Monographs

Medicinal and Pharmaceutical Substances

MEDICINAL AND PHARMACEUTICAL SUBSTANCES

Substances For pharmaceutical use

((Ph. Eur. monograph 2034)

Ph Eur

DEFINITION

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

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

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

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

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

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

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

PRODUCTION

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

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

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

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

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

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

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

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

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

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

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

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

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

CHARACTERS

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

Where a substance may show polymorphism, this may be stated under Characters in order to draw this to the attention of the user who may have to take this characteristic into consideration during formulation of a preparation.

IDENTIFICATION

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

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

TESTS

Polymorphism (*5.9*)

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

Related substances

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

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

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

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

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

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

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

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

For active substances in a new application for a medicinal product for human use, the requirements of the guideline on the limits of genotoxic impurities and the corresponding questions and answers documents published on the website of the European Medicines Agency (or similar evaluation principles for non-European Union member states) must be followed.

Residual solvents

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

Microbiological quality

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

Sterility (*2.6.1*)

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

Bacterial endotoxins (*2.6.14*)

If offered as bacterial endotoxin-free grade, the substance for pharmaceutical use complies with the test for bacterial endotoxins. The limit and test method (if not gelation method A) are stated in the individual monograph. The limit is calculated in accordance with the recommendations in general chapter *5.1.10. Guidelines for using the test for bacterial endotoxins*, unless a lower limit is justified from results from production batches or is required by the competent authority. Where a test for bacterial endotoxins is prescribed, a test for pyrogens is not required.

Pyrogens (*2.6.8*)

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

Additional properties

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

appropriate requirements may be specified in an individual monograph.

ASSAY

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

LABELLING

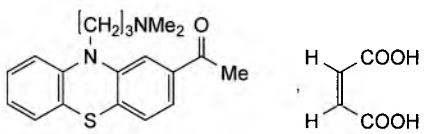
In general, labelling is subject to supranational and national regulation and to international agreements. The statements under the heading Labelling therefore are not comprehensive and, moreover, for the purposes of the Pharmacopoeia only those statements that are necessary to demonstrate compliance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

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

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

Ph Eur

Acepromazine Maleate



$C_{19}H_{22}N_2OS,C_4H_4O_4$ 442.5 *3598-37-6*

Action and use

Dopamine receptor antagonist; neuroleptic.

Preparations

Acepromazine Injection

Acepromazine Tablets

DEFINITION

Acepromazine Maleate is 2-acetyl-10-(3-dimethylaminopropyl) phenothiazine hydrogen maleate. It contains not less than 98.5% and not more than 101.0% of $C_{19}H_{22}N_2OS,C_4H_4O_4$, calculated with reference to the dried substance.

CHARACTERISTICS

A yellow, crystalline powder.

Soluble in *water*; freely soluble in *chloroform*; soluble in *ethanol (96%)*; slightly soluble in *ether*.

IDENTIFICATION

A. Dissolve 20 mg in 2 mL of *water*, add 3 mL of 2M *sodium hydroxide*, extract with 5 mL of *cyclohexane* and evaporate to dryness under reduced pressure. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of acepromazine *(RSV 01)*.

B. Complies with the test for *identification of phenothiazines*, Appendix III A, applying to the plate 1 µL of each solution and using *acepromazine maleate BPCRS* for the preparation of solution (2).

C. Dissolve 0.2 g in a mixture of 3 mL of *water* and 2 mL of 5M *sodium hydroxide* and shake with three 3-mL quantities of *ether*. Add to the aqueous solution 2 mL of *bromine solution*, warm in a water bath for 10 minutes, heat to boiling, cool and add 0.25 mL to a solution of 10 mg of *resorcinol* in 3 mL of *sulfuric acid*. A bluish black colour develops on heating for 15 minutes in a water bath.

TESTS

Acidity

pH of a 1.0% w/v solution, 4.0 to 4.5, Appendix V L.

Melting point

136° to 139°, Appendix V A.

Related substances

Complies with the test for *related substances in phenothiazines*, Appendix III A, but using a mixture of 75 volumes of n-*hexane*, 17 volumes of *butan-2-one* and 8 volumes of *diethylamine* as the mobile phase.

Loss on drying

When dried to constant weight at 105°, loses not more than 1.0% of its weight. Use 1 g.

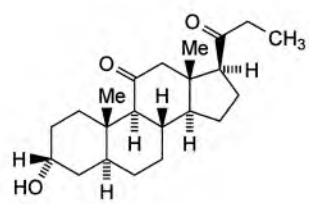
Sulfated ash

Not more than 0.2%, Appendix IX A.

ASSAY

Dissolve 0.4 g in 50 mL of *acetic anhydride* and carry out Method I for *non-aqueous titration*, Appendix VIII A, using *crystal violet solution* as indicator. Each mL of 0.1M *perchloric acid VS* is equivalent to 44.25 mg of $C_{19}H_{22}N_2OS,C_4H_4O_4$.

Alfaxalone



$C_{21}H_{32}O_3$ 332.5 *23930-19-0*

Action and use

Intravenous general anaesthetic.

DEFINITION

Alfaxalone is 3α-hydroxy-5α-pregnane-11, 20-dione. It contains not less than 95.0% and not more than 103.0% of $C_{21}H_{32}O_3$, calculated with reference to the dried substance.

CHARACTERISTICS

A white to creamy white powder.

Practically insoluble in *water*; freely soluble in *chloroform*; soluble in *ethanol (96%)*; practically insoluble in *petroleum spirit (boiling range, 60° to 80°)*.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of alfaxalone *(RSV 03)*.

B. Complies with the test for *identification of steroids*, Appendix III A, using *impregnating solvent II* and *mobile phase D*.

C. In the Assay, the chromatogram obtained with solution (2) shows a peak having the same retention time as the peak due to *alfaxalone BPCRS* in the chromatogram obtained with solution (1).

TESTS

Light absorption

Absorbance of a 0.20% w/v solution in *ethanol (96%)* at 235 nm, not more than 0.20, calculated with reference to the dried substance, Appendix II B.

Related substances

Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of equal volumes of *ethyl acetate* and *toluene* as the mobile phase. Apply separately to the plate 10 µL of each of three solutions of the substance being examined in a mixture of equal volumes of *chloroform* and *methanol* containing (1) 5.0% w/v, (2) 0.15% w/v and (3) 0.050% w/v. After removal of the plate, dry it in a current of air until the solvent has evaporated, spray with a saturated solution of *cerium (IV) sulfate* in *sulfuric acid (50%)* and heat at 110° for 1 hour. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (3%) and not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (1%).

Loss on drying

When dried to constant weight at 105°, loses not more than 1.0% of its weight. Use 1 g.

Sulfated ash

Not more than 0.1%, Appendix IX A.

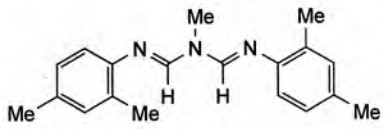
ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. For solution (1) dilute 25 mL of a solution in *propan-2-ol R1* containing 0.2% w/v of *alfaxalone BPCRS*, 0.01% w/v of *alfadolone acetate BPCRS* and 0.03% w/v of *betamethasone BPCRS* (internal standard) to 100 mL with *carbon dioxide-free water*. For solution (2) dilute 25 mL of a solution in *propan-2-ol R1* containing 0.2% w/v of the substance being examined to 100 mL with *carbon dioxide-free water*. For solution (3) dilute 25 mL of a solution in *propan-2-ol R1* containing 0.2% w/v of the substance being examined and 0.03% w/v of the internal standard to 100 mL with *carbon dioxide-free water*.

The chromatographic procedure may be carried out using (a) a stainless steel column (10 cm × 5 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 1 is suitable) and maintained at 60°, (b) as the mobile phase with a flow rate of 1 mL per minute a mixture of *propan-2-ol R1* and *carbon dioxide-free water* adjusted so that the *resolution factor* between the peaks due to alfadolone acetate (retention time about 5 minutes) and alfaxalone (retention time about 6 minutes) is more than 1.0 (a mixture of 25 volumes of *propan-2-ol R1* and 75 volumes of *carbon dioxide-free water* is usually suitable) and (c) a detection wavelength of 205 nm.

Calculate the content of $C_{21}H_{32}O_3$ in the substance being examined using the declared content of $C_{21}H_{32}O_3$ in *alfaxalone BPCRS*; peak areas or peak heights may be used irrespective of the symmetry factor.

Amitraz



$C_{19}H_{23}N_3$ 293.4 *33089-61-1*

Action and use

Topical parasiticide; acaricide.

