MEDICINAL AND PHARMACEUTICAL SUBSTANCES

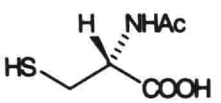
Acetone ☆

Related substances Line 10. For '(0.5 mm)' read '(0.5 µm)'.

Acetylcysteine

Change the monograph to:

Acetylcysteine ☆



$C_5H_9NO_3S$ 163.2 *616-91-1*

Definition Acetylcysteine contains not less than 98.0% and not more than 101.0% of (*R*)-2-acetamido-3-mercaptopropanoic acid, $C_5H_9NO_3S$, calculated with reference to the dried substance.

Characteristics A white, crystalline powder or colourless crystals; freely soluble in *water* and in *ethanol (96%)*; practically insoluble in *dichloromethane*.

Dissolve 1.0 g in carbon dioxide-free water *and dilute to 20 ml with the same solvent (solution S).*

Identification *Identification test C may be omitted if identification tests A, B, D and E are carried out. Identification tests B, D and E may be omitted if identification tests A and C are carried out.*

A. Complies with the test for Specific optical rotation.

B. *Melting point* 104° to 110°, Appendix V A, Method I.

C. Examine by *infrared absorption spectrophotometry*, Appendix II A. The absorption maxima in the spectrum obtained with the substance being examined correspond in position and relative intensity to those in the spectrum obtained with *acetylcysteine EPCRS*. Examine the substances prepared as discs using *potassium bromide*.

D. Examine the chromatograms obtained in the test for Related substances. The retention time and size of the principal peak in the chromatogram obtained with solution (2) are approximately the same as those of the principal peak in the chromatogram obtained with solution (5).

E. To 0.5 ml of solution S add 0.05 ml of a 5% w/v solution of *sodium nitroprusside* and 0.05 ml of 13.5M *ammonia*. A dark violet colour develops.

Appearance of solution Solution S is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method II.

pH To 2 ml of solution S add 8 ml of *carbon dioxide-free water* and mix. The pH of the solution is 2.0 to 2.8, Appendix V L.

Specific optical rotation In a 25-ml volumetric flask, mix 1.25 g with 1 ml of a 1% w/v solution of *disodium edetate*. Add 7.5 ml of a 4% w/v solution of *sodium hydroxide*, mix and dissolve. Dilute to 25 ml with *phosphate buffer solution pH 7.0 R2*. The *specific optical rotation* is +21.0° to +27.0°, Appendix V F, calculated with reference to the dried substance.

Related substances Examine by *liquid chromatography*, Appendix III D. Except where otherwise prescribed, prepare the solutions immediately before use.

Solution (1) Suspend 0.80 g of the substance being examined in 1 ml of 1M *hydrochloric acid* and dilute to 100 ml with *water*.

Solution (2) Dilute 5 ml of solution (1) to 100 ml with *water*. Dilute 5 ml of the solution to 50 ml with *water*.

Solution (3) Use solution (1) after storage for at least 1 hour.

Solution (4) Suspend 4.0 mg of *acetylcysteine EPCRS*, 4.0 mg of *L-cystine*, 4.0 mg of *L-cysteine*, 4.0 mg of N,N'-*diacetyl-L-cystine EPCRS* and 4.0 mg of N,S-*diacetyl-L-cysteine EPCRS* in 1 ml of 1M *hydrochloric acid* and dilute to 100 ml with *water*.

Solution (5) Suspend 4.0 mg of *acetylcysteine EPCRS* in 1 ml of 1M *hydrochloric acid* and dilute to 100 ml with *water*.

The chromatographic procedure may be carried out using:

(a) a stainless steel column (25 cm × 4 mm), packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP18 is suitable),

(b) as mobile phase at a flow rate of 1.0 ml per minute a mixture prepared as follows. Stir 3 volumes of *acetonitrile* and 97 volumes of *water* in a beaker; adjust to pH 3.0 with *orthophosphoric acid*,

(c) as detector a spectrophotometer set at 220 nm.

When the chromatograms are recorded under the prescribed conditions, the retention times are: L-cystine, about 2.2 minutes; L-cysteine, about 2.4 minutes; 2-methyl-2-thiazoline-4-carboxylic acid, originating in solution (3), about 3.3 minutes; acetylcysteine, about 6.4 minutes; *N,N'*-diacetyl-L-cystine, about 12 minutes and *N,S*-diacetyl-L-cysteine, about 14 minutes. Inject 20 µl of solution (4). The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to L-cystine and L-cysteine is at least 1.5 and the *resolution factor* between the peaks corresponding to *N,N'*-diacetyl-L-cystine and *N,S*-diacetyl-L-cysteine is at least 2.0. Inject 20 µl of 0.01M *hydrochloric acid* as a blank. Inject three times 20 µl of solution (4), 20 µl of solution (5) and 20 µl of each of solutions (1), (2) and (3). Continue the chromatography for five times the retention time of acetylcysteine (about 30 minutes).

From the chromatogram obtained with solution (1), calculate the percentage content of the known impurities

and the unknown impurities using the following expressions:

$$\text{known impurity} = \frac{A_1 \times m_2 \times 100}{A_2 \times m_1}$$

$$\text{unknown impurity} = \frac{A_3 \times m_3 \times 100}{A_4 \times m_1}$$

where A_1 = peak area of individual impurity (L-cystine, L-cysteine, *N,N'*-diacetyl-L-cystine and *N,S*-diacetyl-L-cysteine) in the chromatogram obtained with solution (1),

A_2 = peak area of the corresponding individual impurity (L-cystine, L-cysteine, *N,N'*-diacetyl-L-cystine and *N,S*-diacetyl-L-cysteine) in the chromatogram obtained with solution (4);

A_3 = peak area of unknown impurity in the chromatogram obtained with solution (1),

A_4 = peak area of acetylcysteine in the chromatogram obtained with solution (5),

m_1 = weight of the substance being examined in solution (1),

m_2 = weight of the individual impurity in solution (4),

m_3 = weight of acetylcysteine in solution (5).

The percentage content of each known impurity and of each unknown impurity is not greater than 0.5%. The sum of the calculated percentage contents of known and unknown impurities is not greater than 0.5%. Disregard any peak due to the solvent, the peak appearing at retention time of about 3.3 minutes corresponding to 2-methyl-2-thiazoline-4-carboxylic acid and any peak with an area less than 0.1 times that of the principal peak in the chromatogram obtained with solution (5) (0.05%).

Heavy metals 2.0 g complies with *limit test C for heavy metals*, Appendix VII (10 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb)*.

Zinc Not more than 10 ppm of Zn, determined by Method II for *atomic absorption spectrophotometry*, Appendix II D.

Test solution Dissolve 1 g in 0.001M *hydrochloric acid* and dilute to 50 ml with the same acid.

Standard solutions Prepare standard solutions using *zinc standard solution (5 mg/ml Zn)*, diluted with 0.001M *hydrochloric acid*. Measure the *absorbance* at 213.8 nm, using a zinc hollow-cathode lamp as a source of radiation, an air—acetylene flame and a correction procedure for non-specific absorption.

Loss on drying Not more than 1.0%, determined on 1 g by drying in an oven *in vacuo* at 70° for 3 hours, Appendix IX D.

Sulphated ash Not more than 0.2%, determined on 1 g, Appendix IX A, Method II.

Assay Dissolve 0.14 g in 60 ml of *water* and add 10 ml of 2M *hydrochloric acid*. After cooling in iced water, add 10 ml of *potassium iodide solution* and titrate with 0.05M *iodine VS*, using 1 ml of *starch solution* as indicator. Each ml of 0.05M *iodine VS* is equivalent to 16.32 mg of $C_5H_9NO_3S$.

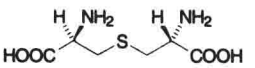
Storage Store in a well-closed container, protected from light.

Action and use Antidote for paracetamol poisoning; mucolytic.

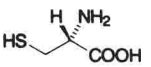
1/96

The impurities limited by the requirements of this monograph include:

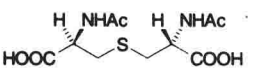
L-cystine



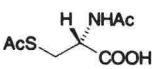
L-cysteine



N,N-diacetyl-L-cystine



N,S-diacetyl-L-cysteine

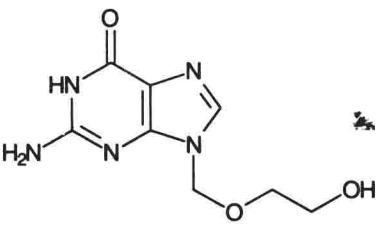


Acyclovir

Change the monograph to:

Aciclovir ☆

Acyclovir



$C_8H_{11}N_5O_3$ 225.2 *59277-89-3*

Definition Aciclovir contains not less than 98.5% and not more than 101.0% of 2-amino-9-[(2-hydroxyethoxy)-methyl]-1,9-dihydro-6*H*-purin-6-one, $C_8H_{11}N_5O_3$, calculated with reference to the anhydrous substance.

Characteristics A white or almost white, crystalline powder; slightly soluble in *water*; freely soluble in *dimethyl sulphoxide*; very slightly soluble in *ethanol (96%)*. It dissolves in dilute solutions of mineral acids and alkali hydroxides.

Identification Examine by *infrared absorption spectrophotometry*, Appendix II A. The absorption maxima in the spectrum obtained with the substance being examined correspond in position and relative intensity to those in the spectrum obtained with *aciclovir EPCRS*.

Appearance of solution Dissolve 0.25 g in 0.1M *sodium hydroxide* and dilute to 25 ml with the same solvent. The solution is *clear*, Appendix IV A, and not more intensely coloured than *reference solution* Y_7, Appendix IV B, Method II.

Related substances A. Examine by *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance. Prepare the solutions immediately before use.

Solution (1) Dissolve 0.1 g of the substance being exam-

ined in *dimethyl sulphoxide* and dilute to 10 ml with the same solvent.
Solution (2) Dissolve 10 mg of *aciclovir impurity A EPCRS* in *dimethyl sulphoxide* and dilute to 20 ml with the same solvent. Dilute 1 ml of the solution to 10 ml with *dimethyl sulphoxide*.

