2 µL.

Chromatographic system B Which may be used for confirmation analysis:

Precolumn:
— *material*: deactivated silica;
— *size*: *l* = 4.5 m, Ø = 0.53 mm.

Column:
— *material*: fused silica;
— *size*: *l* = 60 m, Ø = 0.25 mm;
— *stationary phase*: *cyanopropyl(7)phenyl(7)methyl(86) polysiloxane R* (film thickness 0.25 µm).

Carrier gas *helium for chromatography R.*

Linear velocity 25 cm/s.

Pressure 180 kPa.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 1	75
	1 - 5	75 → 175
	5 - 30	175 → 275
	30 - 40	275 → 285
	40 - 55	285
Injection port		300
Detector		350

Detection Electron capture or thermionic specific detector.

Injection 2 µL.

Chlorides

Maximum 150 ppm.

Boil 1.0 g with 20 mL of *ethanol (90 per cent V/V) R* in a round-bottomed flask fitted with a reflux condenser for 5 min. Cool, add 40 mL of *water R* and 0.5 mL of *nitric acid R* and filter. To the filtrate add 0.15 mL of a 10 g/L solution of *silver nitrate R* in *ethanol (90 per cent V/V) R*. Allow to stand for 5 min protected from light.

Any opalescence in the solution is not more intense than that in a standard prepared at the same time by adding 0.15 mL of a 10 g/L solution of *silver nitrate R* in *ethanol (90 per cent V/V) R* to a mixture of 0.2 mL of *0.02 M hydrochloric acid*, 20 mL of *ethanol (90 per cent V/V) R*, 40 mL of *water R* and 0.5 mL of *nitric acid R*.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 1 h.

Sulfated ash (*2.4.14*)

Maximum 0.15 per cent.

Ignite 5.0 g and use the residue to determine the sulfated ash.

STORAGE

At a temperature not exceeding 25 °C.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for wool fat used in water-emulsifying ointments and lipophilic creams.

Water-absorption capacity

(see Tests).

Drop point (*2.2.17, Method A*)

To fill the metal cup, melt the wool fat on a water-bath, cool to about 50 °C, pour into the cup and allow to stand at 15-20 °C for 24 h. The drop point is typically 38 °C to 44 °C.

Ph Eur

Hydrogenated Wool Fat

(Ph. Eur. monograph 0969)

Ph Eur

DEFINITION

Mixture of higher aliphatic alcohols and sterols obtained from the direct, high-pressure, high-temperature hydrogenation of *wool fat (0134)* during which the esters and acids present are reduced to the corresponding alcohols.

A suitable antioxidant may be added.

CHARACTERS

Appearance

White or pale yellow, unctuous substance.

Solubility

~~Practically insoluble in water, soluble in boiling anhydrous~~ ethanol and in light petroleum.

IDENTIFICATION

First identification: B.

Second identification: A, C.

A. Melting point (see Tests).

B. Examine the chromatograms obtained in the test for fatty alcohols and sterols.

Results The principal peaks in the chromatogram obtained with the test solution are similar in retention time and size to the principal peaks in the chromatogram obtained with reference solution (a).

C. Dissolve 50 mg in 5 mL of *methylene chloride R* and add 1 mL of *acetic anhydride R* and 0.1 mL of *sulfuric acid R*. A green colour is produced.

TESTS

Melting point (*2.2.15*)

45 °C to 55 °C. Allow to stand at 20 °C for 16 h.

Acid value (*2.5.1*)

Maximum 1.0, determined on 5.0 g.

Hydroxyl value (*2.5.3, Method A*)

140 to 180.

Saponification value (*2.5.6*)

Maximum 8.0. Heat under reflux for 4 h.

Fatty alcohols and sterols

Gas chromatography (*2.2.28*).

Test solution Dissolve 0.25 g of the substance to be examined in 60 mL of *anhydrous ethanol R* and dilute to 100.0 mL with the same solvent.

Reference solution (a) Dissolve 0.25 g of *hydrogenated wool fat CRS* in 60 mL of *anhydrous ethanol R* and dilute to 100.0 mL with the same solvent.

Reference solution (b) Dissolve 50 mg of *cetyl alcohol CRS* and 50 mg of *stearyl alcohol CRS* in 60 mL of *anhydrous ethanol R* and dilute to 100.0 mL with the same solvent.

Column:

— *material*: fused silica;

— *size*: *l* = 30 m, Ø = 0.25 mm;

— *stationary phase*: *methylpolysiloxane R* or another non-polar phase (film thickness 0.25 µm).

Carrier gas *helium for chromatography R.*

Flow rate 1 mL/min.

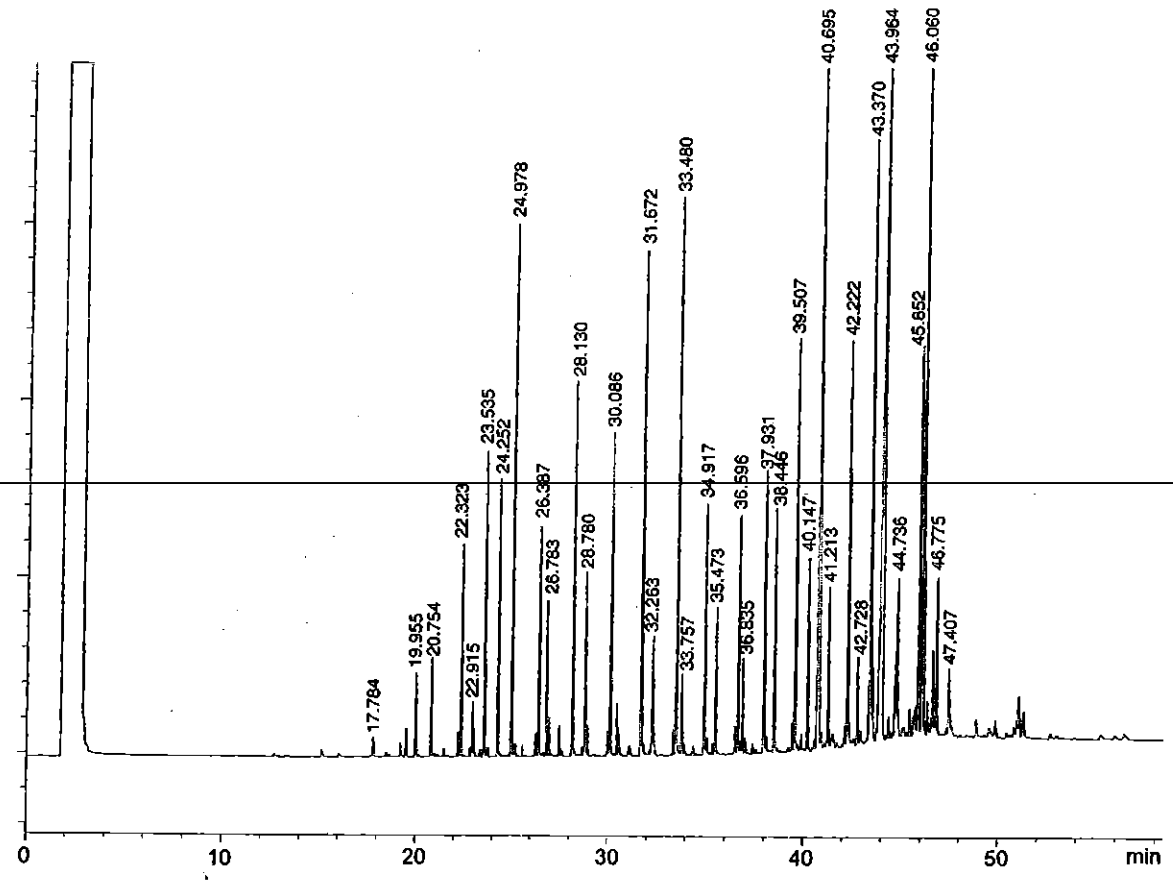


Figure 0969.-1. – *Chromatogram for the test for fatty alcohols and sterols in hydrogenated wool fat: reference solution (a)*

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 5	100
	5 - 45	100 → 300
	45 - 60	300
Injection port		325
Detector		350

Detection Flame ionisation.

Injection 1 µL.

Results The chromatogram obtained with the test solution does not differ significantly from the chromatogram obtained with reference solution (a) (Figure 0969.-1) and it does not show enhanced peaks with retention times corresponding to cetyl alcohol and stearyl alcohol present in the chromatogram obtained with reference solution (b).

Loss on drying (*2.2.32*)
Maximum 3.0 per cent, determined on 2.000 g by drying in an oven at 105 °C for 1 h.

Total ash (*2.4.16*)
Maximum 0.1 per cent, determined on 5.0 g.

STORAGE
In a well-filled container, protected from light.

Ph Eur

Hydrous Wool Fat

Lanolin

(Ph. Eur. monograph 0135)

Ph Eur

DEFINITION
Mixture of 75 per cent *m/m* of wool fat and 25 per cent *m/m* of water. It is obtained by the gradual addition of water to melted wool fat with continuous stirring. A suitable antioxidant may be added.

CHARACTERS
Appearance
Pale yellow, unctuous substance.

IDENTIFICATION
A. In a test-tube, dissolve 0.5 g in 5 mL of *methylene chloride R* and add 1 mL of *acetic anhydride R* and 0.1 mL of *sulfuric acid R*. A green colour develops.

B. Dissolve 50 mg in 5 mL of *methylene chloride R*, add 5 mL of *sulfuric acid R* and shake. A red colour develops and an intense green fluorescence appears in the lower layer when examined in daylight, with illumination from behind the observer.

TESTS
Water-soluble acid or alkaline substances
Melt 6.7 g on a water-bath and shake vigorously for 2 min with 75 mL of *water R* previously heated to 90-95 °C. Allow to cool and filter through filter paper previously rinsed with *water R*. To 60 mL of the filtrate (which may not be clear) add 0.25 mL of *bromothymol blue solution R1*. Not more than 0.2 mL of *0.02 M hydrochloric acid* or 0.15 mL of *0.02 M*

sodium hydroxide is required to change the colour of the indicator.

Drop point (*2.2.17*)
38 °C to 44 °C.

To fill the metal cup, melt the residue obtained in the test for wool-fat content on a water-bath, cool to about 50 °C, pour into the cup and allow to stand at 15-20 °C for 24 h.

Water-absorption capacity
Place 10 g of the residue obtained in the test for wool-fat content in a mortar. Add *water R* in portions of 0.2-0.5 mL from a burette, stirring vigorously after each addition to incorporate the *water R*. The end-point is reached when visible droplets remain which cannot be incorporated. Not less than 20 mL of *water R* is absorbed.

Acid value (*2.5.1*)
Maximum 0.8, determined on 5.0 g dissolved in 25 mL of the prescribed mixture of solvents.

Peroxide value (*2.5.5, Method A*)
Maximum 15.

Saponification value (*2.5.6*)
67 to 79, determined on 2.00 g while heating under reflux for 4 h.

Water-soluble oxidisable substances
To 10 mL of the filtrate obtained in the test for water-soluble acid or alkaline substances add 1 mL of *dilute sulfuric acid R* and 0.1 mL of *0.02 M potassium permanganate*. After 10 min, the solution is not completely decolourised.

Paraffins
Maximum 1.0 per cent.

The tap and cotton plugs used must be free from grease Prepare a column of anhydrous aluminium oxide 230 mm long and 20 mm in diameter by adding a slurry of *anhydrous aluminium oxide R* and *light petroleum R1* to a glass tube fitted with a tap and containing *light petroleum R1*. Allow to settle and reduce the depth of the layer of solvent above the column to about 40 mm. Dissolve 3.0 g of the residue obtained in the test for wool-fat content in 50 mL of warm *light petroleum R1*, cool, pass the solution through the column at a rate of 3 mL/min and wash with 250 mL of *light petroleum R1*. Concentrate the combined eluate and washings to low bulk by distillation, evaporate to dryness on a water-bath and heat the residue at 105 °C for periods of 10 min until 2 successive weighings do not differ by more than 1 mg. The residue weighs a maximum of 30 mg.

Chlorides
Maximum 115 ppm.

Boil 1.3 g with 20 mL of *ethanol (90 per cent V/V) R* under a reflux condenser for 5 min. Cool, add 40 mL of *water R* and 0.5 mL of *nitric acid R* and filter. To the filtrate add 0.15 mL of a 10 g/L solution of *silver nitrate R* in *ethanol (90 per cent V/V) R*. Allow to stand for 5 min, protected from light. Any opalescence in the solution is not more intense than that in a standard prepared at the same time by adding 0.15 mL of a 10 g/L solution of *silver nitrate R* in *ethanol (90 per cent V/V) R* to a mixture of 0.2 mL of *0.02 M hydrochloric acid*, 20 mL of *ethanol (90 per cent V/V) R*, 40 mL of *water R* and 0.5 mL of *nitric acid R*.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent.

Ignite 5.0 g and use the residue.

Wool-fat content
72.5 per cent to 77.5 per cent.

In a suitable tared dish containing a glass rod, heat 30.0 g to constant mass on a water-bath, stirring continuously. Weigh the residue.

STORAGE
At a temperature not exceeding 25 °C.

Ph Eur

Xanthan Gum

(Ph. Eur. monograph 1277)

11138-66-2

Action and use
Excipient.

Ph Eur

DEFINITION
High-molecular-mass anionic polysaccharide produced by fermentation of carbohydrates with *Xanthomonas campestris*. It consists of a principal chain of β(1→4)-linked D-glucose units with trisaccharide side chains, on alternating anhydroglucose units, consisting of 1 glucuronic acid unit included between 2 mannose units. Most of the terminal units contain a pyruvate moiety and the mannose unit adjacent to the principal chain may be acetylated at C-6.

Xanthan gum has a relative molecular mass of approximately 1×10^6. It exists as the sodium, potassium or calcium salt.

Content
Minimum 1.5 per cent of pyruvate acetal groups ($C_3H_3O_2$; M_r 71.1) (dried substance).

CHARACTERS
Appearance
White or yellowish-white, free-flowing powder.

Solubility
Soluble in water giving a highly viscous solution, practically insoluble in organic solvents.

IDENTIFICATION
A. In a flask, suspend 1 g in 15 mL of *0.1 M hydrochloric acid*. Close the flask with a fermentation bulb containing *barium hydroxide solution R* and heat carefully for 5 min. The barium hydroxide solution shows a white turbidity.

B. To 300 mL of *water R*, previously heated to 80 °C and stirred rapidly with a mechanical stirrer in a 400 mL beaker, add, at the point of maximum agitation, a dry blend of 1.5 g of *carob bean gum R* and 1.5 g of the substance to be examined. Stir until the mixture forms a solution, and then continue stirring for 30 min or longer. Do not allow the water temperature to drop below 60 °C during stirring. Discontinue stirring and allow the mixture to stand for at least 2 h. A firm rubbery gel forms after the temperature drops below 40 °C but no such gel forms in a 1 per cent control solution of the sample prepared in the same manner but omitting the carob bean gum.

TESTS
pH (*2.2.3*)
6.0 to 8.0 for a 10.0 g/L solution in *carbon dioxide-free water R*.

2-Propanol
Gas chromatography (*2.2.28*).

Internal standard solution Dilute 0.50 g of *2-methyl-2-propanol R* to 500 mL with *water R*.

Test solution To 200 mL of *water R* in a 1000 mL round-bottomed flask, add 5.0 g of the substance to be examined and 1 mL of a 10 g/L emulsion of *dimeticone R* in *liquid paraffin R*, stopper the flask and shake for 1 h. Distil about 90.0 mL, mix the distillate with 4.0 mL of the internal standard solution and dilute to 100.0 mL with *water R*.

Reference solution Dilute a suitable quantity of *2-propanol R*, accurately weighed, with *water R* to obtain a solution having a known concentration of 2-propanol of about 1 mg/mL. To 4.0 mL of this solution add 4.0 mL of the internal standard solution and dilute to 100.0 mL with *water R*.

Column:
— *size*: *l* = 1.8 m, Ø = 4.0 mm;
— *stationary phase*: *ethylvinylbenzene-divinylbenzene copolymer R* (149-177 µm).

Carrier gas *helium for chromatography R*.

Flow rate 30 mL/min.

Temperature:
— *column*: 165 °C;
— *injection port and detector*: 200 °C.

Detection Flame ionisation.

Injection 5 µL.

Relative retention With reference to 2-propanol: 2-methyl-2-propanol = about 1.5.

Limit:
— *2-propanol*: maximum 750 ppm.

Loss on drying (*2.2.32*)
Maximum 15.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2.5 h.

Total ash (*2.4.16*)
6.5 per cent to 16.0 per cent.

ASSAY

Test solution Dissolve a quantity of the substance to be examined corresponding to 120.0 mg of the dried substance in *water R* and dilute to 20.0 mL with the same solvent.

Reference solution Dissolve 45.0 mg of *pyruvic acid R* in *water R* and dilute to 500.0 mL with the same solvent.

Place 10.0 mL of the test solution in a 50 mL round-bottomed flask, add 20.0 mL of *0.1 M hydrochloric acid* and weigh. Boil on a water-bath under a reflux condenser for 3 h. Weigh and adjust to the initial mass with *water R*. In a separating funnel mix 2.0 mL of the solution with 1.0 mL of *dinitrophenylhydrazine-hydrochloric solution R*. Allow to stand for 5 min and add 5.0 mL of *ethyl acetate R*. Shake and allow the solids to settle. Collect the upper layer and shake with 3 quantities, each of 5.0 mL, of *sodium carbonate solution R*. Combine the aqueous layers and dilute to 50.0 mL with *sodium carbonate solution R*. Mix. Treat 10.0 mL of the reference solution at the same time and in the same manner as for the test solution.

Immediately measure the absorbance (*2.2.25*) of the 2 solutions at 375 nm, using *sodium carbonate solution R* as the compensation liquid.

The absorbance of the test solution is not less than that of the reference solution, which corresponds to a content of pyruvoyl groups of not less than 1.5 per cent.

FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). Some of the characteristics described in the Functionality-related characteristics section may also be present in the mandatory part of the monograph since they also represent mandatory quality criteria. In such cases, a cross-reference to the tests described in the mandatory part is included in the Functionality-related characteristics section. Control of the characteristics can contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristics may be relevant for xanthan gum used as viscosity-increasing agent.