Preparation

Amitraz Dip Concentrate (Liquid)

DEFINITION

Amitraz is *N*-methylbis (2,4-xylyliminomethyl) amine.
It contains not less than 97.0% and not more than 101.0% of $C_{19}H_{23}N_3$, calculated with reference to the anhydrous substance.

CHARACTERISTICS

A white to buff powder.

Practically insoluble in *water*; decomposes slowly in *ethanol (96%)*; freely soluble in *acetone*.

IDENTIFICATION

The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of amitraz *(RSV 04)*.

TESTS

Related substances

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions.

0.010% w/v of *2,4-dimethylaniline*, 0.20% w/v of *form-2',4'-xylidide BPCRS* and 0.20% w/v of *N,N'-bis(2,4-xylyl) formamidine BPCRS* in *methyl acetate* (solution A)

Disperse 30 mg of *N-methyl-N'-(2,4-xylyl)formamidine hydrochloride BPCRS* in 5 mL of *methyl acetate*, add about 32 mg of *triethylamine*, mix with the aid of ultrasound for 2 minutes, filter, wash the filter with a small amount of *methyl acetate* and add sufficient *methyl acetate* to the combined filtrate and washings to produce 25 mL (solution B) (about 0.1% w/v of *N-methyl-N'-(2,4-xylyl)formamidine)*.

(1) 5.0% w/v solution of the substance being examined in *methyl acetate*.

(2) A mixture of equal volumes of solution A and solution B.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *fused silica capillary column* (10 m × 0.53 mm) bonded with a film (5 µm) of *poly [methyl(95)phenyl(5)]siloxane* (Chrompack CP-SIL 8 CB is suitable).

(b) Use *helium* as the carrier gas at 12 mL per minute.

(c) Use gradient conditions at an initial temperature of 125°, maintained at 125° for 5 minutes, increasing linearly to 270° at a rate of 5° per minute and maintained at 270° for 15 minutes.

(d) Use an inlet temperature of 230°.

(e) Use a flame ionisation detector at a temperature of 300°.

(f) Inject 1 µL of each of solutions (1) and (2).

In the chromatogram obtained with solution (2) the peaks following the solvent peak, in order of emergence, are due to 2,4-dimethylaniline, form-2′,4′-xylidide, N-methyl-N′-(2,4-xylyl)formamidine and N,N′-bis(2,4-xylyl)formamidine.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 2,4-dimethylaniline, form-2′,4′-xylidide, N-methyl-N′-(2,4-xylyl)formamidine and N,N′-bis(2,4-xylyl)formamidine is not greater than the area of the corresponding peak in the chromatogram obtained with solution (2) (0.1%, 2%, 1% and 2% respectively);

the area of any other secondary peak is not greater than the area of the peak due to 2,4-dimethylaniline in the chromatogram obtained with solution (2) (0.1%).

Water

Not more than 0.1% w/w, Appendix IX C, Method IA. Use 5 g and a mixture of equal volumes of *chloroform* and *2-chloroethanol* in place of *anhydrous methanol.*

Sulfated ash

Not more than 0.2%, Appendix IX A.

ASSAY

Carry out the method for *gas chromatography*, Appendix III B, using the following solutions.

Prepare a 2% v/v solution of *squalane* (internal standard) in *methyl acetate* (solution C).

(1) 0.15 g of the substance being examined in sufficient *methyl acetate* to produce 30 mL.

(2) 0.15 g of the substance being examined in 10 mL of solution C and add sufficient *methyl acetate* to produce 30 mL.

(3) 1.50% w/v solution of *amitraz BPCRS* in solution C and dilute 1 volume of this solution to 3 volumes with *methyl acetate.*

CHROMATOGRAPHIC CONDITIONS

(a) Use a fused silica capillary column (15 m × 0.53 mm) coated with a 1.5 µm film of methyl silicone gum (Chrompack CP-Sil 5 CB is suitable).

(b) Use *helium* as the carrier gas at 12 mL per minute.

(c) Use isothermal conditions maintained at 220°.

(d) Use an inlet temperature of 230°.

(e) Use a flame ionisation detector at a temperature of 300°.

(f) Inject 1 µL of each solution.

SYSTEM SUITABILITY

The assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to squalane and amitraz is at least 3.0.

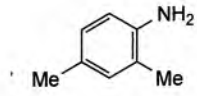
LIMITS

Calculate the content of $C_{19}H_{23}N_3$ from the chromatograms obtained using the declared content of $C_{19}H_{23}N_3$ in *amitraz BPCRS.*

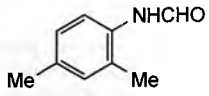
STORAGE

Amitraz should be kept in a well-closed container, which may contain paraformaldehyde, packed in separate sachets as a stabiliser.

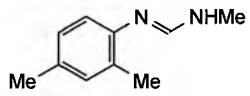
IMPURITIES



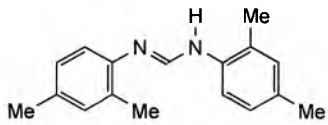
A. 2,4-dimethylaniline (2,4-xylidine),



B. form-2′,4′-xylidide,

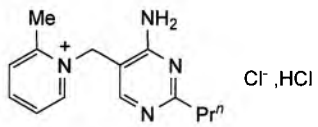


C. *N*-methyl-*N*′-(2,4-xylyl)formamidine,



D. *N,N*′-bis(2,4-xylyl)formamidine.

Amprolium Hydrochloride



$C_{14}H_{19}ClN_4,HCl$ 315.3 *137-88-2*

Action and use

Antiprotozoal; prevention and treatment of coccidiosis (veterinary).

DEFINITION

Amprolium Hydrochloride is 1-(4-amino-2-propylpyrimidin-5-ylmethyl)-2-methylpyridinium chloride hydrochloride. It contains not less than 97.5% and not more than 101.0% of $C_{14}H_{19}ClN_4,HCl$, calculated with reference to the dried substance.

CHARACTERISTICS

A white or almost white powder; odourless or almost odourless.

Freely soluble in *water*; slightly soluble in *ethanol (96%)*; very slightly soluble in *ether*; practically insoluble in *chloroform.*

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of amprolium hydrochloride *(RSV 07).*

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.002% w/v solution in 0.1M *hydrochloric acid* exhibits two maxima, at 246 nm and 262 nm. The *absorbances* at the maxima are about 0.84 and about 0.80, respectively.

C. To 1 mg add 5 mL of *naphthalenediol reagent solution*; a deep violet colour is produced.

D. Yields the reactions characteristic of *chlorides*, Appendix VI.

TESTS

Picoline

Dissolve 1.5 g in 30 mL of *water* in a distillation flask, add 20 mL of a saturated solution of *potassium carbonate sesquihydrate*, connect the flask to a coarse-fritted aerator extending to the bottom of a 100-mL graduated cylinder containing 50 mL of 0.05M *hydrochloric acid*, and pass air, which has previously been passed through *sulfuric acid* and glass wool, through the system for 60 minutes. To 5 mL of the hydrochloric acid solution add sufficient 0.05M *hydrochloric acid* to produce 200 mL. The *absorbance* of the resulting solution at 262 nm is not greater than 0.52, Appendix II B.

Loss on drying

When dried to constant weight at 100° at a pressure not exceeding 0.7 kPa, loses not more than 1.0% of its weight. Use 1 g.

Sulfated ash

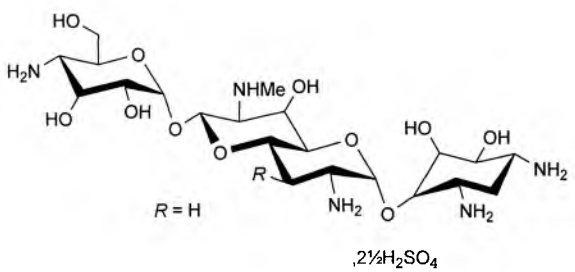
Not more than 0.1%, Appendix IX A.

ASSAY

Carry out Method I for *non-aqueous titration*, Appendix VIII A, using 0.3 g and *1-naphtholbenzein solution* as indicator. Each mL of 0.1M *perchloric acid VS* is equivalent to 15.77 mg of $C_{14}H_{19}ClN_4,HCl$.

Apramycin Sulfate

Apramycin Sulphate



$C_{21}H_{41}N_5O_{11},2½H_2SO_4$ 784.8 *41194-16-5*

Action and use

Aminoglycoside antibacterial.

Preparations

Apramycin Veterinary Oral Powder

Apramycin Premix

DEFINITION

Apramycin Sulfate is the sulfate of 4-*O*-[(2*R*,3*R*,4a*S*,6*R*,7*S*,8*R*,8a*R*)-3-amino-6-(4-amino-4-deoxy-α-D-glucopyranosyloxy)-8-hydroxy-7-methylaminoperhydropyrano[3,2-*b*]pyran-2-yl]-2-deoxystreptamine. It is produced by the growth of certain strains of *Streptomyces tenebrarius* or obtained by any other means. The potency is not less than 450 Units per mg, calculated with reference to the anhydrous substance.