Apply separately to the plate 10 µl of each solution. Keep the spots compact by drying in a current of warm air. Allow the plate to cool and develop over a path of 10 cm with a mixture of 2 volumes of 13.5M *ammonia*, 20 volumes of *methanol* and 80 volumes of *dichloromethane*. Allow the plate to dry in air and examine in *ultraviolet light (254 nm)*. In the chromatogram obtained with solution (1) any *secondary spot* with an Rf value greater than that of the principal spot is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%).

B. Examine by *liquid chromatography*, Appendix III D.
Solution (1) Dissolve 50 mg of the substance being examined in 10 ml of a mixture of 20 volumes of *glacial acetic acid* and 80 volumes of *water* and dilute to 100 ml with the mobile phase.
Solution (2) Dilute 1 ml of solution (1) to 200 ml with the mobile phase.
Solution (3) Dissolve 20 mg of *aciclovir EPCRS* and 20 mg of *aciclovir impurity A EPCRS* in a mixture of 20 volumes of *glacial acetic acid* and 80 volumes of *water* and dilute to 100 ml with the same mixture of solvents. Dilute 1 ml of the solution to 10 ml with the mobile phase.
Solution (4) Dissolve 7 mg of *guanine* in *dimethyl sulphoxide* and dilute to 100 ml with the same solvent. Dilute 1 ml of the solution to 20 ml with the mobile phase.

The chromatographic procedure may be carried out using:
(a) a stainless steel column (0.1 m × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Spherisorb ODS 2 is suitable),
(b) as mobile phase at a flow rate of 2 ml per minute a mixture prepared as follows: dissolve 6 g of *sodium dihydrogen orthophosphate* and 1 g of *sodium decanesulphonate* in 900 ml of *water* and adjust to pH 3 ± 0.1 with *orthophosphoric acid*; add 40 ml of *acetonitrile* and dilute to 1 litre with *water*,
(c) as detector a spectrophotometer set at 254 nm,
(d) a loop injector.

Inject 20 µl of each solution. Record the chromatograms for 7 times the retention time of aciclovir. The test is not valid unless, in the chromatogram obtained with solution (3), the number of *theoretical plates* calculated for the peak due to aciclovir impurity A is at least 1500 and its *capacity factor* is at least 7 (calculate V_0 from the peak corresponding to dimethyl sulphoxide). In the chromatogram obtained with the solution (1) the area of any peak corresponding to guanine is not greater than that of the principal peak in the chromatogram obtained with solution (4) (0.7%), the area of any other *secondary peak* is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.5%) and the sum of the areas of all the *secondary peaks* apart from any peak corresponding to guanine is not greater than twice the area of the peak in the chromatogram obtained with solution (2) (1.0%). Disregard any peak with an area less than 0.05 times that of the principal peak in the chromatogram obtained with solution (2) (0.025%).

Water Not more than 6.0%, determined on 0.5 g, Appendix IX C, Method I.

Sulphated ash Not more than 0.1%, determined on 1 g, Appendix IX A, Method II.

Assay Dissolve 0.15 g in 60 ml of *glacial acetic acid*. Titrate with 0.1M *perchloric acid VS*, determining the end point potentiometrically, Appendix VIII B. Carry out a blank test. Each ml of 0.1M *perchloric acid VS* is equivalent to 22.52 mg of $C_8H_{11}N_5O_3$.

Storage Store in a well-closed container.

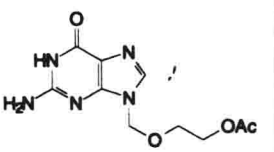
Preparations
Aciclovir Cream
Aciclovir Eye Ointment
Aciclovir Intravenous Infusion
Aciclovir Oral Suspension
Aciclovir Tablets

Action and use Antiviral.

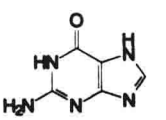
1/96

The impurities limited by the requirements of this monograph include:

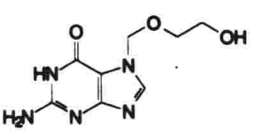
2-amino-9-{[2-(acetyloxy)-ethoxy]methyl}-1,9-dihydro-6*H*-purin-6-one *(aciclovir impurity A)*



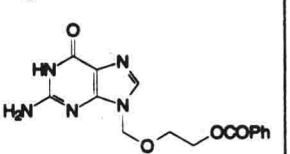
2-amino-1,7-dihydro-6*H*-purin-6-one



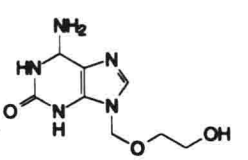
2-amino-7-[(2-hydroxy-ethoxy)methyl]-1,7-dihydro-6*H*-purin-6-one



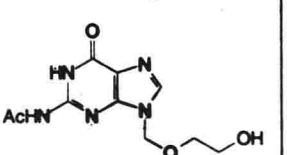
2-amino-9-{[2-(benzoyl-oxy)ethoxy]methyl}-1,9-dihydro-6*H*-purin-6-one



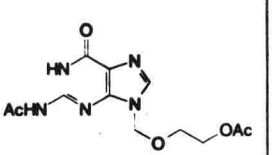
6-amino-9-[(2-hydroxy-ethoxy)methyl]-1,3-dihydro-2*H*-purin-2-one



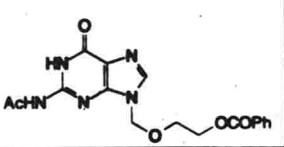
2-acetamido-9-[(2-hydroxyethoxy)methyl]-1,9-dihydro-6*H*-purin-6-one



2-acetamido-9-{[2-(acetyloxy)ethoxy]methyl}-1,9-dihydro-6*H*-purin-6-one



2-acetamido-9-{[2-(benzoyloxy)ethoxy]-methyl}-1,9-dihydro-6*H*-purin-6-one



Barbados Aloes ☆

Assay Line 26. For 'barbaloin' read 'hydroxyanthracene derivatives, as barbaloin'.

7/95

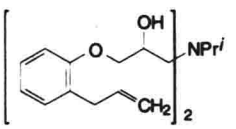
Cape Aloes ☆

Assay Line 26. For 'barbaloin' read 'hydroxyanthracene derivatives, as barbaloin'.

7/95

Alprenolol Hydrochloride ☆

Impurities display box. Replace the fourth structure by:



7/95

Aluminium Chloride Hexahydrate ☆

$AlCl_3,6H_2O$ 241.4 *7784-13-6*

Definition Aluminium Chloride Hexahydrate contains not less than 95.0% and not more than 101.0% of $AlCl_3,6H_2O$.

Characteristics A white or slightly yellow, crystalline powder or colourless crystals; deliquescent; very soluble in *water*; freely soluble in *ethanol (96%)*; soluble in *glycerol*.

Dissolve 10.0 g in distilled water *and dilute to 100 ml with the same solvent (solution S1).*
Dilute 50 ml of solution S1 to 100 ml with water *(solution S2).*

Identification A. Dilute 0.1 ml of solution S2 to 2 ml with *water*. The solution yields reaction A characteristic of *chlorides*, Appendix VI.
B. Dilute 0.3 ml of solution S2 to 2 ml with *water*. The solution yields reaction A characteristic of *aluminium salts*, Appendix VI.

Appearance of solution Solution S2 is *clear*, Appendix IV A, and not more intensely coloured than *reference solution B_7*, Appendix IV B, Method II.

Sulphate 15 ml of solution S1 complies with the *limit test for sulphates*, Appendix VII (100 ppm).

Alkali and alkaline-earth metals To 20 ml of solution S2 add 100 ml of *water* and heat to boiling. To the hot solution add 0.2 ml of *methyl red solution*. Add 6M *ammonia* until the colour of the indicator changes to yellow and dilute to 150 ml with *water*. Heat to boiling and filter. Evaporate 75 ml of the filtrate to dryness on a water bath and ignite to constant weight. The residue weighs not more than 2.5 mg (0.5%).

Heavy metals 12 ml of solution S1 complies with *limit test A for heavy metals*, Appendix VII (20 ppm). Prepare the standard using *lead standard solution (2 ppm Pb)*.

Iron 10 ml of solution S1 complies with the *limit test for iron*, Appendix VII (10 ppm).

Water 42.0 to 48.0%, determined on 50 mg, Appendix IX C, Method I.

Assay Dissolve 0.5 g in 25 ml of *water*. Carry out the *complexometric titration of aluminium*, Appendix VIII D. Titrate with 0.1M *zinc sulphate VS* until the colour of the indicator changes from greyish green to pink. Carry out a blank titration. Each ml of 0.1M *disodium edetate VS* is equivalent to 24.14 mg of $AlCl_3,6H_2O$.

Storage Store in an airtight container.

Action and use Astringent.

1/96

Amitriptyline Hydrochloride ☆

Add a five-pointed star (☆) to the title of the amendment in the *British Pharmacopoeia 1993, Addendum 1995*.

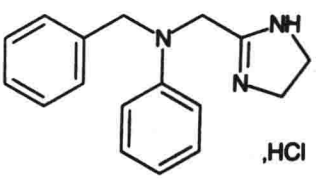
7/95

Amphotericin

Content of tetraenes Change the mathematical expression to:

$$x = F + 100\,(B_1S_2 - B_2S_1) / (N_2B_1 - N_1B_2)$$

Antazoline Hydrochloride ☆



$C_{17}H_{19}N_3,HCl$ 301.8 *2508-72-7*

Definition Antazoline Hydrochloride contains not less than 99.0% and not more than 101.0% of 4,5-dihydro-2-(*N*-benzyl-*N*-phenylaminomethyl)imidazole hydrochloride, $C_{17}H_{19}N_3,HCl$, calculated with reference to the dried substance.

Characteristics A white or almost white, crystalline powder; sparingly soluble in *water*; soluble in *ethanol (96%)*; slightly soluble in *dichloromethane*. It melts at about 240°, with decomposition.

Dissolve 2.0 g in carbon dioxide-free water *prepared from* distilled water, *heating at 60° if necessary. Allow to cool and dilute to 100 ml with the same solvent (solution S).*

Identification *Identification test A may be omitted if identification tests B, C and D are carried out. Identification*

tests B and C may be omitted if identification tests A and D are carried out.