Viscosity
(*2.2.10*): typically minimum 600 mPa·s.

Add 3.0 g (dried substance) within 45-90 s into 250 mL of a 12 g/L solution of *potassium chloride R* in a 500 mL beaker stirring with a low-pitch propeller-type stirrer rotating at 800 r/min. When adding the substance take care that agglomerates are destroyed. Add an additional quantity of 44 mL of *water R*, to rinse any adhering residue from the walls of the beaker. Stir the preparation at 800 r/min for 2 h whilst maintaining the temperature at 24 ± 1 °C. Determine the viscosity within 15 min at 24 ± 1 °C using a rotating viscosimeter set at 60 r/min and equipped with a rotating spindle 1.78 mm high and 12.60 mm in diameter which is attached to a shaft 3.2 mm in diameter. The distance from the top of the cylinder to the lower tip of the shaft should be 25.60 mm and the immersion depth 50.0 mm.

The following characteristics may be relevant for xanthan gum used as matrix former in prolonged-release tablets.

Viscosity
See test above.

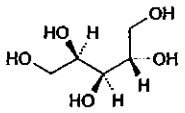
Particle-size distribution (*2.9.31* or *2.9.38*)

Powder flow (*2.9.36*)

Ph Eur

Xylitol

(Ph. Eur. monograph 1381)



$C_5H_{12}O_5$ 152.1 *87-99-0*

Action and use
Sweetening agent.

Ph Eur

DEFINITION
Meso-xylitol.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder or crystals.

Solubility
Very soluble in water, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION
First identification: B.
Second identification: A, C.
A. Melting point (*2.2.14*): 92 °C to 96 °C.
B. Infrared absorption spectrophotometry (*2.2.24*).
Preparation Mulls in *liquid paraffin R.*
Comparison *xylitol CRS.*
C. Thin-layer chromatography (*2.2.27*).
Test solution Dissolve 25 mg of the substance to be examined in *water R* and dilute to 10 mL with the same solvent.
Reference solution (a) Dissolve 25 mg of *xylitol CRS* in *water R* and dilute to 10 mL with the same solvent.
Reference solution (b) Dissolve 25 mg of *mannitol R* and 25 mg of *xylitol R* in *water R* and dilute to 10 mL with the same solvent.
Plate *TLC silica gel plate R.*
Mobile phase *water R, ethyl acetate R, propanol R* (10:20:70 *V/V/V*).
Application 2 µL.
Development Over 3/4 of the plate.
Drying In air.
Detection Spray with *4-aminobenzoic acid solution R*, dry in a current of cold air until the acetone is removed, then heat at 100 °C for 15 min; allow to cool, spray with a 2 g/L solution of *sodium periodate R*, dry in a current of cold air, then heat at 100 °C for 15 min.
System suitability Reference solution (b):
— the chromatogram shows 2 clearly separated spots.
Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution
The solution is not more opalescent than reference suspension IV (*2.2.1*) and not more intensely coloured than reference solution BY_7 (*2.2.2, Method II*).
Dissolve 2.5 g in *water R* and dilute to 50.0 mL with the same solvent.

Conductivity (*2.2.38*)
Maximum 20 µS·cm^{-1}.
Dissolve 20.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100.0 mL with the same solvent. Measure the conductivity of the solution while gently stirring with a magnetic stirrer.

Reducing sugars
Maximum 0.2 per cent, calculated as glucose equivalent.
Dissolve 5.0 g in 6 mL of *water R* with the aid of gentle heat. Cool and add 20 mL of *cupri-citric solution R* and a few glass beads. Heat so that boiling begins after 4 min and maintain boiling for 3 min. Cool rapidly and add 100 mL of a 2.4 per cent *V/V* solution of *glacial acetic acid R* and 20.0 mL of *0.025 M iodine*. With continuous shaking, add 25 mL of a mixture of 6 volumes of *hydrochloric acid R* and 94 volumes of *water R* and, when the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulfate* using 1 mL of *starch solution R*, added towards the end of the titration, as indicator. Not less than 12.8 mL of *0.05 M sodium thiosulfate* is required.

Related substances
Gas chromatography (*2.2.28*).
Internal standard solution Dissolve 5 mg of *erythritol R* in *water R* and dilute to 25.0 mL with the same solvent.
Test solution (a) Dissolve 5.000 g of the substance to be examined in *water R* and dilute to 100.0 mL with the same solvent.
Test solution (b) Dilute 1.0 mL of test solution (a) to 10.0 mL with *water R.*
Reference solution (a) Dissolve 5.0 mg each of *L-arabinitol CRS* (impurity A), *galactitol CRS* (impurity B), *mannitol CRS* (impurity C) and *sorbitol CRS* (impurity D) in *water R* and dilute to 20.0 mL with the same solvent.
Reference solution (b) Dissolve 50.0 mg of xylitol CRS in *water R* and dilute to 10.0 mL with the same solvent.
Pipette 1.0 mL of test solutions (a) and (b) and reference solutions (a) and (b) into 4 separate 100 mL round-bottomed flasks. Add 1.0 mL of the internal standard solution to each of the flasks containing test solution (a) or reference solution (a), and 5.0 mL of the internal standard solution to each of the flasks containing test solution (b) or reference solution (b). Evaporate each mixture to dryness in a water-bath at 60 °C by suitable means. Dissolve each dry residue in 1 mL of *anhydrous pyridine R*, add 1 mL of *acetic anhydride R* to each flask and boil each solution under reflux for 1 h to complete acetylation.
Column:
— *size:* l = 30 m, Ø = 0.25 mm;
— *stationary phase:* *cyanopropyl(7)phenyl(7)methyl(86) polysiloxane R* (0.25 µm).
Carrier gas *nitrogen R.*
Flow rate 1 mL/min.
Split ratio 1:50 to 1:100.
Temperature:

	Time (min)	Temperature (°C)
Column	0 - 1	170
	1 - 6	170 → 230
	6 - 30	230
Injection port		250
Detector		250

Detection Flame ionisation.
Injection 1 µL of test solution (a) and reference solution (a) (solutions obtained after derivatisation).
Relative retention With reference to xylitol (retention time = about 15 min): internal standard = about 0.6; impurity A = about 0.9; impurity C = about 1.4; impurity B = about 1.45; impurity D = about 1.5.
System suitability Reference solution (a):
— *resolution:* minimum 2.0 between the peaks due to impurities B and D.
Calculate the percentage content of each related substance in the substance to be examined using the following expression:

$$100 \times \frac{m_s}{m_u} \times \frac{R_u}{R_s}$$

m_s = mass of the particular component in 1 mL of reference solution (a), in milligrams;

m_u = mass of the substance to be examined in 1 mL of test solution (a), in milligrams;
R_t = ratio of the area of the peak due to the particular derivatised component to the area of the peak due to the derivatised internal standard in the chromatogram obtained with reference solution (a);
R_u = ratio of the area of the peak due to the particular derivatised component to the area of the peak due to the derivatised internal standard in the chromatogram obtained with test solution (a).

The sum of the percentage contents of the related substances in the chromatogram obtained with test solution (a) is not greater than 2.0 per cent. Disregard any peak with an area corresponding to a percentage content of 0.05 per cent or less.

Lead (*2.4.10*)
Maximum 0.5 ppm.

~~Dissolve the substance to be examined in 150.0 mL of the~~ prescribed mixture of solvents.

Nickel (*2.4.15*)
Maximum 1 ppm.

Dissolve the substance to be examined in 150.0 mL of the prescribed mixture of solvents.

Water (*2.5.12*)
Maximum 1.0 per cent, determined on 1.00 g.

Bacterial endotoxins (*2.6.14*)
Less than 4 IU/g if the concentration is less than 100 g/L of xylitol and less than 2.5 IU/g if the concentration is 100 g/L or more of xylitol, when intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY
Gas chromatography (*2.2.28*) as described in the test for related substances with the following modifications.

Injection 1 µL of test solution (b) and reference solution (b) (solutions obtained after derivatisation).

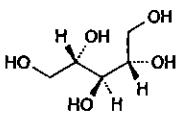
Calculate the percentage content of $C_5H_{12}O_5$ using the following expression:

$$T \times \frac{m_t}{m_v} \times \frac{R_v}{R_t}$$

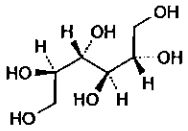
T = declared percentage content of xylitol CRS;
m_t = mass of xylitol CRS in 1 mL of reference solution (b), in milligrams;
m_v = mass of the substance to be examined in 1 mL of test solution (b), in milligrams;
R_t = ratio of the area of the peak due to derivatised xylitol to the area of the peak due to the derivatised internal standard in the chromatogram obtained with reference solution (b);
R_v = ratio of the area of the peak due to derivatised xylitol to the area of the peak due to the derivatised internal standard in the chromatogram obtained with test solution (b).

LABELLING
The label states:
— where applicable, the maximum concentration of bacterial endotoxins;
— where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

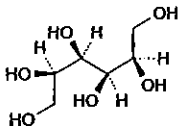
IMPURITIES



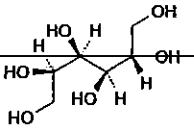
A. L-arabinitol,



B. *meso*-galactitol,



C. D-mannitol,

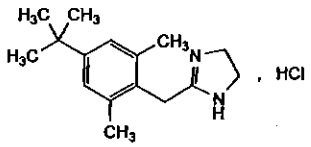


D. D-glucitol (D-sorbitol).

Ph Eur

Xylometazoline Hydrochloride

(Ph. Eur. monograph 1162)



$C_{16}H_{25}ClN_2$ 280.8 *1218-35-5*

Action and use
Alpha-adrenoceptor agonist.

Preparation
Xylometazoline Nasal Drops

Ph Eur

DEFINITION
2-[4-(1,1-Dimethylethyl)-2,6-dimethylbenzyl]-4,5-dihydro-1*H*-imidazole hydrochloride.

Content
99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS
Appearance
White or almost white, crystalline powder.

Solubility
Freely soluble in water, in ethanol (96 per cent) and in methanol.

IDENTIFICATION
First identification: A, E.
Second identification: B, C, D, E.

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *xylometazoline hydrochloride CRS.*

B. Thin-layer chromatography (*2.2.27*).
Test solution Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 5 mL with the same solvent.

Reference solution Dissolve 20 mg of *xylometazoline hydrochloride CRS* in *methanol R* and dilute to 5 mL with the same solvent.

Plate *TLC silica gel G plate R.*

Mobile phase *concentrated ammonia R, methanol R* (5:100 *V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying In air.

Chlorine treatment At the bottom of a chromatographic tank place a beaker containing a mixture of 1 volume of *hydrochloric acid R1*, 1 volume of *water R* and 2 volumes of a 15 g/L solution of *potassium permanganate R*. Close the tank and allow to stand for 15 min. Place the dried plate in the tank and reclose the tank. Leave the plate in contact with the chlorine vapour for 5 min. Withdraw the plate and place it in a current of cold air until the excess of chlorine is removed and an area of the coating below the points of application does not give a blue colour with a drop of *potassium iodide and starch solution R*.

Detection Spray with *potassium iodide and starch solution R*.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. Dissolve about 0.5 mg in 1 mL of *methanol R*. Add 0.5 mL of a freshly prepared 50 g/L solution of *sodium nitroprusside R* and 0.5 mL of a 20 g/L solution of *sodium hydroxide R*. Allow to stand for 10 min and add 1 mL of an 80 g/L solution of *sodium hydrogen carbonate R*. A violet colour develops.

D. Dissolve 0.2 g in 1 mL of *water R*, add 2.5 mL of *ethanol (96 per cent) R* and 2 mL of *1 M sodium hydroxide*. Mix thoroughly and examine in ultraviolet light at 365 nm. The solution shows no fluorescence or at most the same fluorescence as a blank solution prepared in the same manner. The identification is not valid unless a solution prepared in the same manner using *naphazoline hydrochloride CRS* instead of the substance to be examined shows a distinct bluish fluorescence.

E. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Dissolve 2.5 g in *water R* and dilute to 50.0 mL with the same solvent.

Acidity or alkalinity

Dissolve 0.25 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent. Add 0.1 mL of *methyl red solution R* and 0.1 mL of *0.01 M hydrochloric acid*. The solution is red. Not more than 0.2 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to yellow.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 50.0 mL with the same solvent. Allow to stand for 1 h before injection.

Reference solution (a) Dilute 5.0 mL of the test solution to 100.0 mL with *water R*. Dilute 2.0 mL of this solution to 100.0 mL with *water R*.

Reference solution (b) Dissolve 5.0 mg of *xylometazoline impurity A CRS* and 5 mg of the substance to be examined in *water R* and dilute to 50.0 mL with the same solvent. Dilute 10.0 mL of this solution to 50.0 mL with *water R*.

Reference solution (c) Dilute 5.0 mL of reference solution (b) to 50.0 mL with *water R*.

Column:
- *size: l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase: end-capped octadecylsilyl silica gel for chromatography with embedded polar groups R* (5 µm).

Mobile phase:
- *mobile phase A*: 1.36 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*;
- *mobile phase B*: *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	70	30
5 - 20	70 → 15	30 → 85
20 - 35	15	85

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 10 µL.

Relative retention With reference to xylometazoline (retention time = about 7.2 min): impurity A = about 0.79.

System suitability Reference solution (b):
- *resolution*: minimum 2.5 between the peaks due to impurity A and xylometazoline.

Limits:
- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.2 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 25 mL of *anhydrous acetic acid R* and add 10 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 28.08 mg of $C_{16}H_{25}ClN_2$.

STORAGE

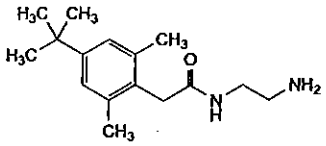
Protected from light.

IMPURITIES

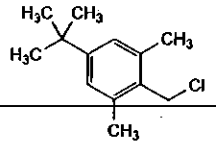
Specified impurities *A*.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is

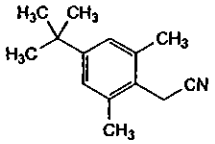
therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D, E, F.*



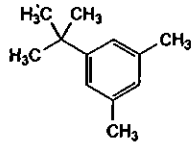
A. *N*-(2-aminoethyl)-2-[4-(1,1-dimethylethyl)-2,6-dimethylphenyl]acetamide,



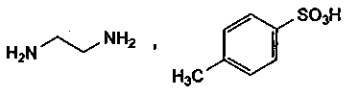
B. 2-(chloromethyl)-5-(1,1-dimethylethyl)-1,3-dimethylbenzene,



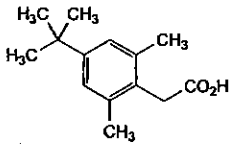
C. [4-(1,1-dimethylethyl)-2,6-dimethylphenyl]acetonitrile,



D. 1-(1,1-dimethylethyl)-3,5-dimethylbenzene,



E. ethane-1,2-diamine mono(4-methylbenzenesulfonate),

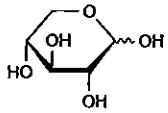


F. [4-(1,1-dimethylethyl)-2,6-dimethylphenyl]acetic acid.

Ph Eur

Xylose

(Ph. Eur. monograph 1278)



$C_5H_{10}O_5$ 150.1 *58-86-6*

Ph Eur

DEFINITION

D-Xylopyranose.

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless needles.

Solubility

Freely soluble in water, soluble in hot ethanol (96 per cent).

IDENTIFICATION

First identification: A.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *xylose CRS.*

B. Thin-layer chromatography (*2.2.27*).

Solvent mixture *water R, methanol R* (2:3 *V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 20 mL with the solvent mixture.

Reference solution (a) Dissolve 10 mg of *xylose CRS* in the solvent mixture and dilute to 20 mL with the solvent mixture.

Reference solution (b) Dissolve 10 mg of *fructose R*, 10 mg of *glucose R* and 10 mg of *xylose R* in the solvent mixture and dilute to 20 mL with the solvent mixture.

Plate *TLC silica gel plate R.*

Mobile phase *water R, methanol R, anhydrous acetic acid R, ethylene chloride R* (10:15:25:50 *V/V/V/V*); measure the volumes accurately since a slight excess of water produces cloudiness.

Application 2 µL; thoroughly dry the points of application.

Development Over a path of 15 cm.

Drying In a current of warm air.

Detection Spray with a 5 g/L solution of *thymol R* in a mixture of 5 volumes of *sulfuric acid R* and 95 volumes of *ethanol (96 per cent) R*. Heat in an oven at 130 °C for 10 min.

System suitability Reference solution (b):

— the chromatogram shows 3 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 0.1 g in 10 mL of *water R*. Add 3 mL of *cupri-tartaric solution R* and heat. An orange or red precipitate is formed.

TESTS

Solution S
Dissolve 10.0 g in *carbon dioxide-free water R* and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity
To 50 mL of solution S add 0.3 mL of *phenolphthalein solution R1*. The solution is colourless. Not more than 0.2 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

Specific optical rotation (*2.2.7*)
+ 18.5 to + 19.5 (dried substance).
Dissolve 10.0 g in 80 mL of *water R*, add 1 mL of *dilute ammonia R2* and dilute to 100.0 mL with *water R*. Allow to stand for 30 min.

Chlorides (*2.4.4*)
Maximum 330 ppm.
Dilute 1.5 mL of solution S to 15 mL with *water R*.

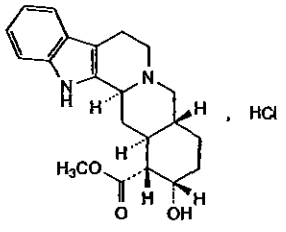
Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C at a pressure not exceeding 0.7 kPa.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

Ph Eur

Yohimbine Hydrochloride

(Ph. Eur. monograph 2172)



$C_{21}H_{27}ClN_2O_3$ 390.9 *65-19-0*

Action and use
Alpha2-adrenoceptor antagonist.

Ph Eur

DEFINITION
Methyl 17α-hydroxyyohimban-16α-carboxylate hydrochloride.

Content
97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
White or slightly yellowish, crystalline powder.