CHARACTERISTICS

A light brown powder or granular material; hygroscopic.

Freely soluble in *water*; practically insoluble in *acetone*, in *ethanol (96%)*, in *ether* and in *methanol*.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *water*.

(1) 0.1% w/v of the substance being examined.

(2) 0.07% w/v of *apramycin BPCRS*.

(3) 0.07% w/v of each of *apramycin BPCRS* and *tobramycin BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 10 cm.

(e) After removal of the plate, allow it to dry in a current of warm air, spray with a mixture of equal volumes of a 46% w/v solution of *sulfuric acid* and a 0.2% w/v solution of *naphthalene-1,3-diol* in *ethanol (96%)* and heat at 150° for 5 to 10 minutes.

MOBILE PHASE

20 volumes of *chloroform*, 40 volumes of 13.5M *ammonia* and 60 volumes of *methanol*, equilibrated for 1 hour before use.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated principal spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in colour and position to that in the chromatogram obtained with solution (2).

B. In the test for Related substances, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

C. Yields reaction A characteristic of *sulfates*, Appendix VI.

TESTS

Sulfate

26.0 to 33.0% of SO_4, calculated with reference to the anhydrous substance, when determined by the following method. Dissolve 0.25 g in 100 mL of *water*, adjust to pH 11 with 13.5M *ammonia* and add 10 mL of 0.1M *barium chloride VS*. Titrate with 0.1M *disodium edetate VS* using 0.5 mg of *phthalein purple* as indicator; add 50 mL of *ethanol (96%)* when the colour of the solution begins to change and continue the titration until the violet-blue colour disappears. Each mL of 0.1M *barium chloride VS* is equivalent to 9.606 mg of SO_4.

Caerulomycin and dipyridyl derivatives

Dissolve 0.5 g in *water* in a 100 mL graduated flask, add 10 mL of *methanol* and dilute to 100 mL with *water*. Place 5 mL in a 25 mL graduated flask and add 5 mL of an acetate buffer prepared by dissolving 8.3 g of *anhydrous sodium acetate* in 25 mL of *water*, adding 12 mL of *glacial acetic acid* and diluting to 100 mL with *water*. Mix, add 1 mL of a 10% w/v solution of *hydroxylamine hydrochloride* in *water* and mix again. Add 5 mL of a 1% w/v solution of *ammonium iron(II) sulfate* and dilute to 25 mL with *water*. Measure the *absorbance* of the resulting solution at 520 nm, Appendix II B, using in the reference cell a solution obtained

by carrying out the same procedure without the substance being examined. The *absorbance* is not greater than that obtained by repeating the test using 5 mg of *2,2'-dipyridyl* dissolved in 10 mL of *methanol* and diluted to 100 mL with *water* and beginning at the words 'Place 5 mL in a 25 mL graduated flask...' (1%).

Related substances

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

(1) 0.50% w/v of the substance being examined.

(2) 0.35% w/v of *apramycin BPCRS*.

(3) Dilute 1 volume of solution (1) to 20 volumes.

(4) Dilute 1 volume of solution (3) to 50 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a column (25 cm × 4 mm) packed with fast cation-exchange polymeric beads (13 µm) with sulfonic acid functional groups (Dionex Fast Cation-1^R is suitable) and a stainless steel post-column reaction coil (380 cm × 0.4 mm) with internal baffles. Use in the reaction coil *ninhydrin reagent I* at a flow rate approximately the same as that for the mobile phase.

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 0.8 mL per minute.

(d) Use a column temperature of 130°. Maintain the post-column reaction coil at the same temperature.

(e) Use a detection wavelength of 568 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A A solution containing 1.961% w/v of *sodium citrate*, 0.08% v/v of *liquefied phenol* and 0.5% v/v of *thiodiglycol*, adjusted to pH 4.25 using *hydrochloric acid*.

Mobile phase B A solution containing 4.09% w/v of *sodium chloride* and 3.922% w/v of *sodium citrate* with 0.08% v/v of *liquefied phenol*, adjusted to pH 7.4 with *hydrochloric acid*.

Equilibrate the column using a mixture containing 75% of mobile phase A and 25% of mobile phase **B**. After each injection elute for 3 minutes using the same mixture and then carry out a linear gradient elution for 6 minutes to 100% of mobile phase B. Elute for a further 21 minutes using 100% of mobile phase **B**, then step-wise re-equilibrate to a mixture of 75% of mobile phase A and 25% of mobile phase B and elute for at least 10 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between the peaks due to compound A and 3-hydroxyapramycin, identified using the reference chromatogram supplied with *apramycin BPCRS*, is at least 0.8.

LIMITS

Multiply the areas of all the *secondary peaks* by 0.5. [NOTE: This is to ensure that the impurities are calculated relative to Apramycin Sulfate (which contains about 50% w/w of apramycin).]

In the chromatogram obtained with solution (1):

the areas of any peaks corresponding to 3-hydroxyapramycin, lividamine/2-deoxystreptamine (combined), compound A and compound B (identified using the reference chromatogram supplied with *apramycin BPCRS*) are not greater than 1.4, 1.0, 0.4 and 0.4 times respectively the area of the principal peak in the chromatogram obtained with solution (3) (7%, 5%, 2% and 2% respectively);

the area of any other *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (3) (2%);

the sum of the areas of all the *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (3) (15%).

Disregard any peak with an area less than the area of the peak in the chromatogram obtained with solution (4) (0.1%).

Sulfated ash

Not more than 1.0%, Appendix IX A, Method II. Use 1 g.

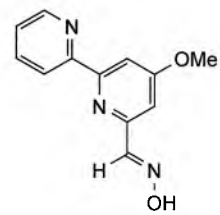
Water

Not more than 14.0% w/w, Appendix IX C. Use 0.2 g and 20 mL of a mixture containing 1 volume of *methanol* and 2 volumes of *formamide* as the solvent. The solvent mixture must be prepared at least 12 hours before use and should be stored in an airtight container.

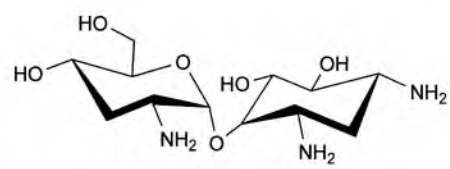
ASSAY

Carry out the *microbiological assay of antibiotics*, Appendix XIV A, Method B. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

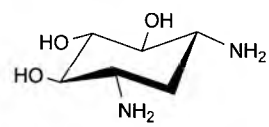
IMPURITIES



A. caerulomycin,



B. lividamine,



C. 2-deoxystreptamine,

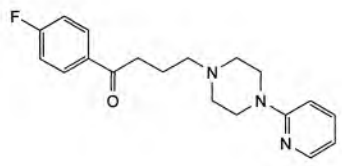
D. 3-hydroxyapramycin; R = OH,

E. 'compound A',

F. 'compound B'.

Azaperone

(Azaperone for Veterinary Use, Ph Eur monograph 1708)



$C_{19}H_{22}FN_3O$ 327.4 *1649-18-9*

Action and use
Dopamine receptor antagonist; neuroleptic (veterinary).

Preparation
Azaperone Injection

Ph Eur

DEFINITION

1-(4-Fluorophenyl)-4-[4-(pyridin-2-yl)piperazin-1-yl] butan-1-one.

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

CHARACTERS

Appearance
White or almost white powder.

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

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison azaperone CRS.

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in *acetone R*, evaporate to dryness and record new spectra using the residues.

TESTS

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

Dissolve 1.0 g in 25 mL of a 14 g/L solution of *tartaric acid R*.

Related substances
Liquid chromatography (*2.2.29*).

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

Reference solution (a) Dissolve 5.0 mg of *azaperone CRS* and 6.0 mg of *benperidol CRS* in *methanol R* and dilute to 200.0 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *methanol R*. Dilute 5.0 mL of the solution to 20.0 mL with *methanol R*.

Column:
— *size*: *l* = 0.10 m, Ø = 4.6 mm;
— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm);
— *temperature*: 25 °C.

Mobile phase:
— *mobile phase A*: dissolve 1.4 g of *anhydrous sodium sulfate R* in 900 mL of *water R*, add 16.0 mL of *0.01 M sulfuric acid* and dilute to 1000 mL with *water R*;
— *mobile phase B*: *methanol R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	95 → 20	5 → 80
15 - 20	20	80

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 10 µL.

Relative retention With reference to azaperone (retention time = about 9 min): impurity A = about 0.9; impurity B = about 1.1; impurity C = about 1.15.