A. Examine by *infrared absorption spectrophotometry*, Appendix II A. The absorption maxima in the spectrum obtained with the substance being examined correspond in position and relative intensity to those in the spectrum obtained with *antazoline hydrochloride EPCRS*. Examine the substances as discs prepared using *potassium chloride*.

B. Examine the chromatograms obtained in the test for Related substances in daylight after spraying. The principal spot in the chromatogram obtained with solution (2) is similar in position, colour and size to the principal spot in the chromatogram obtained with solution (4).

C. To 5 ml of solution S add, drop by drop, 2M *sodium hydroxide* until an alkaline reaction is produced. Filter. Wash the precipitate with two quantities, each of 10 ml, of *water*. Dry the precipitate in a desiccator under reduced pressure. The *melting point* of the dried precipitate, Appendix V A, Method I, is 119° to 123°.

D. Yields reaction A characteristic of *chlorides*, Appendix VI.

Appearance of solution Solution S is *clear*, Appendix IV A, and not more intensely coloured than *reference solution* Y_7, Appendix IV B, Method II.

Acidity or alkalinity To 10 ml of solution S add 0.2 ml of *methyl red solution*. Not more than 0.1 ml of 0.01M *hydrochloric acid VS* or of 0.01M *sodium hydroxide VS* is required to change the colour of the indicator.

Related substances Examine by *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance. Heat the plate at 110° for 15 minutes before using.

Solution (1) Dissolve 0.10 g of the substance being examined in *methanol* and dilute to 5 ml with the same solvent.

Solution (2) Dilute 1 ml of solution (1) to 5 ml with *methanol*.

Solution (3) Dilute 0.5 ml of solution (1) to 100 ml with *methanol*.

Solution (4) Dissolve 20 mg of *antazoline hydrochloride EPCRS* in *methanol* and dilute to 5 ml with the same solvent.

Solution (5) Dissolve 20 mg of *xylometazoline hydrochloride EPCRS* in 1 ml of solution (1) and dilute to 5 ml with *methanol*.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 5 volumes of *diethylamine*, 10 volumes of *methanol* and 85 volumes of *ethyl acetate*. Dry the plate in a current of warm air for 15 minutes. Examine in *ultraviolet light (254 nm)*. The test is not valid unless the chromatogram obtained with solution (5) shows two clearly separated principal spots. Spray with a mixture of equal volumes of a 20% w/v solution of *iron(III) chloride hexahydrate* and a 0.5% w/v solution of *potassium hexacyanoferrate(III)*. Examine immediately in daylight. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%).

Heavy metals 1.0 g complies with *limit test C for heavy metals*, Appendix VII (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb)*.

Loss on drying Not more than 0.5%, determined on 1 g by drying in an oven at 100° to 105° for 3 hours, Appendix IX D.

Sulphated ash Not more than 0.1%, determined on the residue obtained in the test for Loss on drying, Appendix IX A, Method II.

Assay Dissolve 0.25 g in 100 ml of *ethanol (96%)*. Add 0.1 ml of *phenolphthalein solution R1*. Titrate with 0.1M *ethanolic potassium hydroxide VS*. Each ml of 0.1M *ethanolic potassium hydroxide VS* is equivalent to 30.18 mg of $C_{17}H_{19}N_3,HCl$.

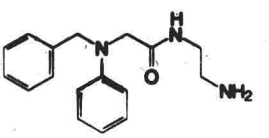
Storage Store in a well-closed container.

Action and use Histamine H_1-receptor antagonist.

1/96

The impurities limited by the requirements of this monograph include:

N-(2-aminoethyl)-2-[(benzyl)(phenyl)amino]-acetamide



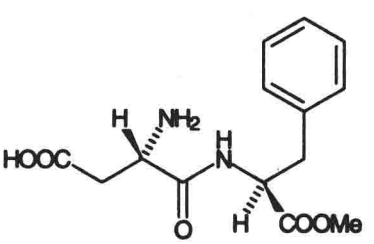
Arachis Oil ☆

Add the following statement.

Preparation

Arachis Oil Enema

Aspartame ☆



$C_{14}H_{18}N_2O_5$ 294.3 *22839-47-0*

Definition Aspartame contains not less than 98.0% and not more than 102.0% of (*S*)-3-amino-*N*-[(*S*)-α-methoxycarbonylphenethyl]succinamic acid, $C_{14}H_{18}N_2O_5$, calculated with reference to the dried substance.

Characteristics A white, crystalline powder; slightly hygroscopic; sparingly soluble to slightly soluble in *water* and in *ethanol (96%)*; practically insoluble in *hexane* and in *dichloromethane*.

Dissolve 0.8 g in carbon dioxide-free water *and dilute to 100 ml with the same solvent (solution S).*

Identification *Identification test B may be omitted if identification tests A, C and D are carried out. Identification tests A, C and D may be omitted if identification test B is carried out.*

A. Dissolve 0.1 g in *ethanol (96%)* and dilute to 100 ml with the same solvent. Examined between 230 nm and 300 nm, Appendix II B, the solution shows absorption maxima at 247 nm, 252 nm, 258 nm and 264 nm.

B. Examine by *infrared absorption spectrophotometry*, Appendix II A. The absorption maxima in the spectrum obtained with the substance being examined correspond in position and relative intensity to those in the spectrum obtained with *aspartame EPCRS*. Examine the substances prepared as discs.

C. Examine by *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance.

Solution (1) Dissolve 15 mg of the substance being examined in 2.5 ml of *water* and dilute to 10 ml with 5M *acetic acid*.

Solution (2) Dissolve 15 mg of *aspartame EPCRS* in 2.5 ml of *water* and dilute to 10 ml with 5M *acetic acid*.

Apply separately to the plate 20 µl of each solution. Develop over a path of 15 cm using a mixture of 2 volumes of *water*, 4 volumes of *anhydrous formic acid*, 30 volumes of *methanol* and 64 volumes of *dichloromethane*. Allow the plate to dry in air. Spray with *ninhydrin solution* and heat at 100° to 105° for 15 minutes. The spot in the chromatogram obtained with solution (1) is similar in position, colour and size to the spot in the chromatogram obtained with solution (2).

D. Dissolve about 20 mg in 5 ml of *methanol* and add 1 ml of *alkaline hydroxylamine solution R1*. Heat on a water bath for 15 minutes. Allow to cool and adjust to about pH 2 with 2M *hydrochloric acid*. Add 0.1 ml of *iron(III) chloride solution R1*. A brownish red colour is produced.

Appearance of solution Solution S is *clear*, Appendix IV A, and not more intensely coloured than *reference solution GY_6*, Appendix IV B, Method II.

Conductivity Not more than 30 µS cm^{-1}. Dissolve 0.8 g in *carbon dioxide-free water* prepared from *distilled water* and dilute to 100 ml with the same solvent. Measure the *conductivity*, Appendix V O, of the solution (C_1) and that of the water used for preparing the solution (C_2). The readings must be stable within 1% over a period of 30 seconds. Calculate the conductivity of the solution of the substance being examined from the expression:

$$C_1 - 0.992C_2$$

Specific optical rotation Dissolve 2 g in a 69% w/v solution of *anhydrous formic acid* and dilute to 50 ml with the same solution. The *specific optical rotation* is +14.5° to +16.5°, Appendix V F, calculated with reference to the dried substance and measured within 30 minutes of the preparation of the solution.

Related substances Examine by *liquid chromatography*, Appendix III D.

Solution (1) Dissolve 0.60 g of the substance being examined in a mixture of 1.5 volumes of *glacial acetic acid* and 98.5 volumes of *water* and dilute to 100 ml with the same mixture of solvents.

Solution (2) Dissolve 9 mg of *aspartame impurity A EPCRS* in a mixture of 1.5 volumes of *glacial acetic acid* and 98.5 volumes of *water* and dilute to 100 ml with the same mixture of solvents.

Solution (3) Dissolve 30 mg of *phenylalanine* in a mixture of 15 volumes of *glacial acetic acid* and 85 volumes of *water* and dilute to 100 ml with the same mixture of solvents. Dilute 1 ml of the solution to 10 ml with *water*.

Solution (4) Dilute 5 ml of solution (1) to 10 ml with *water*. Dilute 3 ml of the solution to 100 ml with *water*.

Solution (5) Dissolve 30 mg of *L-aspartyl-L-phenylalanine* in a mixture of 15 volumes of *glacial acetic acid* and 85 volumes of *water* and dilute to 100 ml with the same mixture of solvents. Dilute 1 ml of the solution to 10 ml with *water*. Mix 1 ml of this solution with 1 ml of solution (3).

The chromatographic procedure may be carried out using:

(a) a stainless steel column (0.25 m × 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm to 10 µm) (Spherisorb ODS 1 is suitable),

(b) as mobile phase at a flow rate of 1 ml per minute a mixture of 10 volumes of *acetonitrile* and 90 volumes of a 0.68% w/v solution of *potassium dihydrogen orthophosphate* previously adjusted to pH 3.7 with *orthophosphoric acid*,

(c) as detector a spectrophotometer set at 220 nm.

Inject 20 µl of solution (4). Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained is not less than 50% of the full scale of the recorder. Inject 20 µl of solution (5). The test is not valid unless, in the chromatogram obtained, the *resolution factor* between the peaks corresponding to phenylalanine and L-aspartyl-L-phenylalanine is at least 3.5. Inject separately 20 µl of each solution. Continue the chromatography for twice the retention time of aspartame. In the chromatogram obtained with solution (1) the area of any peak corresponding to impurity A is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.5%); the area of any peak corresponding to phenylalanine is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (0.5%); and the sum of the areas of any other *secondary peaks* is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (1.5%).

Heavy metals 1.0 g complies with *limit test C for heavy metals*, Appendix VII (10 ppm). Prepare the standard using 1 ml of *lead standard solution (10 ppm Pb)*.

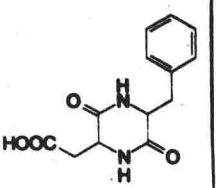
Loss on drying Not more than 4.5%, determined on 1 g by drying in an oven at 100° to 105°, Appendix IX D.