Solubility
Sparingly soluble in water, practically insoluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison *yohimbine hydrochloride CRS*.
B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S
Dissolve 0.500 g in *carbon dioxide-free water R* with heating, allow to cool to room temperature and dilute to 50.0 mL with the same solvent.

pH (*2.2.3*)
3.5 to 5.5 for solution S.

Specific optical rotation (*2.2.7*)
+ 101.0 to + 105.0 (dried substance), determined on solution S.

Related substances
Liquid chromatography (*2.2.29*). *Prepare the solutions protected from light.*

Test solution Dissolve 10.0 mg of the substance to be examined in *methanol R* and dilute to 50.0 mL with the same solvent.

Reference solution (a) Dissolve 5.0 mg of *yohimbine hydrochloride CRS* (containing impurities A, F and G) in *methanol R* and dilute to 25.0 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 100.0 mL with *methanol R*.

Reference solution (c) Dilute 1.0 mL of reference solution (b) to 10.0 mL with *methanol R*.

Column:
— *size*: *l* = 0.125 m, Ø = 4.0 mm;
— *stationary phase*: *octylsilyl silica gel for chromatography R* (4 µm);
— *temperature*: 40 °C.

Mobile phase Mix 50 mL of a 9.08 g/L solution of *potassium dihydrogen phosphate R*, 100 mL of an 11.88 g/L solution of *disodium hydrogen phosphate dihydrate R*, 285 mL of *acetonitrile R*, 4.0 g of *sodium laurilsulfate R* and 355 mL of *water R*.

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 229 nm.

Injection 10 µL.

Run time 3 times the retention time of yohimbine.

Relative retention With reference to yohimbine (retention time = about 7 min): impurity F = about 0.65; impurity G = about 0.70; impurity A = about 0.75.

System suitability Reference solution (a):
— *peak-to-valley ratio*: minimum 1.3, where H_p = height above the baseline of the peak due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity A; and minimum 1.3, where H_p = height above the baseline of the peak due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity F.

Limits:
— *sum of impurities A and G*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
— *impurity F*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.4 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);
— *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{21}H_{27}ClN_2O_3$ from the declared content of *yohimbine hydrochloride CRS*.

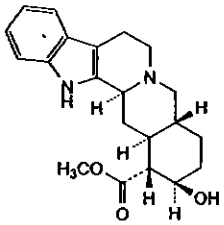
STORAGE

In an airtight container, protected from light.

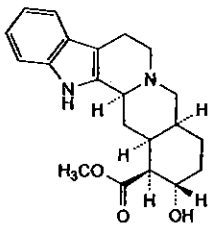
IMPURITIES

Specified impurities *A, F, G.*

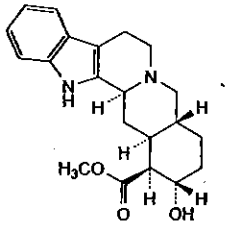
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, C, D, E.*



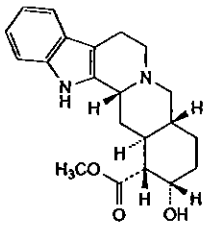
A. methyl 17β-hydroxyyohimban-16α-carboxylate (β-yohimbine),



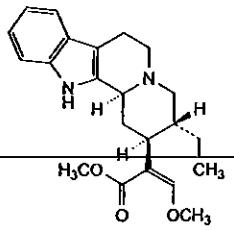
B. methyl 17α-hydroxy-20α-yohimban-16β-carboxylate (α-yohimbine),



C. methyl 17α-hydroxyyohimban-16β-carboxylate (corynantheine),



D. methyl 17α-hydroxy-3β-yohimban-16α-carboxylate (pseudo-yohimbine),



E. methyl (2*Z*)-2-[(2*S*,3*R*,12b*S*)-3-ethyl-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-*a*]quinolizin-2-yl]-3-methoxyprop-2-enoate,

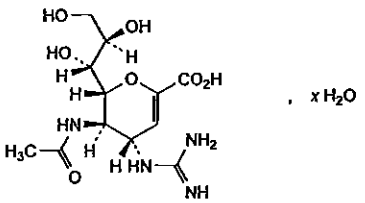
F. unknown structure,

G. unknown structure.

Ph Eur

Zanamivir Hydrate

(Ph. Eur. monograph 2611)



$C_{12}H_{20}N_4O_7,xH_2O$ 332.3 *551942-41-7*
(anhydrous substance)

Action and use
Neuraminidase inhibitor; treatment of influenza.

Ph Eur

DEFINITION

(2*R*,3*R*,4*S*)-3-Acetamido-4-carbamimidamido-2-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-3,4-dihydro-2*H*-pyran-6-carboxylic acid hydrate.

Content
97.0 per cent to 102.0 per cent (dried substance).

It contains a variable quantity of water.

CHARACTERS

Appearance
White or almost white, slightly hygroscopic powder.

Solubility
Slightly soluble in water, practically insoluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *zanamivir hydrate CRS.*

TESTS

Specific optical rotation (*2.2.7*)

+ 36.0 to + 38.5 (dried substance).

Dissolve 0.250 g in 25.0 mL of *water R*; sonicate until dissolution is complete.

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 23.0 mg of the substance to be examined in 20 mL of *water R* and dilute to 50.0 mL with *acetonitrile R1*.

Test solution (b) Dilute 5.0 mL of test solution (a) to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 23.0 mg of *zanamivir for assay CRS* in 20 mL of *water R* and dilute to 50.0 mL with *acetonitrile R1*. Dilute 5.0 mL of the solution to 50.0 mL with the mobile phase.

Reference solution (b) Dissolve 5 mg of *zanamivir for system suitability CRS* (containing impurities A, B, C and E) in 6 mL of *water R* and dilute to 10 mL with *acetonitrile R1*.

Reference solution (c) Dilute 1.0 mL of test solution (a) to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (d) Dissolve 3.0 mg of *zanamivir impurity F CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (e) Dilute 1.0 mL of reference solution (d) to 100.0 mL with the mobile phase. Dilute 3.0 mL of this solution to 20.0 mL with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *amino alkyl vinyl polymer for chromatography R* (5 µm);

— *temperature*: 30 °C.

Mobile phase 0.7 g/L solution of *sulfuric acid R* previously adjusted to pH 5.5 with *dilute ammonia R3*, *acetonitrile R1* (40:60 *V/V*).

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 234 nm.

Preconditioning of the column Prior to first use, rinse with a 0.7 g/L solution of *ammonium sulfate R* at 1.5 mL/min at 30 °C for about 1 h; prior to each use, rinse with the mobile phase for at least 8 h.

Injection 20 µL of test solution (a) and reference solutions (b), (c) and (e).

Run time 3 times the retention time of zanamivir.

Identification of impurities Use the chromatogram supplied with *zanamivir for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C and E; use the chromatogram obtained with reference solution (e) to identify the peak due to impurity F.

Relative retention With reference to zanamivir (retention time = about 9 min): impurity F = about 0.3; impurity B = about 0.6; impurity C = about 0.75; impurity E = about 0.8; impurity A = about 2.6.

System suitability:

— *signal-to-noise ratio*: minimum 10 for the principal peak in the chromatogram obtained with reference solution (e);

— *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity C in the chromatogram obtained with reference solution (b).

Calculation of percentage contents:

— for impurity F, use the concentration of impurity F in reference solution (e);

— for impurities other than F, use the concentration of zanamivir hydrate in reference solution (c).

Limits:

— *impurity A*: maximum 0.5 per cent;

— *impurity B*: maximum 0.3 per cent;

— *impurity C*: maximum 0.2 per cent;

— *impurity F*: maximum 0.01 per cent;

— *unspecified impurities*: for each impurity, maximum 0.10 per cent;

— *total*: maximum 1.2 per cent;

— ~~*reporting threshold*: 0.05 per cent, except for impurity F.~~

Loss on drying (*2.2.32*)

4.0 per cent to 9.0 per cent, determined on 1.000 g by drying *in vacuo* at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (a).

Calculate the percentage content of $C_{12}H_{20}N_4O_7$ taking into account the assigned content of *zanamivir for assay CRS*.

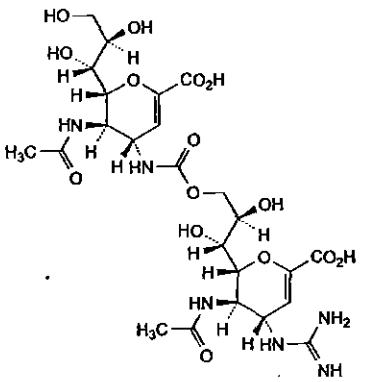
STORAGE

In an airtight container, protected from light.

IMPURITIES

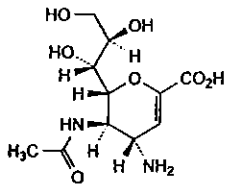
Specified impurities *A, B, C, F.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *D, E, H.*

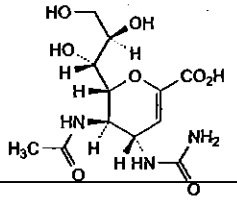


A. (2*R*,3*R*,4*S*)-3-acetamido-2-[(1*R*,2*R*)-3-[[[(2*R*,3*R*,4*S*)-3-acetamido-6-carboxy-2-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-3,4-dihydro-2*H*-pyran-4-yl]carbamoyl]oxy]-1,2-dihydroxypropyl]-4-carbamimidamido-3,4-dihydro-2*H*-pyran-6-carboxylic acid,

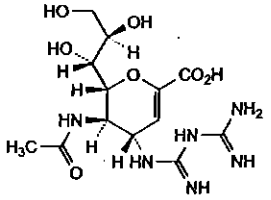
B. unknown structure,



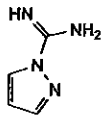
C. (2*R*,3*R*,4*S*)-3-acetamido-4-amino-2-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-3,4-dihydro-2*H*-pyran-6-carboxylic acid,



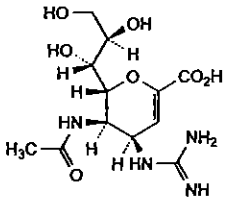
D. (2*R*,3*R*,4*S*)-3-acetamido-4-(carbamoylamino)-2-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-3,4-dihydro-2*H*-pyran-6-carboxylic acid,



E. (2*R*,3*R*,4*S*)-3-acetamido-4-(*N*′-carbamimidoylcarbamimidamido)-2-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-3,4-dihydro-2*H*-pyran-6-carboxylic acid,



F. 1*H*-pyrazole-1-carboximidamide,

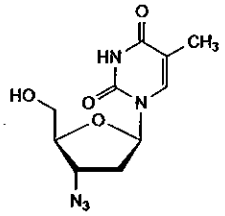


H. (2*R*,3*R*,4*R*)-3-acetamido-4-carbamimidamido-2-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-3,4-dihydro-2*H*-pyran-6-carboxylic acid.

Ph Eur

Zidovudine

(Ph. Eur. monograph 1059)



$C_{10}H_{13}N_5O_4$ 267.2 *30516-87-1*

Action and use
Nucleoside reverse transcriptase inhibitor; antiviral (HIV).

Preparations
Zidovudine Capsules
Zidovudine Tablets
Zidovudine and Lamivudine Tablets
Zidovudine Infusion
Abacavir, Zidovudine and Lamivudine Tablets

Ph Eur

DEFINITION
3′-Azido-3′-deoxythymidine.

Content
97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS
Appearance
White or slightly brownish powder.

Solubility
Sparingly soluble in water, soluble in anhydrous ethanol, practically insoluble in heptane.

It shows polymorphism (*5.9*).

IDENTIFICATION
A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *zidovudine CRS.*

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *water R*, evaporate to dryness in a desiccator, under high vacuum over *diphosphorus pentoxide R* and record new spectra using the residues.

TESTS
Appearance of solution
The solution is not more opalescent than reference suspension I (*2.2.1*) and not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

Dissolve 0.5 g in 50 mL of *water R*, heating if necessary.

Specific optical rotation (*2.2.7*)
+ 60.5 to + 63.0 (dried substance), measured at 25 °C.

Dissolve 0.50 g in *anhydrous ethanol R* and dilute to 50.0 mL with the same solvent.

Related substances
A. Liquid chromatography (*2.2.29*).

Solvent mixture Mix 4 volumes of *acetonitrile R*, 20 volumes of *methanol R* and 76 volumes of a 2 g/L solution of *ammonium acetate R* previously adjusted to pH 6.8 with *dilute acetic acid R*.

Test solution (a) Dissolve 20.0 mg of the substance to be examined in the solvent mixture and dilute to 20.0 mL with the solvent mixture.

Test solution (b) Dilute 10.0 mL of test solution (a) to 50.0 mL with the solvent mixture.

Reference solution (a) Dissolve 2 mg of *zidovudine impurity B CRS* in the solvent mixture and dilute to 50 mL with the solvent mixture. Dilute 1 mL of the solution to 20 mL with the solvent mixture.

Reference solution (b) Dissolve 5 mg of *zidovudine for system suitability A CRS* (containing impurity G) in reference solution (a) and dilute to 5 mL with reference solution (a).

Reference solution (c) Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (d) Dissolve 20.0 mg of *zidovudine CRS* in the solvent mixture and dilute to 20.0 mL with the solvent mixture. Dilute 10.0 mL of the solution to 50.0 mL with the solvent mixture.

Reference solution (e) Dissolve 1 mg of *zidovudine impurity D CRS* in a mixture of 4 volumes of *acetonitrile R*, 40 volumes of *methanol R* and 56 volumes of a 2 g/L solution of *ammonium acetate R* previously adjusted to pH 6.8 with *dilute acetic acid R* and dilute to 50 mL with the same mixture of solvents. Dilute 5 mL of the solution to 10 mL with the same mixture of solvents.

Column:
- *size*: l = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
- *mobile phase A*: 2 g/L solution of *ammonium acetate R* adjusted to pH 6.8 with *dilute acetic acid R*;
- *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	95	5
3 - 18	95 → 85	5 → 15
18 - 28	85 → 30	15 → 70
28 - 43	30	70

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 265 nm.

Injection 20 µL of test solution (a) and reference solutions (b), (c) and (e).

Identification of impurities Use the chromatogram supplied with *zidovudine for system suitability A CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B, and G; use the chromatogram obtained with reference solution (e) to identify the peak due to impurity D.

Relative retention With reference to zidovudine (retention time = about 16 min): impurity B = about 1.05; impurity G = about 1.5; impurity D = about 2.0.

System suitability Reference solution (b):
- *resolution*: minimum 2.0 between the peaks due to zidovudine and impurity B.

Calculation of percentage contents:
- for each impurity, use the concentration of zidovudine in reference solution (c).

Limits:
- *impurity G*: maximum 0.2 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *reporting threshold*: 0.05 per cent; disregard any peak due to impurity D and any peak eluting after this impurity.

B. Liquid chromatography (*2.2.29*).

Test solution Dissolve 0.5 g of the substance to be examined in 10 mL of *acetonitrile R* and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dissolve 5.0 mg of *zidovudine impurity D CRS* in *acetonitrile R* and dilute to 10.0 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 100.0 mL with the mobile phase.

Reference solution (c) Dilute 1 mL of reference solution (a) to 50 mL with the test solution.

Column:
- *size*: l = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *water for chromatography R, acetonitrile R1* (30:70 *V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µL of the test solution and reference solutions (b) and (c).

Run time 10 times the retention time of zidovudine.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity D.

Relative retention With reference to zidovudine (retention time = about 1.5 min): impurity D = about 2.5.

System suitability Reference solution (c):
- *resolution*: minimum 5.0 between the peaks due to zidovudine and impurity D.

Calculation of percentage contents:
- for each impurity, use the concentration of impurity D in reference solution (b).

Limits:
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *reporting threshold*: 0.05 per cent; disregard any peak eluting before impurity D.

Limit:
- *total for tests A and B*: maximum 1.0 per cent.

Loss on drying (*2.2.32*)
Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Liquid chromatography (*2.2.29*) as described in test A for related substances with the following modification.

Injection Test solution (b) and reference solution (d).

Calculate the percentage content of $C_{10}H_{13}N_5O_4$ taking into account the assigned content of *zidovudine CRS*.

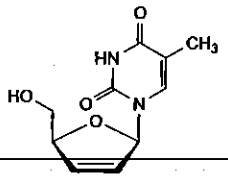
STORAGE
Protected from light.

IMPURITIES
Test A for related substances: A, B, C, E, F, G.

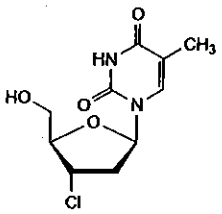
Test B for related substances: D, J, K.

Specified impurities G.

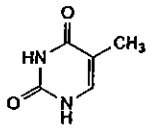
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) A, B, C, D, E, F, J, K.



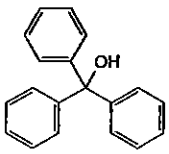
A. 3′-deoxy-2′,3′-didehydrothymidine,



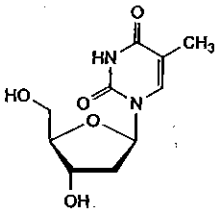
B. 3′-chloro-3′-deoxythymidine,



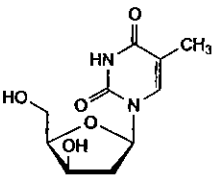
C. 5-methylpyrimidine-2,4(1*H*,3*H*)-dione (thymine),



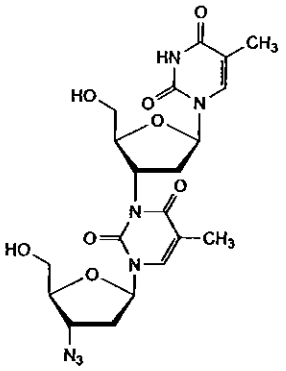
D. triphenylmethanol,



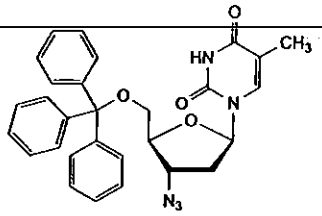
E. thymidine,



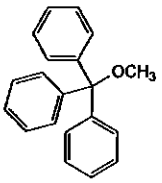
F. 3′-*epi*-thymidine,



G. 3′-(3′-azido-3′-deoxythymidin-3-yl)-3′-deoxythymidine,



J. 3′-azido-3′-deoxy-5′-*O*-(triphenylmethyl)thymidine (tritylzidovudine),



K. 1,1′,1′′-(methoxymethanetriyl)tribenzene (methyl trityl ether).

Ph Eur

Zinc Acetate

(Zinc Acetate Dihydrate, Ph. Eur. monograph 1482)

$C_4H_6O_4Zn,2H_2O$ 219.5 *5970-45-6*

Action and use
Astringent.