System suitability: reference solution (a):
— *resolution*: minimum 8.0 between the peaks due to azaperone and to benperidol.

Limits:
— *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent);
— *unspecified impurities*: for each impurity, not more than 0.8 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.20 per cent);
— *sum of impurities B and C*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.75 per cent);
— *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
— *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 4 h.

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

ASSAY

Dissolve 0.130 g in 70 mL of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Titrate with *0.1 M perchloric acid*, using 0.2 mL of *naphtholbenzein solution R* as indicator.

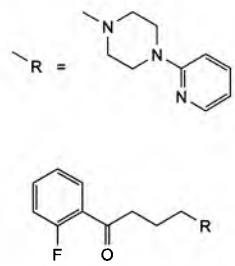
1 mL of *0.1 M perchloric acid* is equivalent to 16.37 mg of $C_{19}H_{22}FN_3O$.

STORAGE

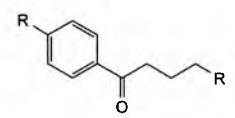
Protected from light.

IMPURITIES

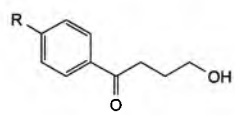
Specified impurities A, B, C



A. 1-(2-fluorophenyl)-4-[4-(pyridin-2-yl)piperazin-1-yl] butan-1-one,



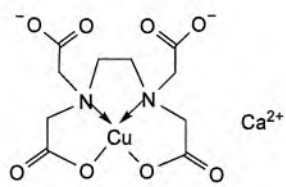
B. 4-[4-(pyridin-2-yl)piperazin-1-yl]-1-[4-[4-(pyridin-2-yl)piperazin-1-yl]phenyl]butan-1-one,



C. 4-hydroxy-1-[4-[4-(pyridin-2-yl)piperazin-1-yl]phenyl]butan-1-one.

Ph Eur

Calcium Copperedetate



$C_{10}H_{12}CaCuN_2O_8,2H_2O$ 427.6

Action and use
Used in the treatment of copper deficiency.

Preparation
Calcium Copperedetate Injection

DEFINITION
Calcium Copperedetate is the dihydrate of calcium [ethylenediaminetetra-acetato(4-)-*N*,*N*′,*O*,*O*′]copper(II). It contains not less than 9.1% and not more than 9.7% of calcium, Ca, and not less than 14.4% and not more than 15.3% of copper, Cu, both calculated with reference to the dried substance.

CHARACTERISTICS
A blue, crystalline powder.

Freely soluble in *water*, the solution gradually precipitating the tetrahydrate; practically insoluble in *ethanol (96%)*.

IDENTIFICATION
A. Dissolve 0.2 g in 5 mL of *water* and add 1 mL of 6M *acetic acid* and 2 mL of *dilute potassium iodide solution*. The solution remains clear and deep blue.

B. Ignite 0.2 g, dissolve the residue in 3 mL of 2M *hydrochloric acid*, neutralise the solution with 5M *ammonia* and add 1 mL of 6M *acetic acid* and 2 mL of *dilute potassium iodide solution*. A white precipitate is produced and iodine is liberated, colouring the supernatant liquid brown.

C. Dissolve 0.5 g in 10 mL of *water*, acidify with 2M *hydrochloric acid*, add 25 mL of a 10% v/v solution of *mercaptoacetic acid* and filter. Make the filtrate alkaline with 5M *ammonia* and add 5 mL of a 2.5% w/v solution of *ammonium oxalate*. A white precipitate is produced which is soluble in *hydrochloric acid* but only sparingly soluble in 6M *acetic acid*.

TESTS
Lead
Not more than 25 ppm of Pb when determined by the following method. Dissolve 1.25 g in 10 mL of *hydrochloric acid*, dilute to 25 mL with *water* and determine by *atomic absorption spectrophotometry*, Appendix II D, measuring at 283.3 nm and using a lead hollow-cathode lamp as the radiation source and *lead standard solution (100 ppm Pb)*, diluted if necessary with *water*, to prepare the standard solutions.

Zinc
Not more than 200 ppm of Zn when determined by the following method. Dissolve 1.0 g in 20 mL of *hydrochloric acid*, dilute to 200 mL with *water* and determine by *atomic absorption spectrophotometry*, Appendix II D, measuring at 213.9 nm and using a zinc hollow-cathode lamp as the radiation source and *zinc standard solution (5 mg/mL Zn)* diluted if necessary with *water*, to prepare the standard solutions.

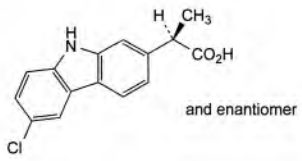
Loss on drying
When dried to constant weight at 105°, loses not more than 2.0% of its weight. Use 1 g.

ASSAY
For copper
Ignite 4 g at 600° to 700°, cool and heat the residue with 12 mL of a mixture of equal volumes of *hydrochloric acid* and *water* on a water bath for 15 minutes. Add 10 mL of *water*, filter and dilute the filtrate to 100 mL with *water* (solution A); reserve a portion for the Assay for calcium. To 25 mL of solution A add 25 mL of *water* and 10 mL of *bromine solution*, boil to remove the bromine, cool and add *dilute sodium carbonate solution* until a faint permanent precipitate is produced. Add 3 g of *potassium iodide* and 5 mL of 6M *acetic acid* and titrate the liberated iodine with 0.1M *sodium thiosulfate VS*, using *starch mucilage* as indicator, until only a faint blue colour remains; add 2 g of *potassium thiocyanate* and continue the titration until the blue colour disappears. Each mL of 0.1M *sodium thiosulfate VS* is equivalent to 6.354 mg of Cu.

For calcium
To 5 mL of solution A add 10 mL of *water* and 10 mL of a 10% v/v solution of *mercaptoacetic acid*, allow to stand until the precipitate has coagulated, dilute to 100 mL with *water*, add 5 mL of 5M *sodium hydroxide* and titrate with 0.05M *disodium edetate VS*, using *methyl thymol blue mixture* as indicator, until the solution becomes a full purple colour, adding the titrant slowly as the end point is approached. Each mL of 0.05M *disodium edetate VS* is equivalent to 2.004 mg of Ca.

Carprofen

(Carprofen for Veterinary Use, Ph Eur monograph 2201)



$C_{15}H_{12}ClNO_2$ 273.7 *53716-49-7*

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

Ph Eur

DEFINITION

(2*RS*)-2-(6-Chloro-9*H*-carbazol-2-yl)propanoic acid.

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

CHARACTERS

Appearance
White or almost white, crystalline powder.

Solubility
Practically insoluble in water, freely soluble in acetone, soluble in methanol, slightly soluble in 2-propanol.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison carprofen CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *acetone R*, evaporate to dryness and record new spectra using the residues.

TESTS

Appearance of solution
The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_3 (*2.2.2, Method II*).

Dissolve 1.0 g in *methanol R* and dilute to 25 mL with the same solvent.

Related substances
Liquid chromatography (*2.2.29*). *Carry out the test protected from light.*

Test solution Dissolve 50 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dissolve 2.5 mg of *carprofen for system suitability CRS* (containing impurity C) in the mobile phase and dilute to 10.0 mL with the mobile phase.

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

Column:
— *size*: l = 0.25 m, Ø = 4.6 mm;
— *stationary phase*: *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer R* (5 µm).

Mobile phase Mix 30 volumes of a 1.36 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R* and 70 volumes of *methanol R2*.

Flow rate 1.3 mL/min.

Detection Spectrophotometer at 235 nm.

Injection 20 µL.

Run time 4 times the retention time of carprofen.

Retention time Carprofen = about 10 min.

System suitability: reference solution (a):
— *resolution*: minimum 1.5 between the peaks due to impurity C and carprofen.

Limits:
— *unspecified impurities*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.20 per cent);
— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Heavy metals (*2.4.8*)
Maximum 20 ppm.

Dissolve 1.0 g in *ethanol (96 per cent) R* and dilute to 20 mL with the same solvent. 12 mL of the solution complies with test B. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*)
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

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

ASSAY

Dissolve 0.200 g in 50 mL of *ethanol (96 per cent) R*. Add 1.0 mL of *0.1 M hydrochloric acid*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Read the volume added between the 2 points of inflexion.

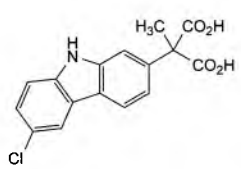
1 mL of *0.1 M sodium hydroxide* is equivalent to 27.37 mg of $C_{15}H_{12}ClNO_2$.

STORAGE

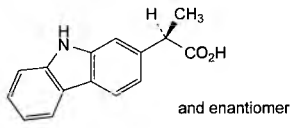
Protected from light.