Sulphated ash Not more than 0.2%, determined on 1 g, Appendix IX A, Method II.

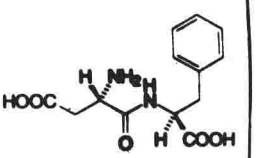
Assay Dissolve 0.25 g in 1.5 ml of *anhydrous formic acid*

The impurities limited by the requirements of this monograph include:

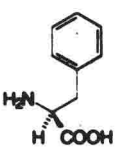
2-(5-benzyl-3,6-dioxopiperazin-2-yl)acetic acid (diketopiperazine) *(aspartame impurity A)*



L-aspartyl-L-phenylalanine



phenylalanine



and 60 ml of *glacial acetic acid*. Titrate immediately with 0.1M *perchloric acid VS*, determining the end point potentiometrically, Appendix VIII B. Each ml of 0.1M *perchloric acid VS* is equivalent to 29.43 mg of $C_{14}H_{18}N_2O_5$

Storage Store in an airtight container.

Action and use Sweetening agent.

1/96

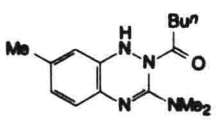
Aspirin ☆

Related substances Lines 1 and 2. For '0.1M *tetrabutylammonium hydroxide* in *propan-2-ol*' read '0.1M *tetrabutylammonium hydroxide in propan-2-ol VS*'.
Line 6. For '*dimethylaminoantipyrine*' read '*4-aminophenazone*'.

7/95

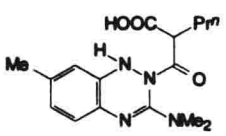
Azapropazone

Impurities display box. Replace the second structure by:



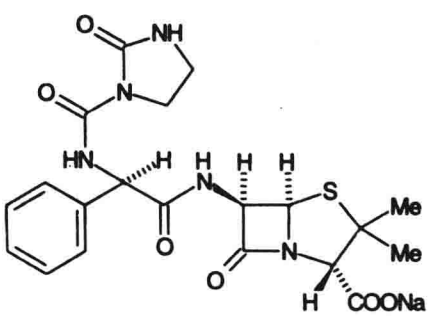
Replace the fourth name and structure by:

α-(3-dimethylamino-7-methyl-1,2-dihydro-1,2,4-benzotriazin-2-ylcarbonyl)valeric acid
(impurity C)



7/95

Azlocillin Sodium



$C_{20}H_{22}N_5NaO_6S$ 483.5 *37091-65-9*

Definition Azlocillin Sodium is sodium (6*R*)-6-[*N*-2-oxoimidazolidin-1-ylcarbonyl)-D-phenylglycylamino]-penicillanate. It contains not less than 92.5% and not more than 102.0% of $C_{20}H_{22}N_5NaO_6S$, calculated with reference to the anhydrous substance.

Characteristics A white to pale yellowish powder; hygroscopic.

Soluble in *water*; very soluble in *methanol*; practically insoluble in *acetone*, in *chloroform* and in *ether*.

Identification A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of azlocillin sodium.
B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as the principal peak in the chromatogram obtained with solution (2).
C. Yields reaction A characteristic of *sodium salts*, Appendix VI.

Acidity or alkalinity pH of a 10% w/v solution, 6.0 to 8.0, Appendix V L.

Clarity and colour of solution A 10.0% w/v solution is *clear*, Appendix IV A, and not more intensely coloured than *reference solution* BY_6, Appendix IV B, Method I.

Specific optical rotation In a 1% w/v solution, +170° to +200°, calculated with reference to the anhydrous substance, Appendix V F.

Related substances Carry out the method for *liquid chromatography*, Appendix III D, using two solutions in *water* containing (1) 0.050% w/v of the substance being examined and (2) 0.0010% w/v of the substance being examined.

The chromatographic procedure may be carried out using (a) a stainless steel column (12.5 cm × 4.6 mm) packed with *stationary phase C* (5 µm) (Hypersil ODS is suitable), (b) as the mobile phase with a flow rate of 1.6 ml per minute a mixture of 145 volumes of *acetonitrile* and 855 volumes of a solution containing 0.58 g of *dipotassium hydrogen orthophosphate* and 4.09 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water* and (c) a detection wavelength of 210 nm. Carry out the procedure at 40°.

Adjust the system so that the height of the principal peak in the chromatogram obtained with solution (2) is at least 20% of the full scale of the recorder. When recorded under the prescribed conditions the retention time of azlocillin is about 5 minutes. If necessary adjust the composition of the mobile phase.

The test is not valid unless the *column efficiency*, determined on the peak due to azlocillin in the chromatogram obtained with solution (2), is at least 24,000 *theoretical plates* per metre and the *symmetry factor* of the principal peak is 0.9 to 2.0.

In the chromatogram obtained with solution (1) the sum of the areas of any peaks due to the 5*R* and 5*S* epimers of azlocillin penicilloate (retention times relative to azlocillin, 0.22 and 0.27) is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (3%); the area of any peak due to ampicillin (retention time relative to azlocillin, 0.29) is not greater than 1.25 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%) and the area of any peak due to the epimers of azlocillin penilloate, which may appear as a doublet, (retention time relative to azlocillin, 0.73) is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (2%).

Water Not more than 2.5% w/w, Appendix IX C. Use 0.25 g.

Assay Carry out the method for *liquid chromatography*, Appendix III D, using two solutions in *water* containing (1) 0.05% w/v of the substance being examined and (2) 0.05% w/v of *azlocillin sodium BPCRS*.

The chromatographic procedure may be carried out using the conditions described under Related substances.

Calculate the content of $C_{20}H_{22}N_5NaO_6S$ from the declared content of $C_{20}H_{22}N_5NaO_6S$ in *azlocillin sodium BPCRS*.

Storage Azlocillin Sodium should be kept in an airtight container and stored at a temperature not exceeding 25°. If the substance is sterile the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

Labelling The label states (1) the date after which the material is not intended to be used; (2) the conditions under which it should be stored; (3) where applicable, that it is sterile; (4) where applicable, that it is apyrogenic.

Preparation
Azlocillin Injection

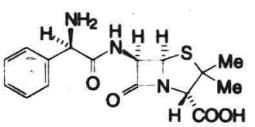
Action and use Antibacterial.

Azlocillin Sodium intended for use in the manufacture of a parenteral dosage form without a further appropriate procedure for the removal of pyrogens complies with the following additional requirement.

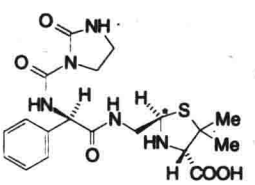
Bacterial endotoxins Carry out the *test for bacterial endotoxins*, Appendix XIV C. Prepare a solution in *water BET* containing 10 mg of Azlocillin Sodium per ml (solution A). The endotoxin limit concentration of solution A is 0.7 Units of endotoxin per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

The impurities limited by the requirements of this monograph include:

ampicillin

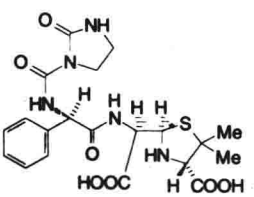


azlocillin penilloate



and epimer at C*

azlocillin penicilloate



and epimer at C*

Azlocillin Sodium intended for use in the manufacture of a parenteral dosage form without a further appropriate sterilisation procedure complies with the following additional requirement.

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

Bearberry Leaf ☆

Definition Bearberry Leaf consists of the whole or cut, dry leaf of *Arctostaphylos uva-ursi* (L.) Spreng. It contains not less than 8.0% of hydroquinone derivatives expressed as anhydrous arbutin, $C_{12}H_{16}O_7$ (molecular weight, 272.3), and calculated with reference to the dried drug.

Characteristics It has the macroscopical and microscopical characters described under Identification tests A and B.

Identification A. The leaf, shiny and dark green on the adaxial surface, lighter on the abaxial surface, is normally 7 mm to 30 mm long × 5 mm to 12 mm wide. The entire leaf is obovate with smooth margins, somewhat reflexed downwards, narrowing at the base into a short petiole. The leaf is obtuse or retuse at its apex. The lamina is thick and coriaceous. The venation, pinnate and finely reticulate, is clearly visible on both surfaces. The adaxial surface is marked with sunken veinlets, giving it a characteristic grainy appearance. Only the young leaf has ciliated margins. Old leaves are glabrous.

B. Reduce to a powder (355). The powder is green to greenish grey or yellowish green. Examine under a microscope using *chloral hydrate solution*. The powder consists of fragments of epidermises which, seen in surface view, show polygonal cells covered by a thick, smooth cuticle and with straight, thick and irregularly pitted walls; *anomocytic stomata*, Appendix XI H, surrounded by five to eleven subsidiary cells and scars of hair bases only on the abaxial epidermis; fragments of palisade parenchyma, with three or four layers of cells of unequal lengths, and spongy parenchyma; groups of lignified fibres from the pericycle, with rows of cells containing prisms of calcium oxalate; occasional conical, unicellular covering trichomes.

C. Examine by *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance.

Solution (1) To 0.5 g of the powdered drug (355) add 5 ml of a mixture of equal volumes of *methanol* and *water* and heat under a reflux condenser for 10 minutes. Filter whilst hot. Wash the flask and the filter with a mixture of equal volumes of *methanol* and *water* and dilute to 5 ml with the same mixture of solvents.

Solution (2) Dissolve 25 mg of *arbutin*, 25 mg of *gallic acid* and 25 mg of *hydroquinone* in *methanol* and dilute to 10 ml with the same solvent.

Apply separately to the plate as bands 10 µl of solution (2) and 20 µl of solution (1). Develop over a path of 15 cm using a mixture of 6 volumes of *anhydrous formic acid*, 6 volumes of *water* and 88 volumes of *ethyl acetate*. Dry the plate at 105° to 110° until the odour of formic acid disappears. Spray with a 1% w/v solution of *2,6-dichloroquinone-4-chloroimide* in *methanol*. Next spray with a 2% w/v solution of *anhydrous sodium carbonate*. The chromatogram obtained with solution (1) shows in its lower third a light blue band similar in position and colour

to one of the bands in the chromatogram obtained with solution (2) (arbutin). In its upper third, it shows two bands similar in position and colour to the other two bands in the chromatogram obtained with solution (2), one is brownish (gallic acid) and the other is blue (hydroquinone). There may also be two or three other blue bands and several brown or brownish grey bands.