Preparation
Erythromycin and Zinc Acetate Lotion

Ph Eur

DEFINITION

Content
99.0 per cent to 101.0 per cent of $C_4H_6O_4Zn,2H_2O$.

CHARACTERS

Appearance
White or almost white crystalline powder or flakes.

Solubility
Freely soluble in water, soluble in ethanol (96 per cent).

IDENTIFICATION

A. It gives reaction (a) of acetates (*2.3.1*).

B. It gives the reaction of zinc (*2.3.1*).

TESTS

Solution S
Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)
5.8 to 7.0.

Dilute 10 mL of solution S to 20 mL with *carbon dioxide-free water R*.

Reducing substances
Boil for 5 min a mixture of 10 mL of solution S, 90 mL of *water R*, 5 mL of *dilute sulfuric acid R* and 1.5 mL of a 0.3 g/L solution of *potassium permanganate R*. The pink colour of the solution remains.

Chlorides (*2.4.4*)
Maximum 50 ppm.

Dilute 10 mL of solution S with 15 mL of *water R*.

Sulfates (*2.4.13*)
Maximum 100 ppm, determined on solution S.

Aluminium
Maximum 5 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Dissolve 2.50 g in 20 mL of a 200 g/L solution of *cadmium- and lead-free nitric acid R* and dilute to 25.0 mL with the same acid solution.

Reference solutions Prepare the reference solutions using *aluminium standard solution (200 ppm Al) R*, diluted with a 200 g/L solution of *cadmium- and lead-free nitric acid R*.

Source Aluminium hollow-cathode lamp.

Wavelength 309.3 nm.

Atomisation device Air-acetylene or acetylene-nitrous oxide flame.

Arsenic (*2.4.2, Method A*)
Maximum 2 ppm, determined on 0.5 g.

Cadmium
Maximum 2 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Use the solution described in the test for aluminium.

Reference solutions Prepare the reference solutions using *cadmium standard solution (0.1 per cent Cd) R*, diluted with a 200 g/L solution of *cadmium- and lead-free nitric acid R*.

Source Cadmium hollow-cathode lamp.

Wavelength 228.8 nm.

Atomisation device Air-acetylene flame.

Copper
Maximum 50 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Use the solution described in the test for iron.

Reference solutions Prepare the reference solutions using *copper standard solution (10 ppm Cu) R*, diluted with a 200 g/L solution of *cadmium- and lead-free nitric acid R*.

Source Copper hollow-cathode lamp.

Wavelength 324.8 nm.

Atomisation device Air-acetylene flame.

Iron
Maximum 50 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Dissolve 1.25 g in 20 mL of a 200 g/L solution of *cadmium- and lead-free nitric acid R* and dilute to 25.0 mL with the same acid solution.

Reference solutions Prepare the reference solutions using *iron standard solution (20 ppm Fe) R*, diluted with a 200 g/L solution of *cadmium- and lead-free nitric acid R*.

Source Iron hollow-cathode lamp.

Wavelength 248.3 nm.

Atomisation device Air-acetylene flame.

Lead
Maximum 10 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Dissolve 5.00 g in 20 mL of a 200 g/L solution of *cadmium- and lead-free nitric acid R* and dilute to 25.0 mL with the same acid solution.

Reference solutions Prepare the reference solutions using *lead standard solution (0.1 per cent Pb) R*, diluting with a 200 g/L solution of *cadmium- and lead-free nitric acid R*.

Source Lead hollow-cathode lamp.

Wavelength 283.3 nm.

Atomisation device Air-acetylene flame.

ASSAY

Dissolve 0.200 g in 5 mL of *dilute acetic acid R*. Carry out the complexometric titration of zinc (*2.5.11*).

1 mL of *0.1 M sodium edetate* is equivalent to 21.95 mg of $C_4H_6O_4Zn,2H_2O$.

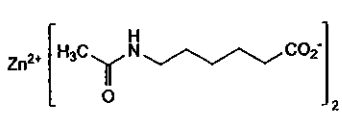
STORAGE

In a non-metallic container.

Ph Eur

Zinc Acexamate

(Ph. Eur. monograph 1279)



$C_{16}H_{28}N_2O_6Zn$ 409.8 *70020-71-2*

Ph Eur

DEFINITION

Zinc bis(6-acetamidohexanoate).

Content
97.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Soluble in water, practically insoluble in acetone and in ethanol (96 per cent). It dissolves in dilute nitric acid.

mp
About 198 °C.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *zinc acexamate CRS*.

B. 5 mL of solution S (see Tests) gives the reaction of zinc (*2.3.1*).

TESTS

Solution S

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 20 mL with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension IV (*2.2.1*) and is colourless (*2.2.2, Method II*).

pH (*2.2.3*)

5.0 to 7.0 for solution S.

Impurity B

Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 0.30 g of the substance to be examined in *water R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 15 mg of *6-aminohexanoic acid R* (impurity B) in *water R* and dilute to 10 mL with the same solvent. Dilute 1 mL of this solution to 10 mL with *water R*.

Plate *TLC silica gel plate R.*

Mobile phase *ammonia R, water R, ethanol (96 per cent) R* (2:30:68 *V/V/V*).

Application 5 µL; allow to dry in air.

Development Over a path of 15 cm.

Drying In a current of warm air.

Detection Spray with *ninhydrin solution R* and heat at 100-105 °C for 15 min.

Limit:

— *impurity B*: any spot due to impurity B is not more intense than the corresponding spot in the chromatogram obtained with the reference solution (0.5 per cent).

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 0.50 g of the substance to be examined in *water R* and dilute to 100.0 mL with the same solvent.

Test solution (b) To 20.0 mL of test solution (a), add 20 mL of the mobile phase and 0.4 mL of a 100 g/L solution of *phosphoric acid R*, then dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 40 mg of *N-acetyl-ε-caprolactam R* (impurity C) in *water R* and dilute to 100.0 mL with the same solvent.

Reference solution (b) Dilute 5.0 mL of reference solution (a) to 100.0 mL with *water R*.

Reference solution (c) Dissolve 20 mg of *zinc acexamate impurity A CRS* in *water R* and dilute to 50.0 mL with the same solvent.

Reference solution (d) Dissolve 40 mg of *ε-caprolactam R* (impurity D) in *water R* and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of this solution to 100.0 mL with *water R*.

Reference solution (e) To 20 mL of test solution (a), add 5 mL of reference solution (b), 5 mL of reference solution (c), 5 mL of reference solution (d) and 0.4 mL of a 100 g/L solution of *phosphoric acid R*, then dilute to 50 mL with the mobile phase.

Reference solution (f) To 5.0 mL of reference solution (c), add 5.0 mL of reference solution (b), 5.0 mL of reference solution (d) and 0.4 mL of a 100 g/L solution of *phosphoric acid R*, then dilute to 50.0 mL with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 0.2 volumes of *phosphoric acid R*, 8 volumes of *acetonitrile R1* and 92 volumes of *water for chromatography R*, then adjust to pH 4.5 with *dilute ammonia R1*.

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 20 µL of test solution (b) and reference solutions (b), (e) and (f).

Run time 8 times the retention time of zinc acexamate.

Elution order Zinc acexamate, impurity D, impurity A, impurity C.

System suitability Reference solution (e):

— *resolution*: minimum 3.0 between the peaks due to zinc acexamate and impurity D; if necessary, adjust the mobile phase to pH 4.7 with *dilute ammonia R1*.

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (2 per cent);

— *impurities C, D*: for each impurity, not more than 1.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (f) (0.15 per cent);

— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the peak due to impurity C in the chromatogram obtained with reference solution (f) (0.05 per cent);

— *sum of impurities other than A*: not more than 5 times the area of the peak due to impurity C in the chromatogram obtained with reference solution (f) (0.5 per cent);

— *disregard limit*: 0.5 times the area of the peak due to impurity C in the chromatogram obtained with reference solution (f) (0.05 per cent).

Iron

Maximum 50 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Dissolve 1.25 g in 20 mL of a 200 g/L solution of *cadmium- and lead-free nitric acid R* and dilute to 25.0 mL with the same acid solution.

Reference solutions Prepare the reference solutions using *iron standard solution (20 ppm Fe) R*, diluting with a 200 g/L solution of *cadmium- and lead-free nitric acid R*.

Source Iron hollow-cathode lamp.

Wavelength 248.3 nm.

Atomisation device Air-acetylene flame.

Loss on drying (*2.2.32*)

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.400 g in 10 mL of *dilute acetic acid R*. Carry out the complexometric titration of zinc (*2.5.11*).

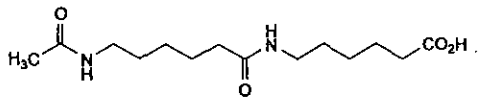
1 mL of *0.1 M sodium edetate* is equivalent to 40.98 mg of $C_{16}H_{28}N_2O_6Zn$.

STORAGE

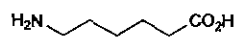
In a non-metallic container.

IMPURITIES

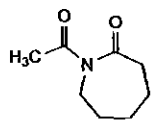
Specified impurities *A, B, C, D.*



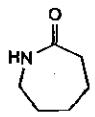
A. 6-(6-acetamidohexanamido)hexanoic acid,



B. 6-aminohexanoic acid (6-aminocaproic acid),



C. 1-acetylazepan-2-one (*N*-acetyl-ε-caprolactam),



D. azepan-2-one (ε-caprolactam).

Ph Eur

Zinc Chloride

(Ph. Eur. monograph 0110)

$ZnCl_2$ 136.3 *7646-85-7*

Ph Eur

DEFINITION

Content

95.0 per cent to 100.5 per cent.

CHARACTERS

Appearance

White or almost white, crystalline powder or cast in white or almost white sticks, deliquescent.

Solubility

Very soluble in water, freely soluble in ethanol (96 per cent) and in glycerol.

IDENTIFICATION

A. Dissolve 0.5 g in *dilute nitric acid R* and dilute to 10 mL with the same acid. The solution gives reaction (a) of chlorides (*2.3.1*).

B. 5 mL of solution S (see Tests) gives the reaction of zinc (*2.3.1*).

TESTS

Solution S

To 2.0 g add 38 mL of *carbon dioxide-free water R* prepared from *distilled water R* and add *dilute hydrochloric acid R* dropwise until dissolution is complete. Dilute to 40 mL with *carbon dioxide-free water R* prepared from *distilled water R*.

pH (*2.2.3*)

4.6 to 5.5.

Dissolve 1.0 g in 9 mL of *carbon dioxide-free water R*, ignoring any slight turbidity.

Oxychlorides

Dissolve 10.0 g in 10 mL of *carbon dioxide-free water R*. The solution is not more opalescent than reference suspension II (*2.2.1*). To 1.5 mL of the solution add 7.5 mL of *ethanol (96 per cent) R*. The solution may become cloudy within 10 min. Any cloudiness disappears on the addition of 0.2 mL of *dilute hydrochloric acid R*.

Sulfates (*2.4.13*)

Maximum 200 ppm.

Dilute 5 mL of solution S to 15 mL with *distilled water R*. Prepare the standard using a mixture of 5 mL of *sulfate standard solution (10 ppm SO_4) R* and 10 mL of *distilled water R*.

Aluminium, calcium, iron, magnesium

To 8 mL of solution S add 2 mL of *concentrated ammonia R* and shake. The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*). Add 1 mL of *disodium hydrogen phosphate solution R*. The solution remains clear for at least 5 min. Add 0.2 mL of *sodium sulfide solution R*. A white precipitate is formed and the supernatant remains colourless.

Ammonium (*2.4.1*)

Maximum 400 ppm.

Dilute 0.5 mL of solution S to 15 mL with *water R*.

ASSAY

Dissolve 0.250 g in 5 mL of *dilute acetic acid R*. Carry out the complexometric titration of zinc (*2.5.11*).

1 mL of *0.1 M sodium edetate* is equivalent to 13.63 mg of $ZnCl_2$.

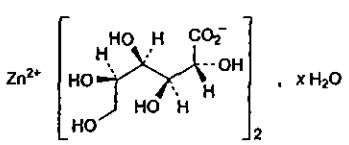
STORAGE

In a non-metallic container.

Ph Eur

Zinc Gluconate

(Ph. Eur. monograph 2164)



$C_{12}H_{22}ZnO_{14},xH_2O$ 455.7
(anhydrous substance)

Ph Eur

DEFINITION

Anhydrous or hydrated zinc D-gluconate.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, hygroscopic, crystalline powder.

Solubility

Soluble in water, practically insoluble in anhydrous ethanol and in methylene chloride.

IDENTIFICATION

A. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 20 mg of the substance to be examined in 1 mL of *water R*.

Reference solution Dissolve 20 mg of *calcium gluconate CRS* in 1 mL of *water R*, heating if necessary in a water-bath at 60 °C.

Plate *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase *concentrated ammonia R, ethyl acetate R, water R, ethanol (96 per cent) R* (10:10:30:50 *V/V/V/V*).

Application 1 µL.

Development Over 3/4 of the plate.

Drying At 100-105 °C for 20 min, then allow to cool to room temperature.

Detection Spray with a solution containing 25 g/L of *ammonium molybdate R* and 10 g/L of *cerium sulfate R* in *dilute sulfuric acid R*, and heat at 100-105 °C for about 10 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

B. Dissolve 0.1 g in 5 mL of *water R*. Add 0.5 mL of *potassium ferrocyanide solution R*. A white precipitate is formed that does not dissolve upon the addition of 5 mL of *hydrochloric acid R*.

TESTS

Solution S

Dissolve 1.0 g in *water R* and dilute to 50 mL with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Sucrose and reducing sugars

Dissolve 0.5 g in a mixture of 2 mL of *hydrochloric acid R1* and 10 mL of *water R*. Boil for 5 min, allow to cool, add 10 mL of *sodium carbonate solution R* and allow to stand for 10 min. Dilute to 25 mL with *water R* and filter. To 5 mL of the filtrate add 2 mL of *cupri-tartaric solution R* and boil for 1 min. Allow to stand for 2 min. No red precipitate is formed.

Chlorides (*2.4.4*)

Maximum 500 ppm.

Dilute 5 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)

Maximum 500 ppm.

Dissolve 2.0 g in a mixture of 10 mL of *acetic acid R* and 90 mL of *distilled water R*.

Cadmium

Maximum 2 ppm.

Atomic absorption spectrometry (*2.2.23, Method II*).

Test solution Dissolve 5.00 g in 20 mL of *deionised distilled water R* with the aid of ultrasound and dilute to 25.0 mL with the same solvent.

Reference solutions Prepare the reference solutions using *cadmium standard solution (0.1 per cent Cd) R*, diluting with *deionised distilled water R*.

Source Cadmium hollow-cathode lamp.

Wavelength 228.8 nm.

Atomisation device Air-acetylene flame.

Water (*2.5.32*)

Maximum 12.0 per cent, determined on 80.0 mg.

Microbial contamination

TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*).

TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

ASSAY

Dissolve 0.400 g in 5 mL of *dilute acetic acid R*. Carry out the complexometric titration of zinc (*2.5.11*).

1 mL of *0.1 M sodium edetate* is equivalent to 45.57 mg of $C_{12}H_{22}ZnO_{14}$.

STORAGE

In a non-metallic, airtight container.

Ph Eur

Zinc Oxide

(Ph. Eur. monograph 0252)

ZnO	81.4	*1314-13-2*

Action and use

Mild astringent.

Preparations

Zinc Cream

Coal Tar and Zinc Ointment

Zinc Ointment

Zinc and Castor Oil Ointment

Compound Zinc Paste

Zinc and Salicylic Acid Paste

Zinc and Coal Tar Paste

Ph Eur

DEFINITION

Content

99.0 per cent to 100.5 per cent (ignited substance).

CHARACTERS

Appearance

Soft, white or faintly yellowish-white, amorphous powder, free from gritty particles.

Solubility

Practically insoluble in water and in ethanol (96 per cent). It dissolves in dilute mineral acids.

IDENTIFICATION

A. It becomes yellow when strongly heated; the yellow colour disappears on cooling.

B. Dissolve 0.1 g in 1.5 mL of *dilute hydrochloric acid R* and dilute to 5 mL with *water R*. The solution gives the reaction of zinc (*2.3.1*).

TESTS

Alkalinity

Shake 1.0 g with 10 mL of boiling *water R*. Add 0.1 mL of *phenolphthalein solution R* and filter. If the filtrate is red, not more than 0.3 mL of *0.1 M hydrochloric acid* is required to change the colour of the indicator.

Carbonates and substances insoluble in acids

Dissolve 1.0 g in 15 mL of *dilute hydrochloric acid R*. It dissolves without effervescence and the solution is not more opalescent than reference suspension II (*2.2.1*) and is colourless (*2.2.2, Method II*).

Arsenic (*2.4.2, Method A*)

Maximum 5 ppm, determined on 0.2 g.

Cadmium

Maximum 10 ppm.

Atomic absorption spectrometry (*2.2.23, Method II*).

Test solution Dissolve 2.0 g in 14 mL of a mixture of equal volumes of *water R* and *cadmium- and lead-free nitric acid R*, boil for 1 min, cool and dilute to 100.0 mL with *water R*.

Reference solutions Prepare the reference solutions using *cadmium standard solution (0.1 per cent Cd) R* and diluting with a 3.5 per cent *V/V* solution of *cadmium- and lead-free nitric acid R*.

Source Cadmium hollow-cathode lamp.

Wavelength 228.8 nm.

Atomisation device Air-acetylene or air-propane flame.

Iron (*2.4.9*)
Maximum 200 ppm.