IMPURITIES

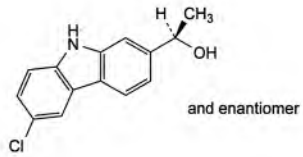
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical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



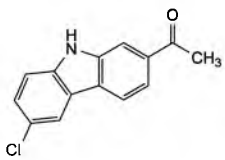
A. 2-(6-chloro-9*H*-carbazol-2-yl)-2-methylpropanedioic acid,



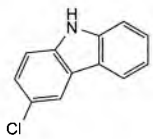
B. (2*RS*)-2-(9*H*-carbazol-2-yl)propanoic acid,



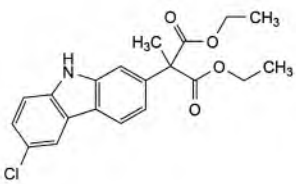
C. (1*RS*)-1-(6-chloro-9*H*-carbazol-2-yl)ethanol,



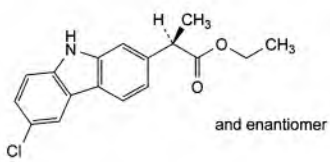
D. 1-(6-chloro-9*H*-carbazol-2-yl)ethanone,



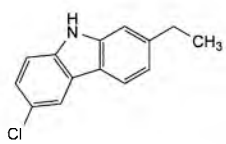
E. 3-chloro-9*H*-carbazole,



F. diethyl 2-(6-chloro-9*H*-carbazol-2-yl)-2-methylpropanedioate,



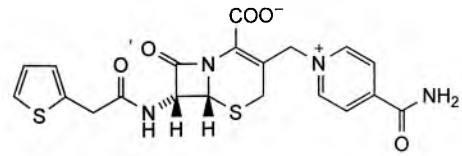
G. ethyl (2*RS*)-2-(6-chloro-9*H*-carbazol-2-yl)propanoate,



H. 6-chloro-2-ethyl-9*H*-carbazole.

Ph Eur

Cefalonium



$C_{20}H_{18}N_4O_5S_2,2H_2O$ 494.5 *5575-21-3 (anhydrous)*

Action and use
Cephalosporin antibacterial.

Preparation
Cefalonium Intramammary Infusion (Dry Cow)

DEFINITION
Cefalonium is 3-(4-carbamoyl-1-pyridiniomethyl)-7-[(2-thienyl)acetamido]-3-cephem-4-carboxylate dihydrate. It contains not less than 95.0% and not more than 103.5% of $C_{20}H_{18}N_4O_5S_2$, calculated with reference to the anhydrous substance.

CHARACTERISTICS
A white or almost white crystalline powder.

Very slightly soluble in *water* and in *methanol*; soluble in *dimethyl sulfoxide*; insoluble in *dichloromethane*, in *ethanol (96%)* and in *ether*. It dissolves in dilute acids and in alkaline solutions.

IDENTIFICATION
A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of cefalonium *(RSV 09)*.

B. The *light absorption*, Appendix II B, in the range 220 to 350 nm of a 0.002% w/v solution in *water* exhibits two maxima, at 235 nm and at 262 nm. The *absorbance* at 235 nm is about 0.76 and at 262 nm is about 0.62.

TESTS
Specific optical rotation
Dissolve 0.25 g with the aid of gentle heat in sufficient *dimethyl sulfoxide* to produce 50 mL. Allow the solution to stand for 30 minutes before measurement of the optical rotation. The *specific optical rotation* of the resulting solution is -50 to -56, calculated with reference to the anhydrous substance, Appendix V F.

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in 8.3M *acetic acid*.

(1) 2.5% w/v of the substance being examined.

(2) 0.05% w/v of the substance being examined.

(3) 0.025% w/v of the substance being examined.

(4) 0.005% w/v of the substance being examined.

(5) 0.05% w/v of each of *cefalotin sodium EPCRS* and *isonicotinamide*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* F_{254}.

(b) Use the mobile phase as described below.

(c) Apply 4 µL of each solution.

(d) Develop the plate to 12 cm.

(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

10 volumes of *glacial acetic acid*, 10 volumes of 1M *sodium acetate* and 30 volumes of *propan-2-ol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (5) shows two clearly separated spots.

LIMITS

Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (2%), not more than one such spot is more intense than the spot in the chromatogram obtained with solution (3) (1%) and not more than three such spots are more intense than the spot in the chromatogram obtained with solution (4) (0.2% each).

Sulfated ash

Not more than 0.2%, Appendix IX A.

Water

6.5 to 8.5% w/w, Appendix IX C. Use 0.5 g.

ASSAY

Measure the *absorbance* of a 0.002% w/v solution at the maximum at 262 nm, Appendix II B. Calculate the content of $C_{20}H_{18}N_4O_5S_2$ from the *absorbance* obtained using a 0.002% w/v solution of *cefalonium BPCRS* and from the declared content of $C_{20}H_{18}N_4O_5S_2$ in *cefalonium BPCRS*.

STORAGE

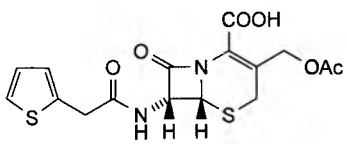
Cefalonium should be protected from light and stored at a temperature not exceeding 30°.

Cefalonium intended for use in the manufacture of either a parenteral dosage form or an intramammary infusion without a further appropriate sterilisation procedure complies with the following additional requirement.

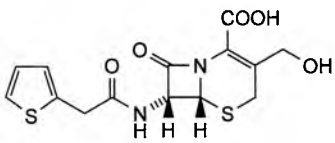
Sterility

Complies with the *test for sterility*, Appendix XVI A.

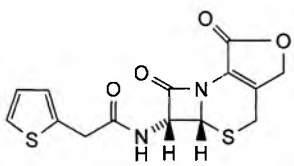
IMPURITIES



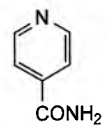
A. cefalotin,



B. 3-hydroxymethyl-7β-(2-thienylacetamido)-3-cephem-4-carboxylic acid,



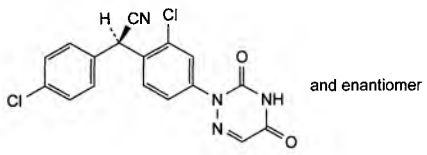
C. 3-hydroxymethyl-7β-(2-thienylacetamido)-3-cephem-4-carboxylic acid lactone,



D. isonicotinamide.

Clazuril

(Clazuril for Veterinary Use, Ph Eur monograph 1714)



$C_{17}H_{10}Cl_2N_4O_2$ 373.2 *101831-36-1*

Action and use

Treatment of coccidiosis; antiprotozoal (veterinary).

Ph Eur

DEFINITION

(2*RS*)-[2-Chloro-4-(3,5-dioxo-4,5-dihydro-1,2,4-triazin-2(3*H*)-yl)phenyl](4-chlorophenyl)acetonitrile.

Content

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

CHARACTERS

Appearance

White or light yellow powder.

Solubility

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

IDENTIFICATION

A. Melting point (*2.2.14*): 199 °C to 203 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison clazuril CRS.

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Solvent mixture tetrahydrofuran R, water R (50:50 *V/V*).

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

Reference solution (a) Dissolve 5 mg of *clazuril for system suitability CRS* (containing impurities A, B, C, D, E, F, G, H and I) in the solvent mixture and dilute to 5.0 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 2.0 mL of this solution to 10.0 mL with the solvent mixture.

Column:

— *size*: *l* = 0.10 m, Ø = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm);

— *temperature*: 35 °C.

Mobile phase:
— *mobile phase A*: mix 100 volumes of a 7.7 g/L solution of *ammonium acetate R* adjusted to pH 6.2 with a 10 per cent *V/V* solution of *anhydrous formic acid R*, 150 volumes of *acetonitrile R* and 750 volumes of *water R*;
— *mobile phase B*: mix 50 volumes of *water R*, 100 volumes of a 7.7 g/L solution of *ammonium acetate R* adjusted to pH 6.2 with a 10 per cent *V/V* solution of *anhydrous formic acid R* and 850 volumes of *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	100 → 0	0 → 100
20 - 25	0	100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 5 µL.

Identification of impurities Use the chromatogram supplied with *clazuril for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D, E, F, G, H and I.

Relative retention With reference to clazuril (retention time = about 16 min): impurity A = about 0.6; impurity B = about 0.78; impurity C = about 0.80; impurity D = about 0.86; impurity E = about 0.9; impurity F = about 0.95; impurity G = about 0.98; impurity H = about 1.1; impurity I = about 1.2.

System suitability: reference solution (a):
— *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to clazuril,
— the chromatogram obtained is similar to the chromatogram supplied with *clazuril for system suitability CRS*.