Foreign matter Not more than 8% of which not more than 5% of stems and not more than 3% of other foreign matter, Appendix XI D, Test B.

Leaves of different colour Not more than 10%, determined in the same manner as Foreign matter.

Loss on drying Not more than 10.0%, determined on 1 g of the powdered drug (355) by drying in an oven at 100° to 105° for 2 hours, Appendix IX D.

Total ash Not more than 5.0%, Appendix XI J, Method II.

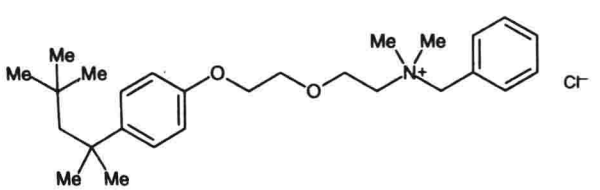
Assay In a 250-ml flask with a ground-glass neck introduce 0.4 g (*m* g) of the powdered drug (250). Add 50 ml of *water* and heat under a reflux condenser for 30 minutes. Allow to cool and dilute to 250 ml with *water*. Allow the particles to settle and introduce 5 ml of the solution into a separating funnel. Add successively, mixing after each addition, 45 ml of *water*, 1 ml of a 2% w/v solution of *4-aminophenazone*, 0.5 ml of 2M *ammonia* and 1 ml of an 8.0% w/v solution of *potassium hexacyanoferrate(III)*. Allow to stand for 5 minutes and shake with 25 ml of *dichloromethane*. Filter the dichloromethane layer through a plug of absorbent cotton soaked in *dichloromethane* into a 100-ml volumetric flask. Shake the aqueous layer again with three quantities, each of 25 ml, of *dichloromethane* and dilute the combined dichloromethane layers to 100 ml with the same solvent. Measure the *absorbance* of the solution at 455 nm using *water* as compensation liquid, Appendix II B. Calculate the percentage content of hydroquinone derivatives, calculated as anhydrous arbutin, from the expression $7.716A/m$ where A is the absorbance at 455 nm and taking the *specific absorbance* of anhydrous arbutin to be 648.

Storage Store in a well-closed container, protected from light.

Action and use Astringent; diuretic.

1/96

Benzethonium Chloride ☆



$C_{27}H_{42}ClNO_2$ 448.1 *121-54-0*

Definition Benzethonium Chloride contains not less than 97.0% and not more than 103.0% of benzyldimethyl-2-{2-[4-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy}ethylammonium chloride, $C_{27}H_{42}ClNO_2$, calculated with reference to the dried substance.

Characteristics A white or yellowish white powder; very soluble in *water* and in *ethanol (96%)*; freely soluble in *dichloromethane*.

An aqueous solution froths copiously when shaken.

Dissolve 5.0 g in carbon dioxide-free water *and dilute to 50 ml with the same solvent (solution S).*

Identification A. *Melting point*, 158° to 164°, after drying at 105° for 4 hours, Appendix V A, Method I.

B. Examine by *thin-layer chromatography*, Appendix III A, using as the coating substance a suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm.

Solution (1) Dissolve 25 mg of the substance being examined in *water* and dilute to 5 ml with the same solvent.

Solution (2) Dissolve 25 mg of *benzethonium chloride EPCRS* in *water* and dilute to 5 ml with the same solvent.

Apply separately to the plate 20 µl of each solution. Develop over a path of 12 cm using a mixture of 5 volumes of *water*, 5 volumes of *glacial acetic acid* and 100 volumes of *methanol*. Dry the plate in a current of warm air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) is similar in position and size to the principal spot in the chromatogram obtained with solution (2).

C. To 5 ml of 2M *sodium hydroxide* add 0.1 ml of *bromophenol blue solution R1* and 5 ml of *dichloromethane* and shake. The lower layer is colourless. Add 0.1 ml of solution S and shake. A blue colour develops in the lower layer.

D. To 2 ml of solution S add 1 ml of 2M *nitric acid*. A white precipitate is formed which dissolves upon addition of 5 ml of *ethanol (96%)*. The solution yields reaction A characteristic of *chlorides*, Appendix VI.

Appearance of solution Solution S is *clear*, Appendix IV A, and not more intensely coloured than *reference solution Y_6*, Appendix IV B, Method II.

Acidity or alkalinity To 25 ml of solution S add 0.1 ml of *phenolphthalein solution*. The solution is colourless. Add 0.3 ml of 0.01M *sodium hydroxide VS*. The solution is pink. Add 0.1 ml of *methyl red solution* and 0.5 ml of 0.01M *hydrochloric acid VS*. The solution is orange-red.

Volatile bases and salts of volatile bases 0.20 g complies with *limit test B for ammonium*, Appendix VII (50 ppm). Prepare the standard using 0.1 ml of *ammonium standard solution (100 ppm NH_4)*. Replace *heavy magnesium oxide* by 2 ml of 10M *sodium hydroxide*.

Loss on drying Not more than 5.0%, determined on 1 g by drying in an oven at 100° to 105° for 4 hours, Appendix IX D.

Sulphated ash Not more than 0.1%, determined on 1 g, Appendix IX A, Method II.

Assay Dissolve 2 g in *water* and dilute to 100 ml with the same solvent. Transfer 25 ml of the solution to a separating funnel, add 10 ml of a 0.4% w/v solution of *sodium hydroxide*, 10 ml of a freshly prepared 5% w/v solution of *potassium iodide* and 25 ml of *dichloromethane*. Shake vigorously, allow to separate and discard the lower layer. Shake the upper layer with three quantities, each of 10 ml, of *dichloromethane* and discard the lower layers. To the upper layer add 40 ml of *hydrochloric acid*, allow to cool and titrate with 0.05M *potassium iodate VS* until the deep brown colour is almost discharged. Add 4 ml of *dichloro-*

methane and continue the titration, shaking vigorously, until the lower layer is no longer brown. Carry out a blank titration using a mixture of 10 ml of a freshly prepared 5% w/v solution of *potassium iodide*, 20 ml of *water* and 40 ml of *hydrochloric acid*. Each ml of 0.05M *potassium iodate VS* is equivalent to 44.81 mg of $C_{27}H_{42}ClNO_2$.

Storage Store in a well-closed container, protected from light.

Action and use Anti-infective.

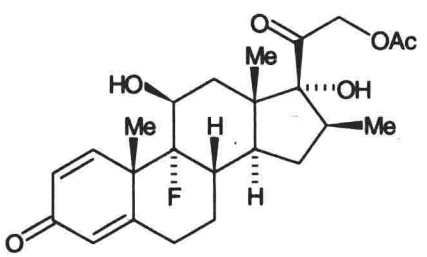
1/96

Benztropine Mesylate

Identification Test A. Change the statement to:

A. Dry the substance at 105° for 3 hours. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of benztropine mesylate.

Betamethasone Acetate ☆



$C_{24}H_{31}FO_6$ 434.5 *987-24-6*

Definition Betamethasone Acetate contains not less than 97.0% and not more than 103.0% of 9α-fluoro-11β,17α-dihydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-21-yl acetate, $C_{24}H_{31}FO_6$, calculated with reference to the anhydrous substance.

Characteristics A white or almost white, crystalline powder; practically insoluble in *water*; freely soluble in *acetone*; soluble in *ethanol (96%)* and in *dichloromethane*.

Identification *Identification tests B and C may be omitted if identification tests A, D, E, F and G are carried out. Identification tests A, D, E, F and G may be omitted if identification tests B and C are carried out.*

A. Dissolve 10 mg in *absolute ethanol* and dilute to 100 ml with the same solvent. Place 2 ml of this solution in a ground-glass-stoppered tube, add 10 ml of *phenylhydrazine—zinc—sulphuric acid solution*, mix and heat in a water bath at 60° for 20 minutes. Cool immediately. The *absorbance* of the solution measured at 419 nm, Appendix II B, is not more than 0.10.

B. Examine by *infrared absorption spectrophotometry*, Appendix II A. The absorption maxima in the spectrum obtained with the substance being examined correspond in position and relative intensity to those in the spectrum obtained with *betamethasone acetate EPCRS*. If the spectra obtained in the solid state show differences, dissolve separately the substance being examined and the reference substance in the minimum volume of *methanol*, evaporate to dryness on a water bath and record new spectra using the residues.

C. Examine by *thin-layer chromatography*, Appendix III A, using as the coating substance a suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm.

Solution (1) Dissolve 10 mg of the substance being examined in a mixture of 1 volume of *methanol* and 9 volumes of *dichloromethane* and dilute to 10 ml with the same mixture of solvents.

Solution (2) Dissolve 20 mg of *betamethasone acetate EPCRS* in a mixture of 1 volume of *methanol* and 9 volumes of *dichloromethane* and dilute to 20 ml with the same mixture of solvents.

Solution (3) Dissolve 10 mg of *prednisolone acetate EPCRS* in solution (2) and dilute to 10 ml with solution (2).

Apply separately to the plate 5 µl of each solution. Prepare the mobile phase by adding a mixture of 1.2 volumes of *water* and 8 volumes of *methanol* to a mixture of 15 volumes of *ether* and 77 volumes of *dichloromethane*. Develop over a path of 15 cm. Allow the plate to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) is similar in position and size to the principal spot in the chromatogram obtained with solution (2). Spray with *ethanolic sulphuric acid (20%)*. Heat at 120° for 10 minutes or until the spots appear. Allow to cool. Examine the plate in daylight and under *ultraviolet light (365 nm)*. The principal spot in the chromatogram obtained with solution (1) is similar in position, colour in daylight, fluorescence in ultraviolet light (365 nm) and size to the principal spot in the chromatogram obtained with solution (2). The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

D. Examine by *thin-layer chromatography*, Appendix III A, using as the coating substance a suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm.