Dissolve 50 mg in 1 mL of *dilute hydrochloric acid R* and dilute to 10 mL with *water R*. Use in this test 0.5 mL of *thioglycollic acid R*.

Lead
Maximum 50 ppm.

Atomic absorption spectrometry (*2.2.23, Method II*).

Test solution Dissolve 5.0 g in 24 mL of a mixture of equal volumes of *water R* and *cadmium- and lead-free nitric acid R*, boil for 1 min, cool and dilute to 100.0 mL with *water R*.

Reference solutions Prepare the reference solutions using *lead standard solution (0.1 per cent Pb) R* and diluting with a 3.5 per cent *V/V* solution of *cadmium- and lead-free nitric acid R*.

Source Lead hollow-cathode lamp.

Wavelength 283.3 nm; 217.0 nm may be used depending on the apparatus.

Atomisation device Air-acetylene flame.

Loss on ignition
Maximum 1.0 per cent, determined on 1.00 g by ignition to constant mass at 500 ± 50 °C.

ASSAY
Dissolve 0.150 g in 10 mL of *dilute acetic acid R*. Carry out the complexometric titration of zinc (*2.5.11*).

1 mL of *0.1 M sodium edetate* is equivalent to 8.14 mg of ZnO.

Ph Eur

Zinc Stearate

(Ph. Eur. monograph 0306)

557-05-1

Action and use
Excipient.

Ph Eur

DEFINITION
Zinc stearate [$(C_{17}H_{35}COO)_2Zn$; M_r 632] may contain varying proportions of zinc palmitate [$(C_{15}H_{31}COO)_2Zn$; M_r 576.2] and zinc oleate [$(C_{17}H_{33}COO)_2Zn$; M_r 628].

Content
10.0 per cent to 12.0 per cent of Zn.

CHARACTERS

Appearance
Light, white or almost white, amorphous powder, free from gritty particles.

Solubility
Practically insoluble in water and in anhydrous ethanol.

IDENTIFICATION
A. Freezing point (*2.2.18*): minimum 53 °C, determined on the residue obtained in the preparation of solution S (see Tests).

B. Neutralise 5 mL of solution S to *red litmus paper R* with *strong sodium hydroxide solution R*. The solution gives the reaction of zinc (*2.3.1*).

TESTS

Solution S
To 5.0 g add 50 mL of *ether R* and 40 mL of a 7.5 per cent *V/V* solution of *nitric acid R* in *distilled water R*. Heat under a reflux condenser until dissolution is complete. Allow to cool. In a separating funnel, separate the aqueous layer and shake the ether layer with 2 quantities, each of 4 mL, of *distilled water R*. Combine the aqueous layers, wash with 15 mL of *ether R* and heat on a water-bath until ether is completely eliminated. Allow to cool and dilute to 50.0 mL with *distilled water R* (solution S). Evaporate the ether layer to dryness and dry the residue at 105 °C.

Appearance of solution
Solution S is not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Appearance of solution of fatty acids
Dissolve 0.5 g of the residue obtained in the preparation of solution S in 10 mL of *chloroform R*. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5 (*2.2.2, Method II*).

Acidity or alkalinity
Shake 1.0 g with 5 mL of *ethanol (96 per cent) R* and add 20 mL of *carbon dioxide-free water R* and 0.1 mL of *phenol red solution R*. Not more than 0.3 mL of *0.1 M hydrochloric acid* or 0.1 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

Acid value of the fatty acids (*2.5.1*)
195 to 210.

Dissolve 0.20 g of the residue obtained in the preparation of solution S in 25 mL of the prescribed mixture of solvents.

Chlorides (*2.4.4*)
Maximum 250 ppm.

Dilute 2 mL of solution S to 15 mL with *water R*.

Sulfates (*2.4.13*)
Maximum 0.6 per cent.

Dilute 1 mL of solution S to 50 mL with *distilled water R*. Dilute 12.5 mL of this solution to 15 mL with *distilled water R*.

ASSAY
To 1.000 g add 50 mL of *dilute acetic acid R* and boil for at least 10 min or until the layer of fatty acids is clear, adding more *water R* as necessary to maintain the original volume. Cool and filter. Wash the filter and the flask with *water R* until the washings are no longer acid to *blue litmus paper R*. Combine the filtrate and washings. Carry out the complexometric titration of zinc (*2.5.11*).

1 mL of *0.1 M sodium edetate* is equivalent to 6.54 mg of Zn.

Ph Eur

Zinc Sulfate Heptahydrate

(Ph. Eur. monograph 0111)

$ZnSO_4,7H_2O$ 287.5 *7446-20-0*

Action and use
Astringent.

Preparations
Zinc Sulfate Eye Drops
Zinc Sulfate Injection
Zinc Sulfate Lotion

Ph Eur

DEFINITION
Content
99.0 per cent to 104.0 per cent.

CHARACTERS
Appearance
White or almost white, crystalline powder or colourless, transparent crystals, efflorescent.

Solubility
Very soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION
A. Solution S (see Tests) gives the reactions of sulfates (*2.3.1*).

B. Solution S gives the reaction of zinc (*2.3.1*).

C. It complies with the limits of the assay.

TESTS
Solution S
Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)
4.4 to 5.6 for solution S.

Chlorides (*2.4.4*)
Maximum 300 ppm.

Dilute 3.3 mL of solution S to 15 mL with *water R*.

Iron (*2.4.9*)
Maximum 100 ppm.

Dilute 2 mL of solution S to 10 mL with *water R*. Use in this test 0.5 mL of *thioglycollic acid R*.

ASSAY
Dissolve 0.200 g in 5 mL of *dilute acetic acid R*. Carry out the complexometric titration of zinc (*2.5.11*).

1 mL of *0.1 M sodium edetate* is equivalent to 28.75 mg of $ZnSO_4,7H_20$.

STORAGE
In a non-metallic, airtight container.

Ph Eur

Zinc Sulfate Hexahydrate

(Ph. Eur. monograph 1683)

$ZnSO_4,6H_2O$ 269.5 *13986-24-8*

Action and use
Astringent.

Ph Eur

DEFINITION
Content
99.0 per cent to 104.0 per cent.

CHARACTERS
Appearance
White or almost white, crystalline powder or colourless transparent crystals, efflorescent.

Solubility
Very soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION
A. Solution S (see Tests) gives the reactions of sulfates (*2.3.1*).

B. Solution S gives the reaction of zinc (*2.3.1*).

C. It complies with the limits of the assay.

TESTS
Solution S
Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)
4.4 to 5.6 for solution S.

Chlorides (*2.4.4*)
Maximum 300 ppm.

Dilute 3.3 mL of solution S to 15 mL with *water R*.

Iron (*2.4.9*)
Maximum 100 ppm.

Dilute 2 mL of solution S to 10 mL with *water R*. Use in this test 0.5 mL of *thioglycollic acid R*.

ASSAY
Dissolve 0.200 g in 5 mL of *dilute acetic acid R*. Carry out the complexometric titration of zinc (*2.5.11*).

1 mL of *0.1 M sodium edetate* is equivalent to 26.95 mg of $ZnSO_4,6H_20$.

STORAGE
In a non-metallic, airtight container.

Ph Eur

Zinc Sulfate Monohydrate

(Ph. Eur. monograph 2159)

$ZnSO_4,H_2O$ 179.5

Action and use
Astringent.

Preparations
Zinc Sulfate Capsules
Zinc Sulfate Tablets

Ph Eur

DEFINITION

Content
99.0 per cent to 101.0 per cent.

CHARACTERS

Appearance
White or almost white, crystalline powder, or colourless, transparent crystals.

Solubility
Very soluble in water, practically insoluble in ethanol (96 per cent).

IDENTIFICATION
A. Solution S (see Tests) gives the reactions of sulfates (*2.3.1*).

B. Solution S gives the reaction of zinc (*2.3.1*).

C. It complies with the limits of the assay.

TESTS

Solution S
Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Appearance of solution
Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*)
4.0 to 5.6 for solution S.

Chlorides (*2.4.4*)
Maximum 300 ppm.

Dilute 3.3 mL of solution S to 15 mL with *water R*.

Iron (*2.4.9*)
Maximum 100 ppm.

Dilute 2 mL of solution S to 10 mL with *water R*.
Use 0.5 mL of *thioglycollic acid R* in this test.

ASSAY
Dissolve 0.160 g in 5 mL of *dilute acetic acid R*. Carry out the complexometric titration of zinc (*2.5.11*).

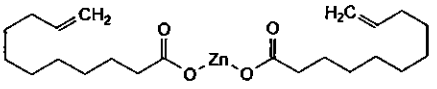
1 mL of *0.1 M sodium edetate* is equivalent to 17.95 mg of $ZnSO_4,H_2O$.

STORAGE
In a non-metallic container.

Ph Eur

Zinc Undecenoate

(Zinc Undercylenate, Ph. Eur. monograph 0539)



$C_{22}H_{38}O_4Zn$ 431.9 *557-08-4*

Action and use
Used topically in treatment of fungal infections.

Ph Eur

DEFINITION
Zinc di(undec-10-enoate).

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance
White or almost white, fine powder.

Solubility
Practically insoluble in water and in ethanol (96 per cent).

mp
116 °C to 121 °C, it may leave a slight solid residue.

IDENTIFICATION
A. To 2.5 g add 10 mL of *water R* and 10 mL of *dilute sulfuric acid R*. Shake with 2 quantities, each of 10 mL, of *ether R*. Reserve the aqueous layer for identification test C. Wash the combined ether layers with *water R* and evaporate to dryness. To the residue add 2 mL of freshly distilled *aniline R* and boil under a reflux condenser for 10 min. Allow to cool and add 30 mL of *ether R*. Shake with 3 quantities, each of 20 mL, of *dilute hydrochloric acid R* and then with 20 mL of *water R*. Evaporate the organic layer to dryness on a water-bath. The residue, after recrystallisation twice from *ethanol (70 per cent V/V) R* and drying *in vacuo* for 3 h, melts (*2.2.14*) at 66 °C to 68 °C.

B. Dissolve 0.1 g in a mixture of 2 mL of *dilute sulfuric acid R* and 5 mL of *glacial acetic acid R*. Add dropwise 0.25 mL of *potassium permanganate solution R*. The colour of the potassium permanganate solution is discharged.

C. A mixture of 1 mL of the aqueous layer obtained in identification test A and 4 mL of *water R* gives the reaction of zinc (*2.3.1*).

TESTS

Alkalinity
Mix 1.0 g with 5 mL of *ethanol (96 per cent) R* and 0.5 mL of *phenol red solution R*. Add 50 mL of *carbon dioxide-free water R* and examine immediately. No reddish colour appears.

Alkali and alkaline-earth metals
Maximum 2.0 per cent.

To 1.0 g add 25 mL of *water R* and 5 mL of *hydrochloric acid R* and heat to boiling. Filter whilst hot. Wash the filter and the residue with 25 mL of hot *water R*. Combine the filtrate and washings and add *concentrated ammonia R* until alkaline. Add 7.5 mL of *thioacetamide solution R* and heat on a water-bath for 30 min. Filter and wash the precipitate with 2 quantities, each of 10 mL, of *water R*. Combine the filtrate and washings, evaporate to dryness on a water-bath and ignite. The residue weighs a maximum of 20 mg.

Sulfates (*2.4.13*)
Maximum 500 ppm.

To 0.1 g add a mixture of 2 mL of *dilute hydrochloric acid R* and 10 mL of *distilled water R* and heat to boiling. Cool, filter and dilute to 15 mL with *distilled water R*. Prepare the standard using 5 mL of *sulfate standard solution (10 ppm SO_4) R* and 10 mL of *distilled water R*.

Loss on drying (*2.2.32*)
Maximum 1.5 per cent, determined on 0.500 g by drying in an oven at 105 °C.

Degree of unsaturation
Dissolve 0.100 g in a mixture of 5 mL of *dilute hydrochloric acid R* and 30 mL of *glacial acetic acid R*. Using 0.05 mL *indigo carmine solution R1*, added towards the end of the titration as indicator. Titrate with *0.0167 M bromide-bromate* until the colour changes from blue to yellow. 9.1 mL to 9.4 mL of *0.0167 M bromide-bromate* is required. Carry out a blank titration.

ASSAY
To 0.350 g add 25 mL of *dilute acetic acid R* and heat to boiling. Carry out the complexometric titration of zinc (*2.5.11*).

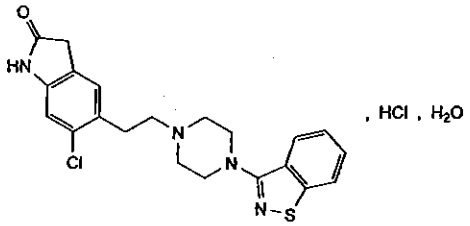
1 mL of *0.1 M sodium edetate* is equivalent to 43.19 mg of $C_{22}H_{38}O_4Zn$.

STORAGE
Protected from light.

Ph Eur

Ziprasidone Hydrochloride Monohydrate

(Ph. Eur. monograph 2421)



$C_{21}H_{22}Cl_2N_4OS,H_2O$ 467.4 *138982-67-9*

Action and use
Dopamine D_2 receptor antagonist; serotonin $5HT_2$ receptor antagonist; neuroleptic.

Ph Eur

DEFINITION
5-[2-[4-(1,2-Benzisothiazol-3-yl)piperazin-1-yl]ethyl]-6-chloro-1,3-dihydro-2*H*-indol-2-one hydrochloride monohydrate.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or slightly pink powder.

Solubility
Practically insoluble in water, slightly soluble in methanol and in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION
A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *ziprasidone hydrochloride monohydrate CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

B. Suspend 30 mg in 2 mL of *water R*, acidify with 0.15 mL of *dilute nitric acid R* and filter. The clear filtrate gives reaction (a) of chlorides (*2.3.1*).

TESTS
Related substances
Liquid chromatography (*2.2.29*). *Carry out the test protected from light and prepare the solutions immediately before use.*

Solvent mixture A *water R, methanol R* (40:60 *V/V*).

Solvent mixture B *hydrochloric acid R, water R, methanol R* (0.04:20:80 *V/V/V*).

Test solution (a) Dissolve 23 mg of the substance to be examined in solvent mixture A and dilute to 100.0 mL with solvent mixture A.

Test solution (b) Dissolve 23 mg of the substance to be examined in solvent mixture B and dilute to 50.0 mL with solvent mixture B.

Reference solution (a) Dissolve 2.5 mg of *ziprasidone for system suitability 1 CRS* (containing impurities A, B and C) in solvent mixture A and dilute to 10.0 mL with solvent mixture A.

Reference solution (b) Dilute 1.0 mL of test solution (b) to 100.0 mL with solvent mixture B. Dilute 1.0 mL of this solution to 10.0 mL with solvent mixture B.

Reference solution (c) Dissolve the contents of a vial of *ziprasidone for system suitability 2 CRS* (containing impurities D and E) in 1.0 mL of solvent mixture B.

A. *Column*:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: spherical *octylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase:
- *mobile phase A*: mix 40 volumes of *methanol R* and 60 volumes of a 6.8 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 2.5 with *phosphoric acid R*;
- *mobile phase B*: *methanol R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	100	0
20 - 21	100 → 0	0 → 100
21 - 24	0	100

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 229 nm.

Injection 20 µL of test solutions (a) and (b) and reference solutions (a) and (b).

Identification of impurities Use the chromatogram supplied with *ziprasidone for system suitability 1 CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B and C.

Relative retention With reference to ziprasidone (retention time = about 7 min): impurity A = about 0.4; impurity B = about 0.8; impurity C = about 0.9.

System suitability Reference solution (a):
- *peak-to-valley ratio*: minimum 1.2, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity B.

Limits:
- *correction factor*: for the calculation of content, multiply the peak area of impurity A by 0.7;
- *impurity B in test solution (b)*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *impurity A in test solution (b)*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
- *impurity C in test solution (a)*: not more than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
- *unspecified impurities in test solution (b)*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- *disregard limit in test solution (b)*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard the peak due to impurity C and any peak with a retention time greater than 20 min.

B. *Column*:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: spherical *octylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 35 °C.

Mobile phase Mix 5 volumes of *methanol R*, 40 volumes of a 6.8 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 6.0 with a 280 g/L solution of *potassium hydroxide R*, and 55 volumes of *acetonitrile R1*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 229 nm.

Injection 20 µL of test solution (b) and reference solutions (b) and (c).

Run time 11 times the retention time of ziprasidone.

Identification of impurities Use the chromatogram supplied with *ziprasidone for system suitability 2 CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities D and E.

Relative retention With reference to ziprasidone (retention time = about 4.5 min): impurity D = about 2.0; impurity E = about 3.0.

System suitability Reference solution (c):
- *resolution*: minimum 6.0 between the peaks due to ziprasidone and impurity D.

Limits:
- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity D = 1.4; impurity E = 0.5;
- *impurities D, E*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak eluting before the peak due to ziprasidone.

Limit:
- *total for tests A and B*: maximum 0.5 per cent.

Water (*2.5.12*)
3.7 per cent to 5.0 per cent, determined on 0.250 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*). *Carry out the test protected from light and prepare the solutions immediately before use.*

Solvent mixture *water R, methanol R* (40:60 *V/V*).

Test solution Dissolve 23.0 mg of the substance to be examined in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Reference solution Dissolve 23.0 mg of *ziprasidone hydrochloride monohydrate CRS* in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: spherical *octylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase Mix 40 volumes of *methanol R* and 60 volumes of a 6.8 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*.

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 229 nm.

Injection 20 µL.

Run time Twice the retention time of ziprasidone.

Retention time Ziprasidone = about 7 min.

System suitability Reference solution:
- *symmetry factor*: maximum 2.0 for the peak due to ziprasidone.

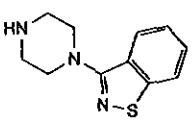
Calculate the percentage content of $C_{21}H_{22}Cl_2N_4OS$ from the declared content of *ziprasidone hydrochloride monohydrate CRS*.

STORAGE

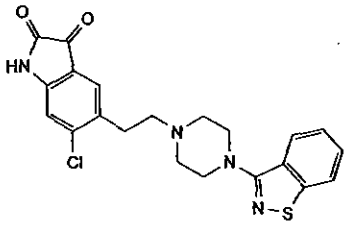
Protected from light.