Limits:
— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity G = 1.4; impurity H = 0.8;
— *impurities A, B, C, D, E, F, G, H, I*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.20 per cent);
— *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.6 per cent);
— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard the peaks due to the solvents.

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

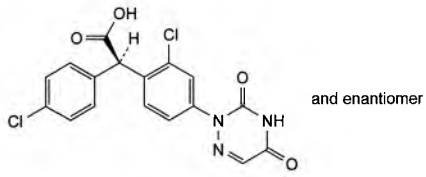
Sulfated ash (*2.4.14*)
Maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve about 0.260 g in 35 mL of *tetrahydrofuran R* and add 35 mL of *water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

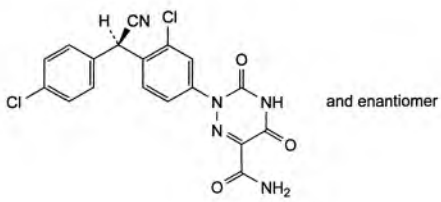
1 mL of *0.1 M sodium hydroxide* is equivalent to 37.32 mg of $C_{17}H_{10}Cl_2N_4O_2$.

STORAGE
Protected from light.

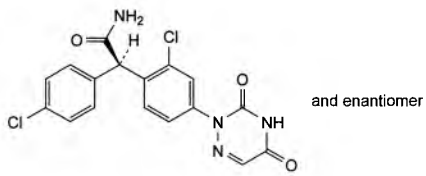
IMPURITIES
Specified impurities A, B, C, D, E, F, G, H, I



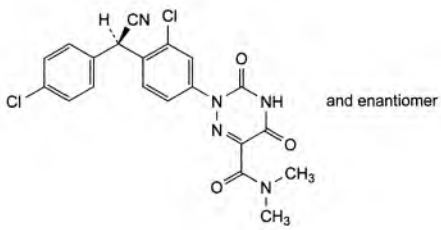
A. (2*RS*)-[2-chloro-4-(3,5-dioxo-4,5-dihydro-1,2,4-triazin-2(3*H*)-yl)phenyl](4-chlorophenyl)acetic acid,



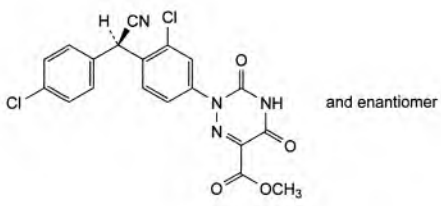
B. 2-[3-chloro-4-[(*RS*)-(4-chlorophenyl)cyanomethyl]phenyl]-3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazine-6-carboxamide,



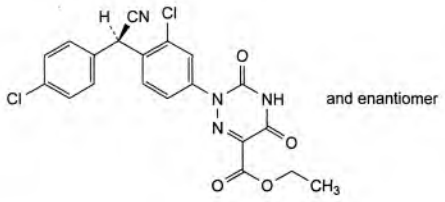
C. (2*RS*)-2-[2-chloro-4-(3,5-dioxo-4,5-dihydro-1,2,4-triazin-2(3*H*)-yl)phenyl]-2-(4-chlorophenyl)acetamide,



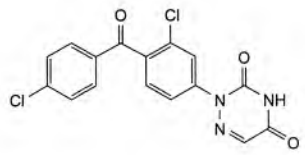
D. 2-[3-chloro-4-[(*RS*)-(4-chlorophenyl)cyanomethyl]phenyl]-*N*,*N*-dimethyl-3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazine-6-carboxamide,



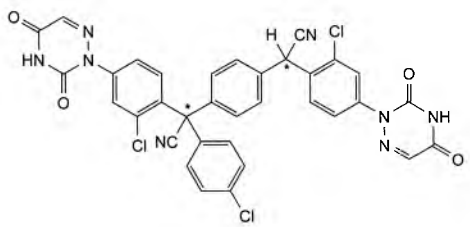
E. methyl 2-[3-chloro-4-[(*RS*)-(4-chlorophenyl)cyanomethyl]phenyl]-3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazine-6-carboxylate,



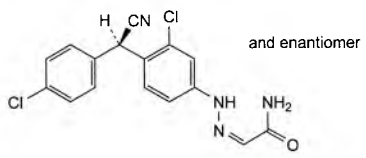
F. ethyl 2-[3-chloro-4-[(*RS*)-(4-chlorophenyl)cyanomethyl]phenyl]-3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazine-6-carboxylate,



G. 2-[3-chloro-4-(4-chlorobenzoyl)phenyl]-1,2,4-triazine3,5(2*H*,4*H*)-dione,



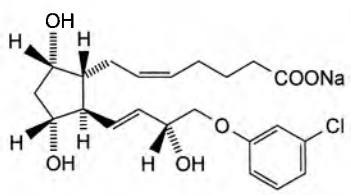
H. [2-chloro-4-(3,5-dioxo-4,5-dihydro-1,2,4-triazin-2(3*H*)-yl)phenyl][4-[[2-chloro-4-(3,5-dioxo-4,5-dihydro-1,2,4-triazin-2(3*H*)-yl)phenyl]cyanomethyl]phenyl](4-chlorophenyl)acetonitrile,



I. (*Z*)-2-[[3-chloro-4-[(*RS*)-(4-chlorophenyl)cyanomethyl]phenyl]diazanylidene]acetamide.

Ph Eur

Cloprostenol Sodium



$C_{22}H_{28}ClNaO_6$ 446.9 *55028-72-3*

Action and use
Prostaglandin ($PGF_{2\alpha}$) analogue.

Preparation
Cloprostenol Injection

DEFINITION
Cloprostenol Sodium is (±)-(5*Z*)-7-(1*R*,3*R*,5*S*)-2-[(1*E*,3*R*)-4-(3-chlorophenoxy)-3-hydroxybut-1-enyl]-3,5-dihydroxycyclopentylhept-5-enoate. It contains not less than 97.5% and not more than 102.5% of $C_{22}H_{28}ClNaO_6$, calculated with reference to the anhydrous substance.

CAUTION *Cloprostenol Sodium is extremely potent and extraordinary care should be taken in any procedure in which it is used.*

CHARACTERISTICS
A white or almost white, amorphous powder; hygroscopic.

Freely soluble in *water*, in *ethanol (96%)* and in *methanol*; practically insoluble in *acetone*.

IDENTIFICATION
A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of cloprostenol sodium *(RSV 11)*.

B. Yields reaction A characteristic of *sodium salts*, Appendix VI.

TESTS
Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *absolute ethanol*.

(1) 2.0% w/v of the substance being examined.

(2) 0.050% w/v of the substance being examined.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Partisil is suitable).

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

(c) Use a flow rate of 1.8 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 5 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of the peak due to Cloprostenol.

MOBILE PHASE

1 volume of *glacial acetic acid*, 70 volumes of *absolute ethanol* and 930 volumes of *hexane*.

LIMITS

In the chromatogram obtained with solution (1):

the sum of the areas of any *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2.5%).

Water
Not more than 3.0% w/w, Appendix IX C. Use 50 mg dissolved in 1 mL of *absolute ethanol*.

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

(1) 0.08% w/v of the substance being examined.

(2) 0.08% w/v of *cloprostenol sodium BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Partisil is suitable).

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

(c) Use a flow rate of 1.8 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 220 nm.

(f) Inject 5 µL of each solution.

MOBILE PHASE

1 volume of *glacial acetic acid*, 100 volumes of *absolute ethanol* and 900 volumes of *hexane*.

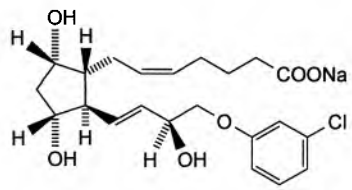
DETERMINATION OF CONTENT

Calculate the content of $C_{22}H_{28}ClNaO_6$ from the chromatograms obtained and using the declared content of $C_{22}H_{28}ClNaO_6$ in *cloprostenol sodium BPCRS*.

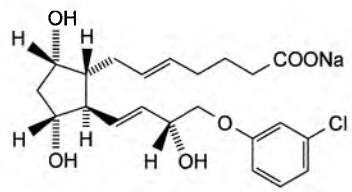
STORAGE

Cloprostenol Sodium should be protected from light and moisture.