Solution (1) Dissolve 25 mg of the substance being examined in *methanol* with gentle heating and dilute to 5 ml with the same solvent (solution A). Dilute 2 ml of the solution to 10 ml with *dichloromethane*.

Solution (2) Transfer 2 ml of solution A to a 15-ml glass tube with a ground-glass stopper or polytetrafluoroethylene cap. Add 10 ml of *saturated methanolic potassium hydrogen carbonate solution* and immediately pass a stream of *nitrogen* briskly through the solution for 5 minutes. Stopper the tube. Heat in a water bath at 45°, protected from light, for 30 minutes. Allow to cool.

Solution (3) Dissolve 25 mg of *betamethasone acetate EPCRS* in *methanol* with gentle heating and dilute to 5 ml with the same solvent (solution B) Dilute 2 ml of the solution to 10 ml with *dichloromethane*.

Solution (4) Transfer 2 ml of solution B to a 15-ml glass tube with a ground-glass stopper or a polytetrafluoroethylene cap. Add 10 ml of *saturated methanolic potassium hydrogen carbonate solution* and immediately pass a stream of *nitrogen* briskly through the solution for 5 minutes. Stopper the tube. Heat in a water bath at 45°, protected from light, for 30 minutes. Allow to cool.

Apply separately to the plate 5 µl of each solution. Prepare the mobile phase by adding a mixture of 1.2 volumes of *water* and 8 volumes of *methanol* to a mixture of 15 volumes of *ether* and 77 volumes of *dichloromethane*. Develop over a path of 15 cm. Allow the plate to dry in air

and examine under *ultraviolet light (254 nm)*. The principal spot in each of the chromatograms obtained with solutions (1) and (2) is similar in position and size to the principal spot in the chromatogram obtained with solutions (3) and (4). Spray with *ethanolic sulphuric acid (20%)*. Heat at 120° for 10 minutes or until the spots appear. Allow to cool. Examine in daylight and under *ultraviolet light (365 nm)*. The principal spot in each of the chromatograms obtained with solutions (1) and (2) is similar in position, colour in daylight, fluorescence in ultraviolet light (365 nm) and size to the principal spot in the chromatograms obtained with solutions (3) and (4). The principal spot in each of the chromatograms obtained with solutions (2) and (4) has an Rf value distinctly lower than that of the principal spots in each of the chromatograms obtained with solutions (1) and (3).

E. Add about 2 mg to 2 ml of *sulphuric acid (96% w/w)* and shake to dissolve. Within 5 minutes, a deep reddish brown colour develops. Add the solution to 10 ml of *water* and mix. The colour is discharged and a clear solution remains.

F. Mix about 5 mg with 45 mg of *heavy magnesium oxide* and ignite in a crucible until an almost white residue is obtained (usually less than 5 minutes). Allow to cool, add 1 ml of *water*, 0.05 ml of *phenolphthalein solution R1* and about 1 ml of 2M *hydrochloric acid* to render the solution colourless. Filter. To a freshly prepared mixture of 0.1 ml of *alizarin S solution* and 0.1 ml of *zirconyl nitrate solution* add 1 ml of the filtrate. Mix, allow to stand for 5 minutes and compare the colour of the solution with that of a blank prepared in the same manner. The test solution is yellow and the blank is red.

G. About 10 mg yields the reaction characteristic of *acetyl groups*, Appendix VI.

Specific optical rotation Dissolve 0.25 g in *1,4-dioxan* and dilute to 25 ml with the same solvent. The *specific optical rotation* is +120° to +128°, Appendix V F, calculated with reference to the anhydrous substance.

Related substances Examine by *liquid chromatography*, Appendix III D.

Solution (1) Dissolve 62.5 mg of the substance being examined in the mobile phase and dilute to 25 ml with the mobile phase.

Solution (2) Dissolve 2 mg of *betamethasone acetate EPCRS* and 2 mg of *dexamethasone acetate EPCRS* in the mobile phase and dilute to 50 ml with the mobile phase.

Solution (3) Dilute 1 ml of solution (1) to 100 ml with the mobile phase.

The chromatographic procedure may be carried out using:

(a) a stainless steel column (0.25 m × 4.0 mm) packed with *silica gel for chromatography* (5 µm) (Hypersil 5µ is suitable),

(b) as mobile phase at a flow rate of 1 ml per minute a mixture prepared as follows: mix 1.54 ml of *water* with 22 ml of *methanol*; add 700 ml of *chloroform stabilised with amylene*, mix carefully and allow to equilibrate; dilute to 1000 ml with *chloroform stabilised with amylene*,

(c) as detector a spectrophotometer set at 254 nm.

Equilibrate the column with the mobile phase at a flow rate of 1 ml per minute for about 45 minutes.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with 20 µl of solution (3) is not less than 50% of the full scale of the recorder.

Inject 20 µl of solution (2). When the chromatograms are recorded in the prescribed conditions, the retention times are dexamethasone acetate about 10 minutes and betamethasone acetate about 13 minutes. The test is not valid unless the *resolution factor* between the peaks corresponding to dexamethasone acetate and betamethasone acetate is at least 5.0; if necessary, adjust slightly the concentrations of methanol and water in the mobile phase, maintaining the ratio of methanol to water and maintaining the homogeneity of the mobile phase.

Inject separately 20 µl of solution (1) and 20 µl of solution (3). Continue the chromatography until the betamethasone acetate peak is completely recorded (about 15 minutes), then modify the flow rate from 1 ml per minute to 2 ml per minute and continue the chromatography for a total of five times the retention time of the principal peak (about 65 minutes). After this time reduce the flow rate to 1 ml per minute for 5 minutes for the successive injection. In the chromatogram obtained with solution (1) the area of any *secondary peak* is not greater than half the area of the principal peak in the chromatogram obtained with solution (3) (0.5%) and the sum of the areas of any *secondary peaks* is not greater than 1.25 times the area of the principal peak in the chromatogram obtained with solution (3) (1.25%). Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (3) (0.05%).

Water Not more than 4.0%, determined on 0.1 g, Appendix IX C, Method I.

Assay Dissolve 50 mg in *ethanol (96%)* and dilute to 100 ml with the same solvent. Dilute 2 ml of the solution to 50 ml with *ethanol (96%)*. Measure the *absorbance*, Appendix II B, at the maximum at 240 nm. Calculate the content of $C_{24}H_{31}FO_6$ taking the *specific absorbance* to be 350.

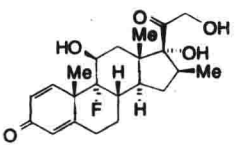
Storage Store in a well-closed container, protected from light.

Action and use Corticosteroid.

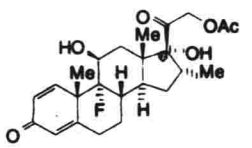
1/96

The impurities limited by the requirements of this monograph include:

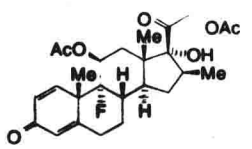
betamethasone



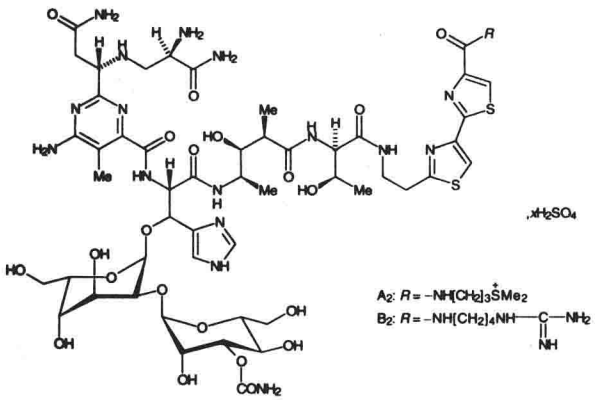
dexamethasone 21-acetate



betamethasone 11,21-diacetate



Bleomycin Sulphate ☆



9041-93-4

Definition Bleomycin Sulphate is the sulphate of a mixture of glycopeptides produced by *Streptomyces verticillus* or by any other means; the two principal components of the mixture are N^1-[3-(dimethylsulphonio)-propyl]bleomycinamide (bleomycin A_2) and N^1-4-(guanidobutyl)bleomycinamide (bleomycin B_2). The potency is not less than 1500 Units per mg, calculated with reference to the dried substance.

Characteristics A white or yellowish white powder; very hygroscopic; very soluble in *water*; slightly soluble in *absolute ethanol*; practically insoluble in *acetone* and in *ether*.

Identification A. Examine the chromatograms obtained in the test for Composition. The retention times and sizes of the two principal peaks in the chromatogram obtained with solution (1) are approximately the same as those of the two principal peaks in the chromatogram obtained with solution (2).

B. Yields the reactions characteristic of *sulphates*, Appendix VI.

Appearance of solution Dissolve 0.20 g in *water* and dilute to 10 ml with the same solvent. The solution is *clear*, Appendix IV A. The *absorbance* measured at 430 nm is not greater than 0.10, Appendix II B.

pH Dissolve 50 mg in *carbon dioxide-free water* and dilute to 10 ml with the same solvent. The pH of the solution is 4.5 to 6.0, Appendix V L.

Composition Examine by *liquid chromatography*, Appendix III D.

Solution (1) Dissolve 25.0 mg of the substance being examined in *water* and dilute to 50 ml with the same solvent.

Solution (2) Dissolve 25.0 mg of *bleomycin sulphate EPCRS* in *water* and dilute to 50 ml with the same solvent.

Solution (3) Dilute 1.5 ml of solution (2) to 100 ml with *water*.

The chromatographic procedure may be carried out using:

(a) a stainless steel column (0.25 m × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (7 µm) (Nucleosil 7 C18 is suitable),

(b) gradient elution at a flow rate of 1.2 ml per minute with a mobile phase initially composed of 10% v/v of *methanol* and 90% v/v of a mixture prepared as follows: dissolve 0.960 g of *sodium pentanesulphonate* in 900 ml of acetic acid (0.48% w/v $C_2H_4O_2$), add 1.86 g of *disodium edetate*, dilute to 1000 ml with the same solvent and adjust to pH 4.3 using 10M *ammonia*; increasing the proportion of *methanol* to 40% v/v over 60 minutes and continuing with the final mixture for about 20 minutes, until demethylbleomycin A_2 is eluted (retention time 1.5 to 2.5, relative to bleomycin A_2),

(c) as detector a spectrophotometer set at 254 nm,

(d) a 20-µl loop injector.