IMPURITIES

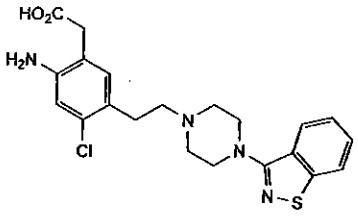
Specified impurities *A, B, C, D, E.*



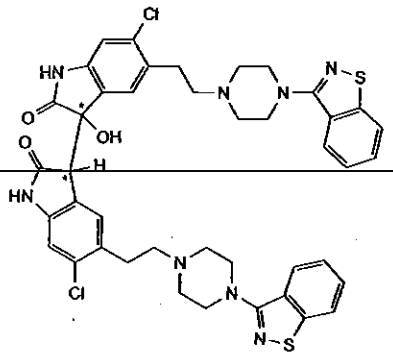
A. 3-piperazin-1-yl-1,2-benzisothiazole,



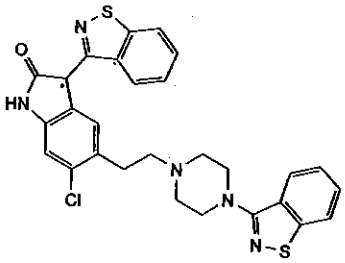
B. 5-[2-[4-(1,2-benzisothiazol-3-yl)piperazin-1-yl]ethyl]-6-chloro-1*H*-indole-2,3-dione,



C. 2-[2-amino-5-[2-[4-(1,2-benzisothiazol-3-yl)piperazin-1-yl]ethyl]-4-chlorophenyl]acetic acid,



D. 5,5′-bis[2-[4-(1,2-benzisothiazol-3-yl)piperazin-1-yl]ethyl]-6,6′-dichloro-3-hydroxy-1,1′,3,3′-tetrahydro-2*H*,2′*H*-3,3′-biindole-2,2′-dione,

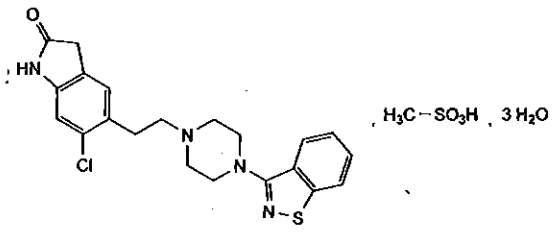


E. 3-(1,2-benzisothiazol-3-yl)-5-[2-[4-(1,2-benzisothiazol-3-yl)piperazin-1-yl]ethyl]-6-chloro-1,3-dihydro-2*H*-indol-2-one.

Ph Eur

Ziprasidone Mesilate Trihydrate

(Ph. Eur. monograph 2649)



$C_{22}H_{25}ClN_4O_4S_2,3H_2O$ 563.1 *199191-69-0*

Action and use
Dopamine D_2 receptor antagonist; serotonin 5HT2 receptor antagonist; neuroleptic.

Ph Eur

DEFINITION
5-[2-[4-(1,2-Benzisothiazol-3-yl)piperazin-1-yl]ethyl]-6-chloro-1,3-dihydro-2*H*-indol-2-one methanesulfonate trihydrate.

Content
98.0 per cent to 102.0 per cent (anhydrous substance).

PRODUCTION
It is considered that alkyl methanesulfonate esters are genotoxic and are potential impurities in ziprasidone mesilate trihydrate. The manufacturing process should be developed taking into consideration the principles of quality risk management, together with considerations of the quality of starting materials, process capability and validation.
The general methods *2.5.37. Methyl, ethyl and isopropyl methanesulfonate in methanesulfonic acid*, *2.5.38. Methyl, ethyl and isopropyl methanesulfonate in active substances* and *2.5.39. Methanesulfonyl chloride in methanesulfonic acid* are available to assist manufacturers.

CHARACTERS
Appearance
White or almost white powder.

Solubility
Very slightly soluble in water, slightly soluble in methanol, practically insoluble in acetonitrile.
It shows polymorphism (*5.9*).

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *ziprasidone mesilate trihydrate CRS.*
If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

TESTS
Related substances
Carry out the tests protected from light and prepare the solutions immediately before use.
A. Liquid chromatography (*2.2.29*).
Solvent mixture *hydrochloric acid R, water R, methanol R* (0.04:40:60 *V/V/V*).
Test solution (a) Dissolve 27.0 mg of the substance to be examined in 35 mL of the solvent mixture, sonicate for about 2 min and shake. Dilute to 50.0 mL with the solvent mixture.
Test solution (b) Dilute 2.0 mL of test solution (a) to 20.0 mL with the solvent mixture.
Reference solution (a) Dissolve 27.0 mg of *ziprasidone hydrochloride monohydrate CRS* in 35 mL of the solvent mixture, sonicate for about 2 min and shake. Dilute to 50.0 mL with the solvent mixture. Dilute 2.0 mL of the solution to 20.0 mL with the solvent mixture.
Reference solution (b) Dissolve 2.5 mg of *ziprasidone for system suitability 1 CRS* (containing impurities B and C) in a mixture of 40 volumes of *water R* and 60 volumes of *methanol R* and dilute to 10 mL with the same mixture of solvents.
Reference solution (c) Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.
Column:
— *size:* l = 0.15 m, Ø = 3.9 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 40 °C.

Mobile phase:
— *mobile phase A*: mix 40 volumes of *methanol R1* and 60 volumes of a 6.8 g/L solution of *potassium dihydrogen phosphate R* previously adjusted to pH 3.0 with *phosphoric acid R*;
— *mobile phase B*: *methanol R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 40	100	0
40 - 41	100 → 0	0 → 100
41 - 50	0	100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 229 nm.

Injection 20 µL of test solution (a) and reference solutions (b) and (c).

Identification of impurities Use the chromatogram supplied with *ziprasidone for system suitability 1 CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B and C.

Relative retention With reference to ziprasidone (retention time = about 6 min): impurity B = about 0.8; impurity C = about 0.9.

System suitability Reference solution (b):
— *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline for the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity B; minimum 2.0, where H_p = height above the baseline for the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to ziprasidone.

Calculation of percentage contents:
— for each impurity, use the concentration of ziprasidone mesilate trihydrate in reference solution (c).

Limits:
— *impurity B*: maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *reporting threshold*: 0.05 per cent; disregard any peak with a retention time greater than 40 min.

B. Liquid chromatography (*2.2.29*).

Solvent mixture *hydrochloric acid R, water R, methanol R* (0.04:20:80 *V/V/V*).

Test solution Dissolve 27 mg of the substance to be examined in 35 mL of the solvent mixture, sonicate for about 2 min and shake. Dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dissolve the contents of a vial of *ziprasidone for system suitability 2 CRS* (containing impurities D and E) in 1 mL of the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.15 m, Ø = 3.9 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 35 °C.

Mobile phase Mix 8 volumes of *methanol R1*, 42 volumes of *acetonitrile for chromatography R* and 50 volumes of a solution prepared as follows: dissolve 3.4 g of *potassium dihydrogen phosphate R* and 21.6 g of *sodium octanesulfonate R* in 900 mL of *water for chromatography R*, adjust to pH 3.0 with *phosphoric acid R* and dilute to 1000 mL with *water for chromatography R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 229 nm.

Injection 20 µL.

Run time 15 times the retention time of ziprasidone.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peaks due to impurities D and E.

Relative retention With reference to ziprasidone (retention time = about 3 min): impurity E = about 2.6; impurity D = about 7.8.

System suitability Reference solution (a):
— *resolution*: minimum 5.0 between the peaks due to impurities E and D.

Calculation of percentage contents:
— for each impurity, use the concentration of ziprasidone mesilate trihydrate in reference solution (b).

Limits:
— *impurity D*: maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *reporting threshold*: 0.05 per cent; disregard any peak eluting before the peak due to ziprasidone.

Limit:
— *total for tests A and B*: maximum 0.2 per cent.

Water (*2.5.12*)
8.5 per cent to 10.1 per cent, determined on 0.050 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

Bacterial endotoxins (*2.6.14*)
Less than 17.5 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*) as described in test A for related substances with the following modifications.

Injection 20 µL of test solution (b) and reference solution (a).

System suitability Reference solution (a):
— *symmetry factor*: maximum 1.7 for the peak due to ziprasidone.

Calculate the percentage content of $C_{22}H_{25}ClN_4O_4S_2$ taking into account the assigned content of *ziprasidone hydrochloride monohydrate CRS* and a conversion factor of 1.133.

STORAGE

Protected from light. If the substance is sterile, store in a sterile, airtight, tamper-evident container.

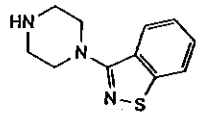
IMPURITIES

Test A for related substances
A, B, C.

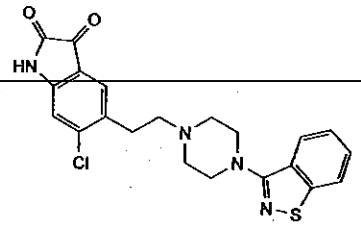
Test B for related substances
D, E.

Specified impurities *B, D.*

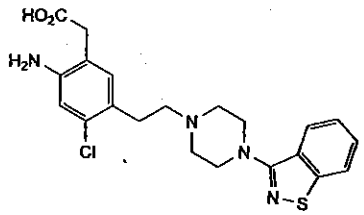
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, C, E.*



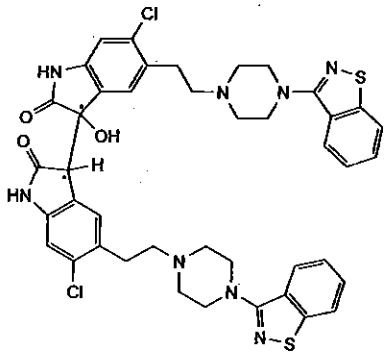
A. 3-piperazin-1-yl-1,2-benzisothiazole,



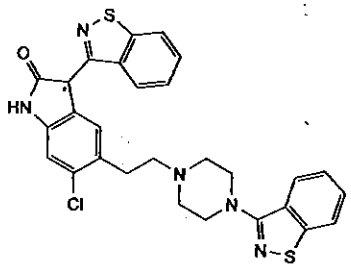
B. 5-[2-[4-(1,2-benzisothiazol-3-yl)piperazin-1-yl]ethyl]-6-chloro-1*H*-indole-2,3-dione,



C. 2-[2-amino-5-[2-[4-(1,2-benzisothiazol-3-yl)piperazin-1-yl]ethyl]-4-chlorophenyl]acetic acid,



D. 5,5′-bis[2-[4-(1,2-benzisothiazol-3-yl)piperazin-1-yl]ethyl]-6,6′-dichloro-3-hydroxy-1,1′,3,3′-tetrahydro-2*H*,2′*H*-3,3′-biindole-2,2′-dione,

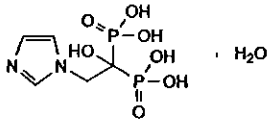


E. 3-(1,2-benzisothiazol-3-yl)-5-[2-[4-(1,2-benzisothiazol-3-yl)piperazin-1-yl]ethyl]-6-chloro-1,3-dihydro-2*H*-indol-2-one.

Ph Eur

Zoledronic Acid Monohydrate

(Ph. Eur. monograph 2743)



$C_5H_{10}N_2O_7P_2,H_2O$ 290.1 *165800-06-6*

Action and use
Bisphosphonate; treatment of osteolytic lesions, hypercalcaemia.

Ph Eur

DEFINITION

[1-Hydroxy-2-(1*H*-imidazol-1-yl)ethane-1,1-diyl] bis(phosphonic acid) monohydrate.

Content
99.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Slightly soluble in water, practically insoluble in anhydrous ethanol and in heptane.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *zoledronic acid monohydrate CRS.*

B. Water (see Tests).

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution B_7 or BY_7 (*2.2.2, Method II*).

Dissolve 0.5 g in a 4 g/L solution of *sodium hydroxide R* and dilute to 50.0 mL with the same solution.

pH (*2.2.3*)
1.8 to 2.8.

Dissolve 0.150 g in *carbon dioxide-free water R* and dilute to 50.0 mL with the same solvent. Sonicate for 10 min, if necessary.

Related substances
Liquid chromatography (*2.2.29*).

Solution A Dissolve 10.8 g of *sodium octanesulfonate R* and 37 mg of *sodium edetate R* in *water for chromatography R*, add 10 mL of *perchloric acid R* and 2 mL of *phosphoric acid R* and dilute to 1000 mL with *water for chromatography R*.

Test solution Dissolve 40.0 mg of the substance to be examined in the mobile phase, sonicate for 30 min, and dilute to 20.0 mL with the mobile phase.

Reference solution (a) Dissolve 2 mg of *zoledronic acid impurity A CRS*, 5 mg of *zoledronic acid impurity B CRS* and 2 mg of *sodium nitrate R* in the mobile phase and dilute to 50 mL with the mobile phase. To 1 mL of the solution add 7 mL of the mobile phase and dilute to 20 mL with the test solution.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:
— *size: l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase: end-capped phenylhexylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 20 °C.

Mobile phase *acetonitrile R1*, solution A (4:96 *V/V*).

Flow rate 0.6 mL/min.

Detection Spectrophotometer at 215 nm.

Preconditioning Precondition the instrument and the column once before each series of injections as follows:
— *instrument*: rinse the instrument without a column with a 25 per cent *V/V* solution of *acetic acid R* at 5 mL/min for about 20 min. Then, rinse with *water for chromatography R* at 5 mL/min for about 2 h;
— *column*: rinse the column with the mobile phase at 0.6 mL/min for about 1 h. During the rinsing, inject the test solution 15 times, applying a run time of about 3 min for each injection.

Injection 10 µL.

Run time 5 times the retention time of zoledronic acid.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A and B and the nitrate ion.

Relative retention With reference to zoledronic acid (retention time = about 6 min): nitrate = about 0.6; impurity B = about 0.7; impurity A = about 0.9.

System suitability Reference solution (a):
— *resolution*: minimum 1.5 between the peaks due to impurity A and zoledronic acid; minimum 1.5 between the peaks due to the nitrate ion and impurity B.

Calculation of percentage contents:
— *correction factor*: multiply the peak area of impurity B by 1.9;
— for each impurity, use the concentration of zoledronic acid monohydrate in reference solution (b).

Limits:
— *impurity B*: maximum 0.5 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.5 per cent;
— *reporting threshold*: 0.05 per cent, disregard the peak due to the nitrate ion.

Impurities E and F

Liquid chromatography (*2.2.29*).

Test solution Dissolve 50.0 mg of the substance to be examined in mobile phase B, sonicate if necessary, and dilute to 10.0 mL with mobile phase B.

Reference solution (a) Dissolve 95.0 mg of *anhydrous sodium dihydrogen phosphate R* and 79.0 mg of *phosphorous acid R* (impurity E) in *water R* and dilute to 100.0 mL with the same solvent.

Reference solution (b) Dissolve 34.0 mg of *sodium chloride R* in *water R* and dilute to 100.0 mL with the same solvent.

Reference solution (c) To 1.0 mL of reference solution (a) add 0.5 mL of reference solution (b) and dilute to 100.0 mL with mobile phase B.

Reference solution (d) Dilute 0.1 mL of reference solution (a) to 100 mL with mobile phase B.

Precolumn:
— *size: l* = 0.05 m, Ø = 4.0 mm;
— *stationary phase: anion-exchange resin R* (13 µm).

Column:
— *size: l* = 0.25 m, Ø = 4.0 mm;
— *stationary phase: anion-exchange resin R* (9 µm);
— *temperature*: 30 °C.

Mobile phase:
— *mobile phase A: carbon dioxide-free water R*;
— *mobile phase B*: 4.0 g/L solution of *sodium hydroxide R* in *carbon dioxide-free water R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 13	80 → 70	20 → 30
13 - 17	70 → 60	30 → 40
17 - 29	60	40

Flow rate 1.0 mL/min.

Detection Conductivity detector; use a self-regenerating anion suppressor.

Injection 20 µL.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peaks due to impurities E and F; use the chromatogram obtained with reference solution (b) to identify the peak due to the chloride ion.

Relative retention With reference to chloride (retention time = about 5 min): impurity E = about 1.2; impurity F = about 3.4.

System suitability:
— *resolution*: minimum 1.5 between the peaks due to the chloride ion and impurity E in the chromatogram obtained with reference solution (c);
— *signal-to-noise ratio*: minimum 10 for the peak due to impurity F in the chromatogram obtained with reference solution (d).

Calculation of percentage contents:
— for each impurity, use the concentration of the corresponding impurity in reference solution (c).

Limits:
— *impurities E, F*: for each impurity, maximum 0.15 per cent.

Water (*2.5.12*)

5.0 per cent to 7.5 per cent, determined on 0.100 g.

ASSAY

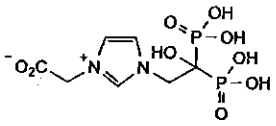
Dissolve 0.150 g in 50 mL of *carbon dioxide-free water R*. Sonicate for 10 min, if necessary. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Read the volume added at the 3rd inflexion point.

1 mL of *0.1 M sodium hydroxide* is equivalent to 9.07 mg of $C_5H_{10}N_2O_7P_2$.

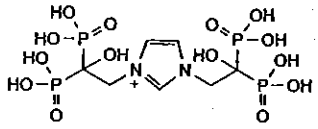
IMPURITIES

Specified impurities: *B, E, F.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, C, D.*

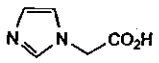


A. [1-(2-hydroxy-2,2-diphosphonoethyl)-1*H*-imidazol-3-ium-3-yl]acetate,

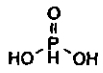


B. 1,3-bis(2-hydroxy-2,2-diphosphonoethyl)-1*H*-imidazol-3-ium,

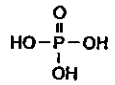
C. 1*H*-imidazole,



D. (1*H*-imidazol-1-yl)acetic acid,



E. phosphonic acid (phosphorous acid),

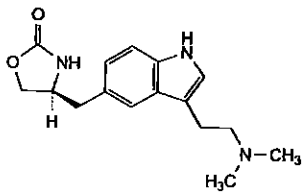


F. phosphoric acid.

Ph Eur

Zolmitriptan

(Ph. Eur. monograph 2737)



$C_{16}H_{21}N_3O_2$ 287.4 *139264-17-8*

Action and use
Serotonin 5HT1 receptor agonist; treatment of migraine.