IMPURITIES



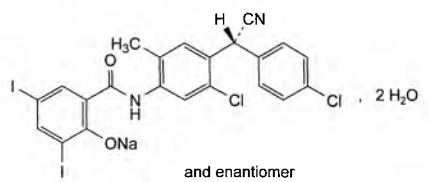
A. (±)-(5*Z*)-7-(1*R*,3*R*,5*S*)-2-[(1*E*,3*S*)-4-(3-chlorophenoxy)-3-hydroxybut-1-enyl]-3,5-dihydroxycyclopentylhept-5-enoate *(epimer)*,



B. (±)-(5*E*)-7-(1*R*,3*R*,5*S*)-2-[(1*E*,3 *R*)-4-(3-chlorophenoxy)-3-hydroxybut-1-enyl]-3,5-dihydroxycyclopentylhept-5-enoate *(trans-isomer)*.

Closantel Sodium Dihydrate

(Closantel Sodium Dihydrate for Veterinary Use, Ph Eur monograph 1716)



and enantiomer

$C_{22}H_{13}Cl_2I_2N_2NaO_2,2H_2O$ 721 *61438-64-0*

Action and use

Antihelminthic.

Ph Eur

DEFINITION

N-[5-Chloro-4-[(*RS*)-(4-chlorophenyl)cyanomethyl]-2-methylphenyl]-2-hydroxy-3,5-diiodobenzamide sodium salt dihydrate.

Content

98.5 per cent to 101.5 per cent (anhydrous substance).

CHARACTERS

Appearance

Yellow powder, slightly hygroscopic.

Solubility

Very slightly soluble in water, freely soluble in ethanol (96 per cent), soluble in methanol.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs without recrystallisation.

Comparison closantel sodium dihydrate CRS.

B. Dissolve 0.1 g in 2 mL of *ethanol (96 per cent) R*. The solution gives reaction (a) of sodium (*2.3.1*).

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution GY_4 (*2.2.2, Method II*).

Dissolve 0.50 g in *ethanol (96 per cent) R* and dilute to 50 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use and protect from light.*

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

Reference solution (a) Dissolve 10 mg of *closantel for system suitability CRS* (containing impurities A to J) in *methanol R* and dilute to 1.0 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *methanol R*. Dilute 5.0 mL of this solution to 25.0 mL with *methanol R*.

Column:

— *size*: $l = 0.10$ m, Ø = 4.6 mm,

— *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm),

— *temperature*: 35 °C.

Mobile phase:

— *mobile phase A*: to 100 mL of a 7.7 g/L solution of *ammonium acetate R* previously adjusted to pH 4.3 with *acetic acid R*, add 50 mL of *acetonitrile R* and 850 mL of *water R*;

— *mobile phase B*: to 100 mL of a 7.7 g/L solution of *ammonium acetate R* previously adjusted to pH 4.3 with *acetic acid R*, add 50 mL of *water R* and 850 mL of *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2	50	50
2 - 22	50 → 20	50 → 80
22 - 27	20	80

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 240 nm.

Injection 10 µL.

Relative retention With reference to closantel (retention time = about 16 min): impurity A = about 0.07; impurity B = about 0.48; impurity C = about 0.62; impurity D = about 0.65; impurity E = about 0.82; impurity F = about 0.89; impurity G = about 0.93; impurity H = about 1.13; impurity I = about 1.16; impurity J = about 1.55.

System suitability: reference solution (a):

— *resolution*: baseline separation between the peaks due to impurity G and closantel,

— the chromatogram obtained is similar to the chromatogram supplied with *closantel for system suitability CRS*.

Limits:

— *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.5; impurity B = 1.3;

— *impurity G*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *impurities F, H, I*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *impurities A, B, C, D, E, J*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);

— *total*: not more than 7.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent);

— *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.12*)

4.8 per cent to 5.8 per cent, determined on 0.250 g.

Use a mixture of 1 volume of *dimethylformamide R* and 4 volumes of *methanol R* as the solvent.

ASSAY

Dissolve 0.500 g in 50 mL of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

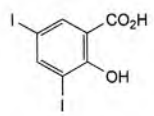
1 mL of *0.1 M perchloric acid* is equivalent to 68.5 mg of $C_{22}H_{13}Cl_2I_2N_2NaO_2$.

STORAGE

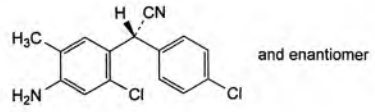
In an airtight container, protected from light.

IMPURITIES

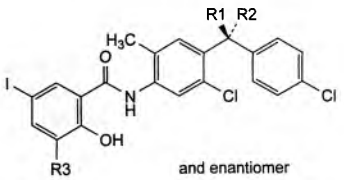
Specified impurities: A, B, C, D, E, F, G, H, I, J.



A. 2-hydroxy-3,5-diiodobenzoic acid,



B. (2*RS*)-(4-amino-2-chloro-5-methylphenyl)(4-chlorophenyl)ethanenitrile,



C. R1 = H, R2 = CO_2H, R3 = I: (2*RS*)-[2-chloro-4-[(2-hydroxy-3,5-diiodobenzoyl)amino]-5-methylphenyl](4-chlorophenyl)acetic acid,

D. R1 = H, R2 = $CONH_2$, R3 = I: *N*-[4-[(1*RS*)-2-amino-1-(4-chlorophenyl)-2-oxoethyl]-5-chloro-2-methylphenyl]-2-hydroxy-3,5-diiodobenzamide,

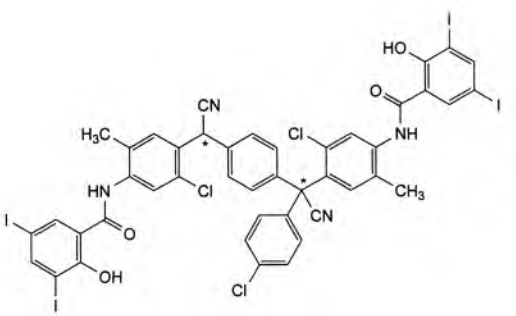
E. R1 = H, R2 = CN, R3 = Cl: 3-chloro-*N*-[5-chloro-4-[(*RS*)-(4-chlorophenyl)cyanomethyl]-2-methylphenyl]-2-hydroxy-5-iodobenzamide,

F. R1 + R2 = O, R3 = I: *N*-[5-chloro-4-(4-chlorobenzoyl)-2-methylphenyl]-2-hydroxy-3,5-diiodobenzamide,

G. R1 = H, R2 = $C(=NH)OCH_3$, R3 = I: methyl (2*RS*)-2-[2-chloro-4-[(2-hydroxy-3,5-diiodobenzoyl)amino]-5-methylphenyl]-2-(4-chlorophenyl)acetimidate,

H. R1 = H, R2 = $CO\text{-}OCH_3$, R3 = I: methyl (2*RS*)-[2-chloro-4-[(2-hydroxy-3,5-diiodobenzoyl)amino]-5-methylphenyl](4-chlorophenyl)acetate,

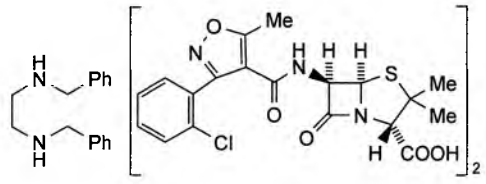
I. R1 = R3 = H, R2 = CN: *N*-[5-chloro-4-[(*RS*)-(4-chlorophenyl)cyanomethyl]-2-methylphenyl]-2-hydroxy-5-iodobenzamide,



J. *N*-[5-chloro-4-[[4-[[2-chloro-4-[(2-hydroxy-3,5-diiodobenzoyl)amino]-5-methylphenyl]cyanomethyl]phenyl](4-chlorophenyl)cyanomethyl]-2-methylphenyl]-2-hydroxy-3,5-diiodobenzamide.

Ph Eur

Cloxacillin Benzathine



$C_{16}H_{20}N_2,(C_{19}H_{18}ClN_3O_5S)_2$ 1112.1 *32222-55-2*

Action and use
Penicillin antibacterial.

Preparations
Cloxacillin Benzathine Intramammary Infusion (Dry Cow)

Ampicillin Trihydrate and Cloxacillin Benzathine Intramammary Infusion (Dry Cow)

DEFINITION
Cloxacillin Benzathine is *N,N'*-dibenzylethylenediammonium bis[(6*R*)-6-(3-*o*-chlorophenyl-5-methylisoxazole-4-carboxamido)penicillanate]. It contains not less than 92.0% of $C_{16}H_{20}N_2,(C_{19}H_{18}ClN_3O_5S)_2$ and not less than 20.0% and not more than 22.0% of benzathine, $C_{16}H_{20}N_2$, each calculated with reference to the anhydrous substance.

CHARACTERISTICS
A white or almost white powder.

Slightly soluble in *water*; freely soluble in *methanol*; slightly soluble in *ethanol (96%)* and in *propan-2-ol*.

IDENTIFICATION
A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of cloxacillin benzathine *(RSV 12)*.