Inject solution (2). The test is not valid unless the *resolution factor* between the two principal peaks is at least 5. Inject solution (3). The test is not valid unless the *signal-to-noise* ratio calculated for the principal peak is at least 20. Inject solution (2) six times. The test is not valid unless the relative standard deviation of the area of the principal peak is at most 2%.

Inject solution (1). The composition, calculated by *normalisation* and disregarding any peak with an area less than 0.1% of the total, is: bleomycin A_2 (first principal peak) 55% to 70%; bleomycin B_2 (second principal peak) 25% to 32%; sum of bleomycin A_2 and bleomycin B_2 not less than 85%; demethylbleomycin A_2 (retention time relative to bleomycinA_2, 1.5 to 2.5) not more than 5.5%; other related substances not greater than 9.5%.

Copper Not more than 200 ppm of Cu, determined by *atomic absorption spectrophotometry*, Appendix II D.

Test solution Dissolve 50 mg in *water* and dilute to 10 ml with the same solvent.

Standard solution Dilute 1 ml of *copper standard solution (10 ppm Cu)* to 10 ml with *water*.

Measure the absorbance at 324.7 nm using a copper hollow-cathode lamp as source of radiation and an air—acetylene flame.

Loss on drying Not more than 3.0%, determined on 50 mg by drying at 60° at a pressure not exceeding 670 Pa for 3 hours, Appendix IX D.

Assay Carry out Method A for the *biological assay of antibiotics*, Appendix XIV A. 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.

Storage Store in an airtight container, at a temperature of 2° to 8°. If the substance is sterile, store in a sterile, airtight, tamper-evident container.

Labelling The label states (1) where applicable, that the substance is sterile; (2) where applicable, that the substance is free from bacterial endotoxins.

Action and use Antineoplastic.

The impurities limited by the requirements of this monograph include:

bleomycinic acid	R = -OH
bleomycin A_5	R = -NH[CH$_2$]$_3$NH[CH$_2$]$_4$NH$_2$
bleomycin B_4	R = -NH[CH$_2$]$_4$NHC(=NH)NH[CH$_2$]$_4$NHC(=NH)NH$_2$
demethylbleomycin A_2	R = -NH[CH$_2$]$_4$SMe

Bleomycin Sulphate intended for use in the manufacture of parenteral dosage forms without a further appropriate sterilisation procedure complies with the following additional requirement.

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

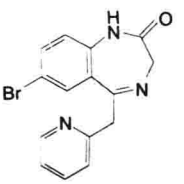
Bleomycin Sulphate intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins Not more than 5 Units of endotoxin per mg, Appendix XIV C.

1/96

Bromazepam ☆

Impurities displa/ box. Replace the third structure by:

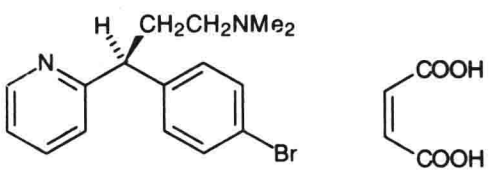


7/95

Brompheniramine Maleate

Change the monograph to:

Brompheniramine Maleate ☆



and enantiomer

$C_{16}H_{19}BrN_2,C_4H_4O_4$ 435.3 *980-71-2*

Definition Brompheniramine Maleate contains not less than 98.0% and not more than 101.0% of (*RS*)-3-(4-bromophenyl)-3-(2-pyridyl)propyldimethylamine (*Z*)-butenedioate, $C_{16}H_{19}BrN_2,C_4H_4O_4$, calculated with reference to the dried substance.

Characteristics A white, crystalline powder; soluble in *water*; freely soluble in *ethanol (96%)*, in *methanol* and in *dichloromethane*.

Identification *Identification test F may be omitted if identification tests A, B, C, D and E are carried out. Identification tests C and D may be omitted if identification tests A, B, E and F are carried out.*

A. *Melting point*, 130° to 135° Appendix V A, Method I.

B. Dissolve 65 mg in 0.1M *hydrochloric acid* and dilute to 100 ml with the same acid. Dilute 5 ml of this solution to 100 ml with 0.1M *hydrochloric acid*. Examined between 220 nm and 320 nm, Appendix II B, the solution shows an absorption maximum at 265 nm. The *specific absorbance* at the maximum is 190 to 210.

C. Examine by *infrared absorption spectrophotometry*, Appendix II A. The absorption maxima in the spectrum obtained with the substance being examined correspond in position and relative intensity to those in the spectrum obtained with *brompheniramine maleate EPCRS*. Examine the substances prepared as discs using *potassium bromide*.

D. Examine the chromatograms obtained in the test for Related substances. The retention time and size of the principal peak in the chromatogram obtained with solution (1) are approximately the same as those of the principal peak in the chromatogram obtained with solution (2). The chromatogram obtained with solution (4) shows two principal peaks with retention times corresponding to the retention time of the peak obtained with solution (2) and solution (3).

E. To 0.2 g add 3 ml of *water* and 1 ml of 10M *sodium hydroxide*. Shake with three quantities, each of 5 ml, of *ether*. To 0.1 ml of the aqueous layer add a solution of 10 mg of *resorcinol* in 3 ml of *sulphuric acid (96% w/w)*. Heat on a water bath for 15 minutes. No colour develops. To the remainder of the aqueous layer add 2 ml of *bromine solution*. Heat in a water bath for 15 minutes, heat to boiling and cool. To 0.2 ml of the solution add a solution of 10 mg of *resorcinol* in 3 ml of *sulphuric acid (96% w/w)* and heat on a water bath for 15 minutes. A blue colour develops.

F. To 0.15 g in a porcelain crucible add 0.5 g of *anhydrous sodium carbonate*. Heat over an open flame for 10 minutes. Allow to cool. Take up the residue in 10 ml of 2M *nitric acid* and filter. To 1 ml of the filtrate add 1 ml of *water*. The solution yields reaction A characteristic of *bromides*, Appendix VI.

Appearance of solution Dissolve 2.0 g in *methanol* and dilute to 20 ml with the same solvent. The solution is *clear*, Appendix IV A, and not more intensely coloured than *reference solution* BY_6, Appendix IV B, Method II.

pH Dissolve 0.20 g in 20 ml of *carbon dioxide-free water*. The pH of the solution is 4.0 to 5.0, Appendix V L.

Optical rotation Dissolve 2.5 g in *water* and dilute to 25 ml with the same solvent. The angle of *optical rotation* measured in a 2-dm tube is –0.2° to +0.2°, Appendix V F.

Related substances Examine by *gas chromatography*, Appendix III B.

Solution (1) Dissolve 0.1 g of the substance being examined in 10 ml of *dichloromethane*.

Solution (2) Dissolve 10 mg of *brompheniramine maleate EPCRS* in *dichloromethane* and dilute to 1 ml with the same solvent.

Solution (3) Dissolve 5 mg of *chlorphenamine maleate EPCRS* in *dichloromethane* and dilute to 1 ml with the same solvent.

Solution (4) To 0.5 ml of solution (1) add 0.5 ml of solution (3).

The chromatographic procedure may be carried out using:

(a) a glass column (2.3 m × 2 mm) packed with *acid- and base-washed, silanised diatomaceous support* (135 µm to 175 µm) (Chromosorb W AW-DMCS is suitable) impregnated with 3% w/w of *polymethylphenylsiloxane* (OV 17 is suitable),

(b) *nitrogen for chromatography* as the carrier gas at a flow rate of 20 ml per minute,
(c) a flame-ionisation detector,
maintaining the temperature of the column at 205° and that of the injection port and of the detector at 250°.

Inject 1 µl of each solution. The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to brompheniramine and chlorpheniramine is at least 1.5. After injecting solution (1), continue the chromatography for at least 2.5 times the retention time of the principal peak. In the chromatogram obtained with solution (1) the sum of the areas of any *secondary peaks* is not greater than 1% of the area of the principal peak and no *secondary peak* has an area greater than 0.4% of the area of the principal peak. Disregard any peak with an area less than 0.1% of that of the peak corresponding to brompheniramine in the chromatogram obtained with solution (1).

Heavy metals 1.0 g complies with *limit test C for heavy metals*, Appendix VII (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb)*.

Loss on drying Not more than 0.5%, determined on 1 g by drying in an oven at 100° to 105° for 3 hours, Appendix IX D.

Sulphated ash Not more than 0.1%, determined on 1 g, Appendix IX A, Method II.

Assay Dissolve 0.26 g in 50 ml of *anhydrous acetic acid*. Titrate with 0.1M *perchloric acid VS*, determining the end point potentiometrically, Appendix VIII B. Each ml of 0.1M *perchloric acid VS* is equivalent to 21.77 mg of $C_{16}H_{19}BrN_2,C_4H_4O_4$.

Storage Store in a well-closed container, protected from light.

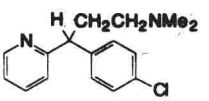
Preparation
Brompheniramine Tablets

Action and use Histamine H_1-receptor antagonist.

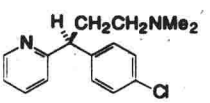
1/96

The impurities limited by the requirements of this monograph include:

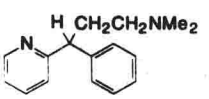
chlorpheniramine
(chlorphenamine)



dexchlorpheniramine



pheniramine



Bumetanide

Definition For 'not less than 99.0% and not more than 100.5%' read 'not less than 99.0% and not more than 101.5%'.

7/94

Add the following statement.

Preparations
Bumetanide Injection
Bumetanide Oral Solution
Bumetanide Tablets

7/95

Preparations Add the following.
Bumetanide and Slow Potassium Tablets

Butylated Hydroxyanisole ☆

Change the molecular formula to $C_{11}H_{16}O_2$.
Line 2. For '$C_{11}H_{16}O$' read '$C_{11}H_{16}O_2$'.