Ph Eur

DEFINITION
(4*S*)-4-[[3-[2-(Dimethylamino)ethyl]-1*H*-indol-5-yl]methyl]-1,3-oxazolidin-2-one.

Content
97.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS
Appearance
White or almost white powder.

Solubility
Slightly soluble or very slightly soluble in water, freely soluble in methanol, sparingly soluble in acetone.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).
Comparison *zolmitriptan CRS*.

TESTS
Enantiomeric purity
Liquid chromatography (*2.2.29*).
Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 mL with the mobile phase.
Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.
Reference solution (b) Dissolve 5 mg of *zolmitriptan impurity A CRS* in the mobile phase and dilute to 5.0 mL with the mobile phase. To 1.0 mL of the solution add 1.0 mL of the test solution and dilute to 10.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 50.0 mL with the mobile phase.
Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *amylose derivative of silica gel for chiral separation R* (5 µm);
— *temperature*: 35 °C.
Mobile phase *diethylamine R, 2-propanol R, methanol R, heptane R* (0.1:10:15:75 *V/V/V/V*).
Flow rate 1.0 mL/min.
Detection Spectrophotometer at 285 nm.
Injection 10 µL.
Run time Twice the retention time of zolmitriptan.
Relative retention With reference to zolmitriptan (retention time = about 7 min): impurity A = 0.7.
System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the peaks due to impurity A and zolmitriptan.
Calculation of percentage content:
— for impurity A, use the concentration of zolmitriptan in reference solution (a).
Limit:
— *impurity A*: maximum 0.10 per cent.

Related substances
Liquid chromatography (*2.2.29*).
Solvent mixture Mobile phase B, mobile phase A (10:90 *V/V*).
Test solution (a) Dissolve 10.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.
Test solution (b) Dilute 1.0 mL of test solution (a) to 5.0 mL with the solvent mixture.
Reference solution (a) Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.
Reference solution (b) Dissolve 5 mg of *zolmitriptan for system suitability CRS* (containing impurities C, H and I) in the solvent mixture and dilute to 5.0 mL with the solvent mixture.
Reference solution (c) Dissolve 10.0 mg of *zolmitriptan CRS* in the solvent mixture and dilute to 10.0 mL with the solvent

mixture. Dilute 1.0 mL of the solution to 5.0 mL with the solvent mixture.

Column:
— *size*: *l* = 0.10 m, Ø = 3.0 mm;
— *stationary phase*: *end-capped solid core phenylhexylsilyl silica gel for chromatography R* (2.7 µm);
— *temperature*: 20 °C.

Mobile phase A Dissolve 2.72 g of *potassium dihydrogen phosphate R* and 0.94 g of *sodium hexanesulfonate R* in *water for chromatography R*, adjust to pH 2.0 with *phosphoric acid R* and dilute to 1000 mL with *water for chromatography R*;

Mobile phase B *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 0.5	90	10
0.5 - 4	90 → 85	10 → 15
4 - 8	85	15
8 - 9	85 → 80	15 → 20
9 - 10	80	20
10 - 12	80 → 70	20 → 30
12 - 13	70	30

Flow rate 0.8 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 2 µL of test solution (a) and reference solutions (a) and (b).

Identification of impurities Use the chromatogram supplied with *zolmitriptan for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities C, H and I.

Relative retention With reference to zolmitriptan (retention time = about 5 min): impurity H = about 0.97; impurity I = about 1.1; impurity C = about 2.0.

System suitability Reference solution (b):
— *peak-to-valley ratio*: minimum 8, where H_p = height above the baseline of the peak due to impurity H and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to zolmitriptan; minimum 1.5, where H_p = height above the baseline of the peak due to impurity I and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to zolmitriptan.

Calculation of percentage contents:
— *correction factor*: multiply the peak area of impurity C by 2.0;
— for each impurity, use the concentration of zolmitriptan in reference solution (a).

Limits:
— *impurity C*: maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.5 per cent;
— *reporting threshold*: 0.05 per cent.

Water (*2.5.12*)
Maximum 0.5 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Detection Spectrophotometer at 283 nm.

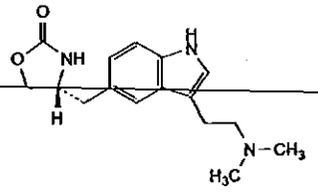
Injection 2 µL of test solution (b) and reference solution (c).

Calculate the percentage content of $C_{16}H_{21}N_3O_2$ taking into account the assigned content of *zolmitriptan CRS*.

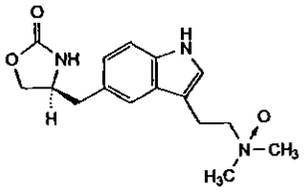
IMPURITIES

Specified impurities *A, C.*

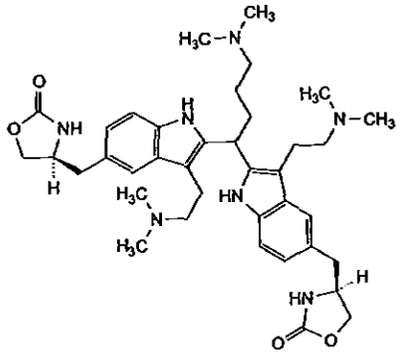
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *B, D, E, F, G, H, I.*



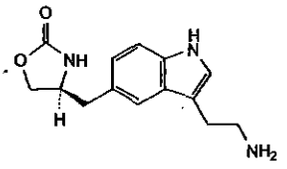
A. (4*R*)-4-[[3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl] methyl]-1,3-oxazolidin-2-one,



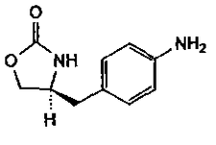
B. *N*,*N*-dimethyl-2-[5-[[(4*S*)-2-oxo-1,3-oxazolidin-4-yl] methyl]-1*H*-indol-3-yl]ethan-1-amine *N*-oxide,



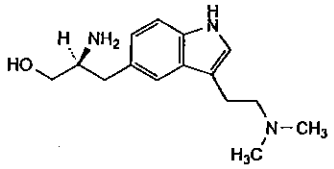
C. (4*S*,4′*S*)-4,4′-[[4-(dimethylamino)butane-1,1-diyl]bis[[3-[2-(dimethylamino)ethyl]-1*H*-indole-2,5-diyl]methylene]] bis(1,3-oxazolidin-2-one),



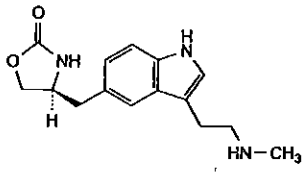
D. (4*S*)-4-[[3-(2-aminoethyl)-1*H*-indol-5-yl]methyl]-1,3-oxazolidin-2-one,



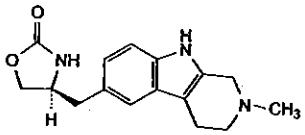
E. (4*S*)-4-[(4-aminophenyl)methyl]-1,3-oxazolidin-2-one,



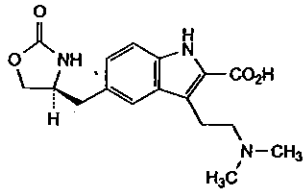
F. (2*S*)-2-amino-3-[3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl]propan-1-ol,



G. (4*S*)-4-[[3-[2-(methylamino)ethyl]-1*H*-indol-5-yl]methyl]-1,3-oxazolidin-2-one,



H. (4*S*)-4-[(2-methyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indol-6-yl)methyl]-1,3-oxazolidin-2-one,

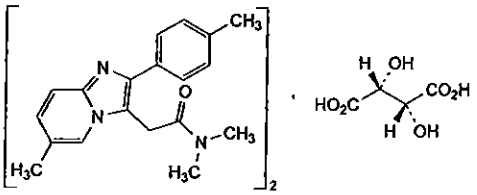


I. 3-[2-(dimethylamino)ethyl]-5-[[(4*S*)-2-oxo-1,3-oxazolidin-4-yl]methyl]-1*H*-indole-2-carboxylic acid.

Ph Eur

Zolpidem Tartrate

(Ph. Eur. monograph 1280)



$C_{42}H_{48}N_6O_8$ 765 *99294-93-6*

Action and use
Non-benzodiazepine hypnotic.

Preparation
Zolpidem Tablets

Ph Eur

DEFINITION

Bis[*N*,*N*-dimethyl-2-[6-methyl-2-(4-methylphenyl)imidazo[1,2-*a*]pyridin-3-yl]acetamide] (2*R*,3*R*)-2,3-dihydroxybutanedioate.

Content
98.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance
White or almost white, hygroscopic, crystalline powder.

Solubility
Slightly soluble in water, sparingly soluble in methanol, practically insoluble in methylene chloride.

IDENTIFICATION

First identification: A, C.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Dissolve 0.10 g in 10 mL of a 10.3 g/L solution of *hydrochloric acid R*. Add 10 mL of *water R*. Add dropwise with stirring 1 mL of *dilute ammonia R2*. Filter and collect the resulting precipitate. Wash the precipitate with *water R* and then dry at 105 °C for 2 h. Examine the precipitate as a disc.

Comparison Repeat the operations using 0.10 g of *zolpidem tartrate CRS*.

B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 50 mg of the substance to be examined in 5 mL of *methanol R*, add 0.1 mL of *diethylamine R* and dilute to 10 mL with *methanol R*.

Reference solution (a) Dissolve 50 mg of *zolpidem tartrate CRS* in 5 mL of *methanol R*, add 0.1 mL of *diethylamine R* and dilute to 10 mL with *methanol R*.

Reference solution (b) Dissolve 50 mg of *flunitrazepam CRS* in 5 mL of *methylene chloride R* and dilute to 10 mL with the same solvent. Mix 1 mL of this solution and 1 mL of reference solution (a).

Plate *TLC silica gel F_{254} plate R.*

Mobile phase *diethylamine R, cyclohexane R, ethyl acetate R* (10:45:45 *V/V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Retardation factors Zolpidem = about 0.3; flunitrazepam = about 0.5.

System suitability Reference solution (b):
— the chromatogram shows 2 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve about 0.1 g in 1 mL of *methanol R*, heating gently. 0.1 mL of this solution gives reaction (b) of tartrates (*2.3.1*).

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 or BY_6 (*2.2.2, Method II*). *Prepare the solutions protected from light and carry out the test as rapidly as possible.*

Triturate 0.25 g with 0.125 g of *tartaric acid R*. Dissolve the mixture in 20 mL of *water R* and dilute to 25 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*).

Test solution Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 2.5 mg of *zolpidem for system suitability CRS* (containing impurities A and B) in the mobile phase and dilute to 5 mL with the mobile phase.

Column:
— *size*: *l* = 0.15 m, Ø = 3.9 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (4 µm).

Mobile phase Mix 18 volumes of *acetonitrile R*, 23 volumes of *methanol R* and 59 volumes of a 5.6 g/L solution of *phosphoric acid R* previously adjusted to pH 5.5 with *triethylamine R*.

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL.

Run time 4 times the retention time of zolpidem.

Identification of impurities Use the chromatogram supplied with *zolpidem for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and B.

Relative retention With reference to zolpidem (retention time = about 7 min): tartaric acid = about 0.1; impurity A = about 0.8; impurity B = about 3.6.

System suitability Reference solution (b):
— *resolution*: minimum 2.0 between the peaks due to impurity A and zolpidem.

Calculation of percentage contents:
— for each impurity, use the concentration of zolpidem tartrate in reference solution (a).

Limits:
— *impurity B*: maximum 0.15 per cent;
— *unspecified impurities*: for each impurity, maximum 0.10 per cent;
— *total*: maximum 0.2 per cent;
— *reporting threshold*: 0.05 per cent; disregard the peak due to tartaric acid.

Water (*2.5.12*)
Maximum 3.0 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in a mixture of 20 mL of *anhydrous acetic acid R* and 20 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 mL of *0.1 M perchloric acid* is equivalent to 38.24 mg of $C_{42}H_{48}N_6O_8$.

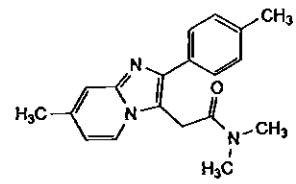
STORAGE

In an airtight container, protected from light.

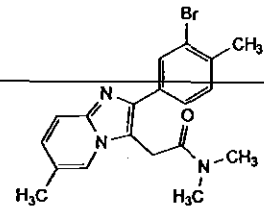
IMPURITIES

Specified impurities *B*.

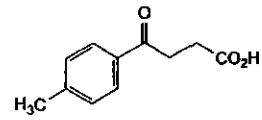
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, C, D, E, F*.



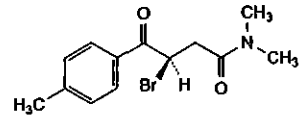
A. *N,N*-dimethyl-2-[7-methyl-2-(4-methylphenyl)imidazo [1,2-*a*]pyridin-3-yl]acetamide,



B. 2-[2-(3-bromo-4-methylphenyl)-6-methylimidazo[1,2-*a*] pyridin-3-yl]-*N,N*-dimethylacetamide,

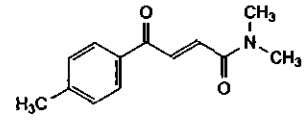


C. 4-(4-methylphenyl)-4-oxobutanoic acid,

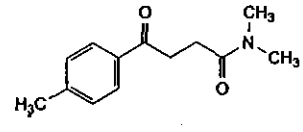


and enantiomer

D. (3*RS*)-3-bromo-*N,N*-dimethyl-4-(4-methylphenyl)-4-oxobutanamide,



E. (2*E*)-*N,N*-dimethyl-4-(4-methylphenyl)-4-oxobut-2-enamide,

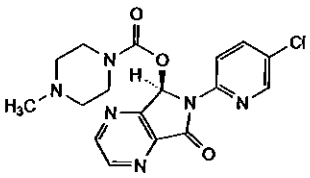


F. *N,N*-dimethyl-4-(4-methylphenyl)-4-oxobutanamide.

Ph Eur

Zopiclone

(Ph. Eur. monograph 1060)



and enantiomer

$C_{17}H_{17}ClN_6O_3$ 388.8 *43200-80-2*

Action and use
Non-benzodiazepine hypnotic.

Preparation
~~Zopiclone Tablets~~

Ph Eur

DEFINITION

(5*RS*)-6-(5-Chloropyridin-2-yl)-7-oxo-6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyrazin-5-yl 4-methylpiperazine-1-carboxylate.

Content
98.5 per cent to 100.5 per cent.

CHARACTERS

Appearance
White or slightly yellowish powder.

Solubility
Practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in acetone, practically insoluble in ethanol (96 per cent). It dissolves in dilute mineral acids.

IDENTIFICATION

First identification: B.

Second identification: A, C.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution Dissolve 50.0 mg in a 3.5 g/L solution of *hydrochloric acid R* and dilute to 100.0 mL with the same solvent. Dilute 2.0 mL of this solution to 100.0 mL with a 3.5 g/L solution of *hydrochloric acid R*.

Spectral range 220-350 nm.

Absorption maximum At 303 nm.

Specific absorbance at the absorption maximum 340 to 380.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *zopiclone CRS*.

C. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 10 mg of the substance to be examined in *methylene chloride R* and dilute to 10 mL with the same solvent.

Reference solution Dissolve 10 mg of *zopiclone CRS* in *methylene chloride R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel GF_{254} plate R.*

Mobile phase *triethylamine R, acetone R, ethyl acetate R* (2:50:50 *V/V/V*).

Application 10 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Solution S
Dissolve 1.0 g in *dimethylformamide R* and dilute to 20.0 mL with the same solvent.

Appearance of solution
Solution S is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than intensity 5 of the range of reference solutions of the most appropriate colour (*2.2.2, Method II*).

Optical rotation (*2.2.7*)
−0.05° to + 0.05°.

Dilute 10.0 mL of solution S to 50.0 mL with *dimethylformamide R*.

~~Related substances~~
Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution Dissolve 40.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a) Dilute 2.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b) Dissolve 4.0 mg of *zopiclone oxide CRS* (impurity A) in the mobile phase and dilute to 10.0 mL with the mobile phase. To 5.0 mL of the solution add 0.5 mL of the test solution and dilute to 50.0 mL with the mobile phase.

Reference solution (c) Dissolve 2.0 mg of *zopiclone impurity B CRS* in the mobile phase and dilute to 5.0 mL with the mobile phase. Dilute 1.0 mL of the solution to 100.0 mL with the mobile phase.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 30 °C.

Mobile phase Mix 38 volumes of *acetonitrile R* and 62 volumes of a solution containing 8.1 g/L of *sodium laurilsulfate R* and 1.6 g/L of *sodium dihydrogen phosphate R*, previously adjusted to an apparent pH of 3.5 with a 10 per cent *V/V* solution of *phosphoric acid R*.

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 303 nm.

Injection 20 µL.

Run time 1.5 times the retention time of zopiclone.

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peak due to impurity B.

Retention time Zopiclone = 27 min to 31 min; if necessary, adjust the concentration of acetonitrile in the mobile phase.

Relative retention With reference to zopiclone: impurity B = about 0.1; impurity A = about 0.9.

System suitability Reference solution (b):
— *resolution*: minimum 3.0 between the peaks due to impurity A and zopiclone; if necessary, adjust the mobile phase to pH 4.0 with a 10 per cent *V/V* solution of *phosphoric acid R*.

Limits:
- — *impurity B*: not more than twice the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.2 per cent);
- — *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
- — *total (excluding impurity B)*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
- — *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

2-Propanol

Gas chromatography (*2.2.28*).

Internal standard solution Dilute 5 mL of *ethanol R1* to 100 mL with *ethylene chloride R*. Dilute 1 mL of the solution to 10 mL with *ethylene chloride R*.

Test solution Dissolve 0.25 g of the substance to be examined in *ethylene chloride R*, add 0.5 mL of the internal standard solution and dilute to 5.0 mL with *ethylene chloride R*.