B. Shake 0.1 g with 1 mL of 1M *sodium hydroxide* for 2 minutes, add 2 mL of *ether*, shake for 1 minute and allow to separate. Evaporate 1 mL of the ether layer to dryness, dissolve the residue in 2 mL of *glacial acetic acid* and add 1 mL of *dilute potassium dichromate solution*. A golden yellow precipitate is produced.

C. Shake 50 mg with 10 mL of *water* and filter. To 5 mL of the filtrate add a few drops of *silver nitrate solution*. No precipitate is produced. Heat 50 mg with 2 mL of *alcoholic potassium hydroxide solution* on a water bath for 15 minutes, add 15 mg of *activated charcoal*, shake and filter. Acidify the filtrate with 2M *nitric acid*. The solution yields reaction A characteristic of *chlorides*, Appendix VI.

TESTS
Water
Not more than 5.0% w/w, Appendix IX C. Use 0.5 g.

ASSAY
For cloxacillin benzathine
To 60 mg add 40 mL of *methanol*, shake to dissolve, add 25 mL of 1M *sodium hydroxide* and allow to stand for 30 minutes. Add 27.5 mL of 1M *hydrochloric acid* and sufficient *water* to produce 100 mL, mix, transfer 20 mL of the solution to a stoppered flask, add 30 mL of 0.01M *iodine VS*, close the flask with a wet stopper and allow to stand for 15 minutes protected from light. Titrate the excess of iodine with 0.02M *sodium thiosulfate VS*, using *starch mucilage*, added towards the end of the titration, as indicator. Add a further 12 mg of the substance being examined to 10 mL of *water*, swirl to disperse, add 30 mL of 0.01M *iodine VS* and titrate immediately with 0.02M *sodium thiosulfate VS*, using *starch mucilage*, added towards the end of the titration, as indicator. The difference between the titrations represents the volume of 0.01M *iodine VS* equivalent to the total penicillins present. Calculate the content of $C_{16}H_{20}N_2,(C_{19}H_{18}ClN_3O_5S)_2$ from the difference obtained by carrying out the assay simultaneously using *cloxacillin benzathine BPCRS* and from the declared content of $C_{16}H_{20}N_2,(C_{19}H_{18}ClN_3O_5S)_2$ in *cloxacillin benzathine BPCRS*.

For benzathine
To 1 g add 30 mL of a saturated solution of *sodium chloride* and 10 mL of 5M *sodium hydroxide*, shake well, and extract with four 50 mL quantities of *ether*. Wash the combined extracts with three 10 mL quantities of *water*, extract the combined washings with 25 mL of *ether* and add the extract to the main ether solution. Evaporate the ether solution to low bulk, add 2 mL of *absolute ethanol* and evaporate to dryness. To the residue add 50 mL of *anhydrous acetic acid* and titrate with 0.1M *perchloric acid VS*, using 0.1 mL of *1-naphtholbenzein solution* as indicator. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of perchloric acid required to neutralise the liberated base. Each mL of 0.1M *perchloric acid VS* is equivalent to 12.02 mg of $C_{16}H_{20}N_2$.

STORAGE
Cloxacillin Benzathine should be kept in an airtight container. If the material is sterile, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

LABELLING
The label states, where applicable, that the material is sterile.

Cloxacillin Benzathine intended for use in the manufacturer of either a parenteral dosage form or an intramammary infusion without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility
Complies with the *test for sterility*, Appendix XVI A.

Cobalt Oxide

Co_3O_4 240.8 *1307-96-9*

Action and use
Used in the prevention of cobalt deficiency in ruminants.

DEFINITION
Cobalt Oxide consists of cobalt(II,III) oxide (tricobalt tetraoxide) with a small proportion of cobalt(III) oxide (dicobalt trioxide). It contains not less than 70.0% and not more than 75.0% of Co, calculated with reference to the substance ignited at about 600°.

CHARACTERISTICS
A black powder.

Practically insoluble in *water*. It dissolves in mineral acids and in solutions of the alkali hydroxides.

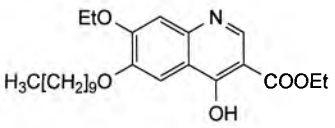
IDENTIFICATION
A. Dissolve 50 mg, with warming, in 5 mL of *hydrochloric acid* and add 10 mL of *water*. To 2 mL of the solution add 1 mL of 5M *sodium hydroxide*. A blue precipitate which becomes pink on warming is produced. Reserve the remainder of the solution for use in test B.

B. Neutralise 10 mL of the solution reserved in test A with 5M *sodium hydroxide* and add 0.5 mL of 6M *acetic acid* and 10 mL of a 10% w/v solution of *potassium nitrite*. A yellow crystalline precipitate is produced.

Loss on ignition
When ignited at about 600°, loses not more than 1.0% of its weight. Use 1 g.

ASSAY
Dissolve 0.1 g in 20 mL of *hydrochloric acid*, by repeated evaporation if necessary. Add 300 mL of *water*, 4 g of *hydroxylamine hydrochloride* and 25 mL of 13.5M *ammonia*. Warm to 80° and titrate with 0.05M *disodium edetate VS*, using *methyl thymol blue mixture* as indicator, until the colour changes from blue to purple. Each mL of 0.05M *disodium edetate VS* is equivalent to 2.946 mg of Co.

Decoquinate



$C_{24}H_{35}NO_5$ 417.6 *18507-89-6*

Action and use
Antiprotozoal (veterinary).

Preparation
Decoquinate Premix

DEFINITION
Decoquinate is ethyl 6-decyloxy-7-ethoxy-4-hydroxyquinoline-3-carboxylate. It contains not less than 99.0% and not more than 101.0% of $C_{24}H_{35}NO_5$, calculated with reference to the dried substance.

CHARACTERISTICS
A cream to buff-coloured, microcrystalline powder; odourless or almost odourless.

Insoluble in *water*; very slightly soluble in *chloroform* and in *ether*; practically insoluble in *ethanol (96%)*.

IDENTIFICATION
A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of decoquinate *(RSV 14)*.

B. The *light absorption*, Appendix II B, in the range 230 to 350 nm of the solution used in the test for Light absorption exhibits a well-defined maximum only at 265 nm.

TESTS
Light absorption
Dissolve 40 mg in 10 ml of hot *chloroform* and, keeping the solution warm, dilute slowly with 70 ml of *absolute ethanol*. Cool and dilute to 100 ml with *absolute ethanol*. Immediately dilute 10 ml to 100 ml with *absolute ethanol*. To 10 ml of the solution add 10 ml of 0.1M *hydrochloric acid* and dilute to 100 ml with *absolute ethanol*. The *absorbance* of the resulting solution at the maximum at 265 nm is 0.38 to 0.42, calculated with reference to the dried substance, Appendix II B.

Related substances
Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) 1.0% w/v of the substance being examined in *chloroform*, prepared with the aid of heat.

(2) 0.0050% w/v of *diethyl 4-decyloxy-3-ethoxyanilinomethylenemalonate BPCRS* in *chloroform*.

(3) 0.010% w/v of the substance being examined in *chloroform*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel* F_{254} (Merck silica gel 60 F_{254} plates are suitable).

(b) Use the mobile phase as described below.

(c) Apply 10 µl of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

5 volumes of *anhydrous formic acid*, 10 volumes of *absolute ethanol* and 85 volumes of *chloroform*.

LIMITS

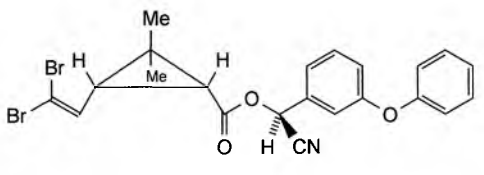
Any *secondary spot* corresponding to diethyl 4-decyloxy-3-ethoxyanilinomethylenemalonate in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%) and any other *secondary spot* is not more intense than the spot in the chromatogram obtained with solution (3) (1%).

Loss on drying
When dried to constant weight at 105°, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash
Not more than 0.1%, Appendix IX A.

ASSAY
Dissolve 1 g in a mixture of 50 ml of *chloroform* and 50 ml of *anhydrous acetic acid* and carry out Method I for *non-aqueous titration*, Appendix VIII A, using *crystal violet solution* as indicator. Each ml of 0.1M *perchloric acid VS* is equivalent to 41.76 mg of $C_{24}H_{35}NO_5$.

Deltamethrin



$C_{22}H_{19}Br_2NO_3$ 505.2 *52918-63-5*

Action and use
Insecticide (veterinary).

Preparation
Deltamethrin Pour-on

DEFINITION
Deltamethrin is (*S*)-α-cyano-3-phenoxybenzyl-(1*R*,3*R*)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylate. It contains not less than 97.0% and not more than 101.0% of $C_{22}H_{19}Br_2NO_3$.