Reference solution Dilute 4.5 mL of *2-propanol R* to 100.0 mL with *ethylene chloride R*. To 1.0 mL of the solution add 10.0 mL of the internal standard solution and dilute to 100.0 mL with *ethylene chloride R*.

Column:
- — *material*: fused silica;
- — *size*: *l* = 10 m, Ø = about 0.53 mm;
- — *stationary phase*: *styrene-divinylbenzene copolymer R* (film thickness 20 µm).

Carrier gas *helium for chromatography R*.

Flow rate 4 mL/min.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 5	50
	5 - 10	50 → 70
	10 - 14	70
	14 - 20.5	70 → 200
	20.5 - 27.5	200
Injection port		150
Detector		250

Detection Flame ionisation.

Injection 1 µL.

Calculate the percentage content *m/m* of 2-propanol taking its density to be 0.785 g/mL at 20 °C.

Limit:
- — *2-propanol*: maximum 0.7 per cent *m/m*.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.300 g in a mixture of 10 mL of *anhydrous acetic acid R* and 40 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 38.88 mg of $C_{17}H_{17}ClN_6O_3$.

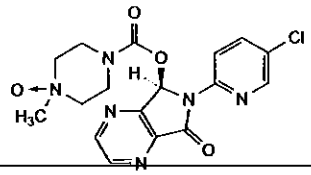
STORAGE

Protected from light.

IMPURITIES

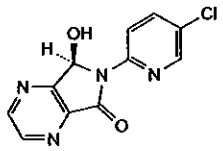
Specified impurities *B*.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, C*.



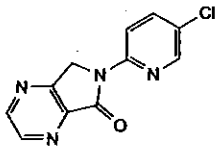
and enantiomer

A. (5*RS*)-6-(5-chloropyridin-2-yl)-7-oxo-6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyrazin-5-yl 4-methylpiperazine-1-carboxylate 4-oxide (zopiclone oxide),



and enantiomer

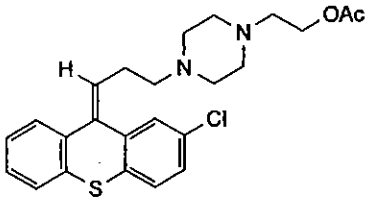
B. (7*RS*)-6-(5-chloropyridin-2-yl)-7-hydroxy-6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyrazin-5-one,



C. 6-(5-chloropyridin-2-yl)-6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyrazin-5-one.

Ph Eur

Zuclopenthixol Acetate



$C_{24}H_{27}ClN_2O_2S$ 443.0 *85721-05-7*

Action and use

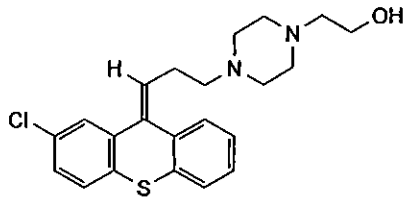
Dopamine receptor antagonist; neuroleptic.

Preparation

Zuclopenthixol Acetate Injection

DEFINITION

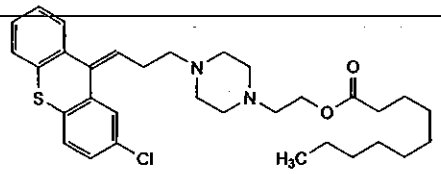
Zuclopenthixol Acetate is (*Z*)-2-4-[3-(2-chlorothioxanthene-9-ylidene)propyl]piperazin-1-ylethyl acetate. It contains not less than 98.0% and not more than 102.0% of $C_{24}H_{27}ClN_2O_2S$, calculated with reference to the dried substance.



C. zuclopenthixol.

Zuclopenthixol Decanoate

(Ph. Eur. monograph 1707)



$C_{32}H_{43}ClN_2O_2S$ 555.2 *64053-00-5*

Action and use
Dopamine receptor antagonist; neuroleptic.

Preparation
Zuclopenthixol Decanoate Injection

Ph Eur

DEFINITION
2-[4-[3-[(9*Z*)-2-Chloro-9*H*-thioxanthen-9-ylidene]propyl]piperazin-1-yl]ethyl decanoate.

Content
98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS
Appearance
Yellow, viscous, oily liquid.

Solubility
Very slightly soluble in water, very soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION
Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of zuclopenthixol decanoate.*

TESTS
Appearance of solution
The solution is clear (*2.2.1*).

Using an ultrasonic bath, dissolve 1.0 g in *ethanol (96 per cent) R* and dilute to 20.0 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*). *Carry out the test protected from light and prepare the solutions immediately before use.*

Solution A Dissolve 8.89 g of *docusate sodium R* in *water for chromatography R*, stirring for about 6-8 h, and dilute to 1000 mL with the same solvent.

Test solution Dissolve 25.0 mg of the substance to be examined in *acetonitrile R* and dilute to 100.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with *acetonitrile R*.

Reference solution (b) Dissolve 5.0 mg of *zuclopenthixol impurity B CRS* in *acetonitrile R* and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of this solution to 100.0 mL with *acetonitrile R*.

Reference solution (c) Dissolve the contents of a vial of *zuclopenthixol for system suitability CRS* (containing impurities A, B and C) in 1 mL of *methanol R*.

Column:
— *size*: *l* = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
— *temperature*: 40 °C.

Mobile phase Mix 25 volumes of solution A and 75 volumes of *anhydrous ethanol R*, then add 0.1 volumes of *phosphoric acid R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 270 nm.

Injection 20 µL.

Run time Twice the retention time of zuclopenthixol decanoate.

Identification of impurities Use the chromatogram supplied with *zuclopenthixol for system suitability CRS* and the chromatograms obtained with reference solutions (b) and (c) to identify the peaks due to impurities A, B and C.

Relative retention With reference to zuclopenthixol decanoate (retention time = about 12 min): impurity C = about 0.4; impurity B = about 0.5; impurity A = about 1.1.

System suitability Reference solution (c):
— *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity B; and minimum 2.5, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to zuclopenthixol decanoate.

Limits:
— *impurity A*: not more than 1.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.3 per cent);
— *impurity B*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
— *impurity C*: not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
— *unspecified impurities*: for each impurity, not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
— *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent);
— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 3 h.

Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

CHARACTERISTICS

A yellowish, viscous oil.

Very slightly soluble in *water*; very soluble in *dichloromethane*, in *ethanol (96%)* and in *ether*.

IDENTIFICATION

A. The *light absorption* of a 0.0015% w/v solution in *ethanol (96%)*, Appendix II B, in the range 210 to 350 nm exhibits two maxima at 230 and 268 nm. The *absorbances* at the maxima are about 1.18 and 0.51 respectively.

B. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of zuclopenthixol acetate *(RS 363)*.

TESTS

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions protected from light.

(1) 0.250% w/v of the substance being examined in *dichloromethane*.

(2) Dilute 1 volume of solution (1) to 100 volume with *dichloromethane*, further dilute 1 volume of this solution to 10 volumes with the same solvent.

(3) Dilute 1 volume of solution (2) to 2 volumes with *dichloromethane*.

(4) 0.00050% w/v of *2- chlorothioxanthone BPCRS* in *dichloromethane*.

(5) 0.000625% w/v of *zuclopenthixol hydrochloride BPCRS* in a solution containing 3 drops of *diethylamine* in *dichloromethane*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* F_{254} (Merck silica gel 60 F254 plates are suitable).

(b) Use an unlined tank and the mobile phase as described below.

(c) Apply 4 µL of each solution.

(d) Develop the plate to 10 cm.

(e) After removal of the plate, allow it to dry in air, spray with a mixture of equal volumes of *sulfuric acid* and *absolute ethanol*, heat at 110° for 5 minutes and examine under *ultraviolet light (365 nm)* immediately.

MOBILE PHASE

10 volumes of *diethylamine*, 40 volumes of *dichloromethane* and 50 volumes of *cyclohexane*.

LIMITS

In the chromatogram obtained with solution (1):

any spot corresponding to 2-chlorothioxanthone is not more intense than the spot in the chromatogram obtained with solution (4) (0.2%);

any spot corresponding to zuclopenthixol is not more intense than the spot in the chromatogram obtained with solution (5) (0.25%);

any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (2) (0.1%);

not more than three other secondary spots are more intense than the spot in the chromatogram obtained with solution (3) (0.05%).

trans-Isomer

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *dichloromethane* protected from light.

(1) 0.040% w/v of the substance being examined.

(2) 0.00046% w/v of *trans-clopenthixol acetate dihydrochloride BPCRS* (equivalent to 0.00040% w/v of *trans*-clopenthixol acetate).

(3) 0.020% w/v of the substance being examined and 0.023% w/v of trans-*clopenthixol acetate dihydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use stainless steel column (25 cm x 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Spherisorb S 5W is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use ambient temperature.

(e) Use a detection wavelength of 254 nm.

(f) Inject 15 µL of each solution.

MOBILE PHASE

0.03 volume of 13.5M *ammonia*, 45 volumes of *dichloromethane*, 45 volumes of *heptane* and 50 volumes of *acetonitrile*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the principal peaks is at least 2.6.

LIMITS

In the chromatogram obtained with solution (1), the area of any peak corresponding to *trans*-clopenthixol acetate is not greater than the area of the peak in the chromatogram obtained with solution (2) (1%).

Loss on drying

When dried at 60° at a pressure not exceeding 0.7 kPa for 3 hours, loses not more than 0.4% of its weight, Appendix XI D. Use 1 g.

Sulfated ash

Not more than 0.1%, Appendix IX A.

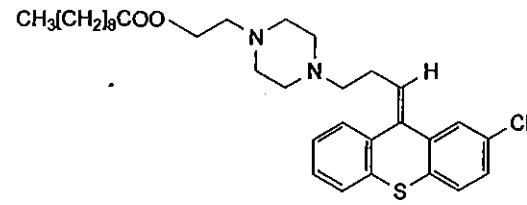
ASSAY

Dissolve 0.2 g in 50 mL of *anhydrous acetic acid* and carry out Method I for *non-aqueous titration*, Appendix VIII A, determining the end point potentiometrically. Each mL of *0.1M perchloric acid VS* is equivalent to 22.15 mg of $C_{24}H_{27}ClN_2O_2S$.

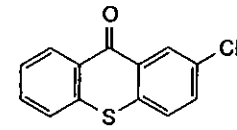
STORAGE

Zuclopenthixol Acetate should be protected from light and stored at a temperature not exceeding -20°.

IMPURITIES



A. *trans*-clopenthixol acetate *(trans-isomer)*,



B. 2-chlorothioxanthone,

ASSAY

Dissolve 0.250 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

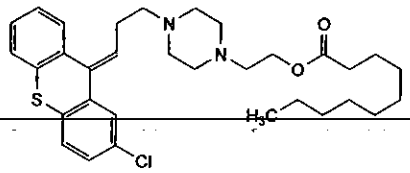
1 mL of *0.1 M perchloric acid* is equivalent to 27.76 mg of $C_{32}H_{43}ClN_2O_2S$.

STORAGE

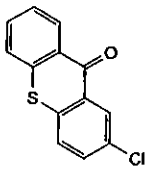
Under an inert gas in an airtight container, protected from light, at −20 °C or below.

IMPURITIES

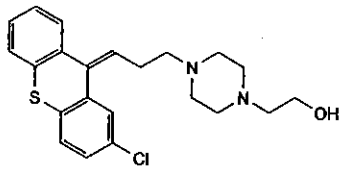
Specified impurities *A, B, C.*



A. 2-[4-[3-[(9*E*)-2-chloro-9*H*-thioxanthen-9-ylidene] propyl]piperazin-1-yl]ethyl decanoate,



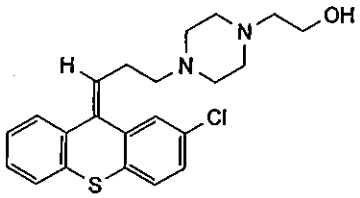
B. 2-chloro-9*H*-thioxanthen-9-one,



C. 2-[4-[3-[(9*Z*)-2-chloro-9*H*-thioxanthen-9-ylidene] propyl]piperazin-1-yl]ethanol.

Ph Eur

Zuclopenthixol Hydrochloride



,2HCl

$C_{22}H_{25}ClN_2OS,2HCl$ 473.9 *633-59-0*

Action and use

Dopamine receptor antagonist; neuroleptic.

Preparation

Zuclopenthixol Tablets

DEFINITION

Zuclopenthixol Hydrochloride is (*Z*)-2-4-[3-(2-chlorothioxanthene-9-ylidene)propyl]piperazin-1-ylethanol dihydrochloride. It contains not less than 98.0% and not more than 101.0% of $C_{22}H_{25}ClN_2OS,2HCl$, calculated with reference to the anhydrous substance.

CHARACTERISTICS

An off-white, granular powder.

Very soluble in *water*; sparingly soluble in *ethanol (96%)*; very slightly soluble in *ether*.

IDENTIFICATION

A. The *light absorption* of a 0.0015% w/v solution in *ethanol (96%)*, Appendix II B, in the range 210 to 350 nm exhibits two maxima at 230 and 268 nm. The *absorbances* at the maxima are about 1.0 and 0.4, respectively.

B. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of zuclopenthixol hydrochloride *(RS 365)*.

C. Yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS

Acidity

pH of a 1.0% w/v solution, 2.0 to 3.0, Appendix V L.

Free amine

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions protected from light.

Solvent A Shake 0.6 mL of *diethylamine* with 150 mL of *ethanol (96%)* and add sufficient *ethanol (96%)* to produce 200 mL.

(1) 0.25% w/v solution of the substance being examined in solvent A.

(2) Dilute 1 volume of solution (1) to 400 volumes with solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel F_{254} plate* (Merck plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 4 µL of each solution.

(d) Develop the plate to 10 cm.

(e) After removal of the plate, allow it to dry in air, spray with a mixture of equal volumes of *sulfuric acid* and *absolute ethanol*, heat at 110° for 5 minutes and examine under *ultraviolet light (365 nm)* immediately.

MOBILE PHASE

2 volumes of *water*, 10 volumes of 13.5M *ammonia*, 20 volumes of *butan-1-ol* and 65 volumes of *acetone*.

LIMITS

In the chromatogram obtained with solution (1) any *secondary spot* of the same colour and at an Rf value lower than that of the principal spot is not more intense than the spot in the chromatogram obtained solution (2) (0.25%).

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

Solution A 18 volumes of *acetonitrile* and 82 volumes of *water*.

(1) Dissolve 20 mg of the substance being examined in 200 mL of solution A.

(2) Dilute 1 volume of solution (1) to 100 volumes with solution A and further dilute 1 volume of this solution to 10 volumes with solution A.

(3) Dilute 3 volumes of solution (1) to 100 volumes with solution A.

(4) 0.002% w/v each of *zuclopenthixol hydrochloride BPCRS* and *trans-clopenthixol hydrochloride BPCRS* (impurity B) in solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (5 cm × 2.1 mm) packed with *octadecylsilyl silica gel for chromatography* (3.5 µm) (Symmetry Shield RP18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.6 mL per minute.

(d) Use a column temperature 40°.

(e) Use a detection wavelength of 270 nm.

(f) Inject 5 µL of each solution.

MOBILE PHASE

Mobile phase A 1 volume of *trifluoroacetic acid* and 25 volumes of *water*.

Mobile phase B 1 volume of *trifluoroacetic acid* and 25 volumes of *acetonitrile*.

Mobile phase C *methanol*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Mobile phase C (% v/v)	Comment
0-8	82	18	0	isocratic
8-17	82→5	18→10	0→85	linear gradient
17-19	5→82	10→18	85→0	linear gradient
19-30	82	18	0	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the retention times relative to zuclopenthixol (retention time about 6 minutes) are: impurity A, about 0.1; impurity B, about 1.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution* between the peaks corresponding to zuclopenthixol and impurity B is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

The area of any peak due to trans-zuclopenthixol is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (3%);

The area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

The sum of the areas of any *secondary peak*, excluding trans-clopenthixol hydrochloride, is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than half the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Water

Not more than 2.5%, determined on 0.5 g, Appendix IX C.

Sulfated ash

Not more than 0.1%, Appendix IX A.

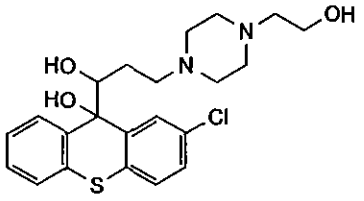
ASSAY

Dissolve 0.5 g in 50 mL of *anhydrous acetic acid* and carry out Method I for *non-aqueous titration*, Appendix VIII A, using *crystal violet solution* as indicator. Each mL of *0.1M perchloric acid VS* is equivalent to 23.70 mg of $C_{22}H_{27}Cl_3N_2OS$.

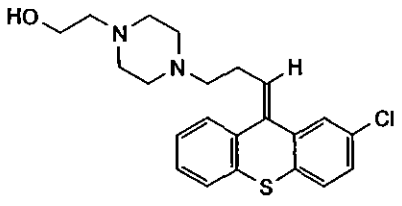
STORAGE

Zuclopenthixol Hydrochloride should be protected from light.

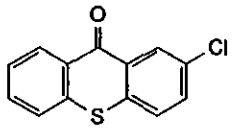
IMPURITIES



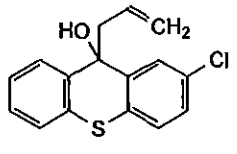
A. 2-chloro-9-(1-hydroxy-3-(4-(2-hydroxyethyl)]piperazin-1-ylpropyl)thioxanthen9-ol,



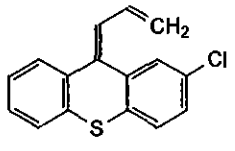
B. *trans*-clopenthixol,



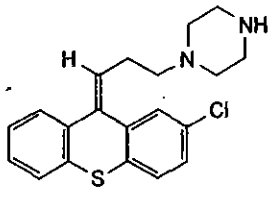
C. 2-chlorothioxanthone,



D. 9-allyl-2-chlorothioxanthen-9-ol,



E. 9-allylidene-2-chlorothioxanthene,



F. 2-chloro-9-(3-piperazin-1-ylpropylidene)thioxanthene.