Identification Delete the italicised statement and test A. Relabel tests B, C and D as tests A, B and C, respectively.

Impurities display box. Change to read 'The impurities limited by the requirements of this monograph include hydroquinone.'.

7/95

Calcitriol ☆

Impurities display box. Third structure. Add missing '-OH' at C-25.

7/95

Absorbance Change the statement to:

Absorbance Dilute one volume of solution (1) obtained in the Assay with an equal volume of the mobile phase. The *absorbance* of the resulting solution, Appendix II B, measured at the maximum at 265 nm is 0.38 to 0.42.

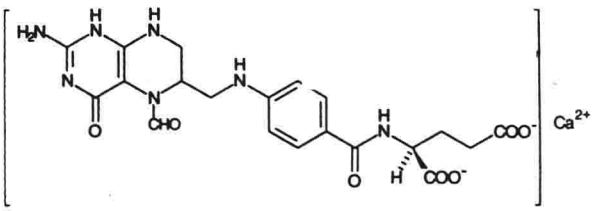
10/95

Calcium Chloride ☆

Add the following statement.

Preparation
Calcium Chloride Intravenous Infusion

Calcium Folinate ☆



$C_{20}H_{21}CaN_7O_7,xH_2O$ 511.5 *(anhydrous)* *1492-18-8*

Definition Calcium Folinate contains not less than 97.0% and not more than 102.0% of calcium 5-formyltetrahydropteroylglutamate, $C_{20}H_{21}CaN_7O_7$, and not less than 7.54% and not more than 8.14% of Ca, both calculated with reference to the anhydrous and solvent-free substance.

Characteristics A white or light yellow, amorphous or crystalline powder; sparingly soluble in *water*; practically insoluble in *acetone* and in *ethanol (96%)*. The amorphous form may produce supersaturated solutions in *water*.

Dissolve 1.25 g in carbon dioxide-free water *heating at 40° if necessary and dilute to 50 ml with the same solvent (solution S).*

Identification *Identification test B may be omitted if identification tests A, C and D are carried out. Identification tests A and C may be omitted if identification tests B and D are carried out.*

A. Complies with the test for Specific optical rotation.

B. Examine by *infrared absorption spectrophotometry*, Appendix II A. The absorption maxima in the spectrum obtained with the substance being examined correspond in position and relative intensity to those in the spectrum obtained with *calcium folinate EPCRS*. Examine the substances prepared as discs. If the spectra obtained show differences, dissolve the substance being examined and the reference substance separately in the minimum quantity of *water* and add dropwise sufficient *acetone* to produce a precipitate. Allow to stand for 15 minutes, collect the precipitate by centrifugation, wash the precipitate with two small quantities of *acetone* and dry. Record new spectra using the residues.

C. Examine by *thin-layer chromatography*, Appendix III A, using *cellulose F_{254}* as the coating substance.

Solution (1) Dissolve 15 mg of the substance being examined in a 3% v/v solution of 10M *ammonia* and dilute to 5 ml with the same solvent.

Solution (2) Dissolve 15 mg of *calcium folinate EPCRS* in a 3% v/v solution of 10M *ammonia* and dilute to 5 ml with the same solvent.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using the lower layer of a mixture of 1 volume of *3-methylbutan-2-ol* and 10 volumes of a 5% w/v solution of *citric acid* previously adjusted to pH 8 with 10M *ammonia*. Allow the plate to dry in air and examine under *ultraviolet light (254 nm)*. The principal spot in the chromatogram obtained with solution (1) is similar in position and size to the principal spot in the chromatogram obtained with solution (2).

D. Yields reaction B characteristic of *calcium salts*, Appendix VI.

Carry out the following tests and the assay as rapidly as possible, protected from bright light.

Appearance of solution Solution S is *clear*, Appendix IV A. The *absorbance* of solution S, Appendix II B, measured at 420 nm using *water* as the compensation liquid, is not greater than 0.60.

pH The pH of solution S is 6.8 to 8.0, Apppendix V L.

Specific optical rotation The *specific optical rotation* is +14.4° to +18.0°, Appendix V F, determined on solution S and calculated with reference to the anhydrous and solvent-free substance.

Acetone, ethanol and methanol Not more than 0.5% w/v of acetone, not more than 3.0% w/v of ethanol and not more than 0.5% w/v of methanol. Examine by *headspace gas chromatography* using the standard addition method, Appendix III B.

Solution (1) Dissolve 0.25 g of the substance being examined in *water* and dilute to 10 ml with the same solvent.

Solution (2) Dilute 0.125 g of *acetone*, 0.75 g of *absolute ethanol* and 0.125 g of *methanol* in *water* and dilute to 1000 ml with the same solvent.

The chromatographic procedure may be carried out using:

(a) a fused-silica column (10 m × 0.32 mm) coated with *styrene—divinylbenzene co-polymer*,

(b) *nitrogen for chromatography* as the carrier gas at a flow rate of 4 ml per minute,

(c) a flame-ionisation detector,

raising the temperature of the column from 80° to 220° at a rate of 10° per minute and maintaining the temperature of the injection port at 110° and that of the detector at 270°. Place the samples in a thermostatically controlled chamber at 80° for 20 minutes and pressurise them for 30 seconds. Repeat the injections at least three times.

Related substances Examine the chromatograms obtained in the Assay for calcium folinate. In the chromatogram obtained with solution (1) the area of any peak corresponding to formylfolic acid is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (1%), the area of any other *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1%) and the sum of the areas of any *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (3) (2.5%). Disregard any peak with an area less than that of the principal peak in the chromatogram obtained with solution (5) (0.1%).

Chlorides Dissolve 72 mg in 10 ml of *water* and add 3 ml of 5M *acetic acid*. Filter and wash the precipitate with five quantities, each of 5 ml, of *water*. Collect the filtrate and washings and dilute to 100 ml with *water*. 15 ml of this solution complies with the *limit test for chlorides*, Appendix VII (0.5%).

Heavy metals To 0.80 g in a suitable crucible, add a sufficient quantity of *sulphuric acid (96% w/w)* to wet the substance and carefully ignite at a low temperature until thoroughly charred. To the residue add 2 ml of *nitric acid* and 0.25 ml of *sulphuric acid (96% w/w)* and heat cautiously until white fumes are no longer evolved. Ignite at 500° to 600° until the carbon is completely burnt off.

Allow to cool and add 4 ml of 7M *hydrochloric acid*. Cover the crucible and heat on a water bath for 15 minutes, uncover and evaporate slowly to dryness on a water bath. To the residue add 0.05 ml of *hydrochloric acid* and 10 ml of hot *water*. Allow to stand for 2 minutes and add dropwise sufficient 6M *ammonia* to make the solution just alkaline to *red litmus paper*. Dilute to 25 ml with *water* and adjust to pH 3.0 to 4.0 with 2M *acetic acid*. Filter if necessary, rinse the crucible and the filter with 10 ml of *water*, combine the filtrate and washings and dilute to 40 ml with *water*.

To 12 ml of the above solution add 2 ml of *acetate buffer pH 3.5*. Mix and add 1.2 ml of *thioacetamide reagent*. Mix immediately. The solution is not more intensely coloured than a standard prepared as follows: dilute 4 ml of *lead standard solution (10 ppm Pb)* to 25 ml with *water*, adjust to pH 3.0 to 4.0 using 2M *acetic acid* and dilute to 40 ml with *water*; to 12 ml of this solution add 2 ml of *acetate buffer pH 3.5*, mix and add 1.2 ml of *thioacetamide reagent* (50 ppm).

Platinum Not more than 20 ppm, determined by *X-ray fluorescence spectrophotometry*, Appendix II F.

Water Not more than 17.0%, Appendix IX C, Method I, determined on 0.2 g of the substance being examined, ground to a very fine powder. Stir the substance being examined in the titration solvent for about 6 minutes before titrating and use a suitable titrant that does not contain pyridine.

Assay ***For calcium*** Dissolve 0.4 g in 150 ml of *water* and dilute to 300 ml with the same solvent. Carry out the *complexometric titration of calcium*, Appendix VIII D. Each ml of 0.1M *disodium edetate VS* is equivalent to 4.008 mg of Ca.

For calcium folinate Examine by *liquid chromatography*, Appendix III D.

Solution (1) Dissolve 10 mg of the substance being examined in *water* and dilute to 10 ml with the same solvent.

Solution (2) Dissolve 10 mg of *calcium folinate EPCRS* in *water* and dilute to 10 ml with the same solvent.

Solution (3) Dilute 1 ml of solution (2) to 100 ml with *water*.

Solution (4) Dissolve 10 mg of *formylfolic acid EPCRS* in *water* and dilute to 100 ml with the same solvent. Dilute 1 ml of this solution to 10 ml with *water*.

Solution (5) Dilute 1 ml of solution (3) to 10 ml with *water*.

Solution (6) Dilute 5 ml of solution (4) to 10 ml with solution (3).

The chromatographic procedure may be carried out using:

(a) a stainless steel column (0.25 m × 4 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm),
(b) as mobile phase at a flow rate of 1 ml per minute a mixture prepared as follows: mix 220 ml of *methanol* and 780 ml of a solution containing 2 ml of *tetrabutylammonium hydroxide solution* and 2.2 g of *disodium hydrogen orthophosphate* and previously adjusted to pH 7.5 with *orthophosphoric acid*; if necessary, adjust the concentration of methanol to achieve the prescribed resolution,
(c) as detector a spectrophotometer set at 280 nm,
(d) a loop injector,

maintaining the temperature of the column at 40° to 45°.

Inject separately 10 µl of each solution. Record the chromatograms for 2.5 times the retention time of the principal peak in the chromatogram obtained with solution (1). The test is not valid unless, in the chromatogram obtained with solution (6), the *resolution factor* between the peaks corresponding to calcium folinate and formylfolic acid is at least 2.2 and the relative standard deviation of the area of the principal peak for six replicate injections of solution (2) is at most 2.0%.

Calculate the percentage content of $C_{20}H_{21}CaN_7O_7$ from the peak areas and the declared content of *calcium folinate EPCRS*.

Storage Store in an airtight container, protected from light. If the substance is sterile, store in a sterile, airtight, tamper-evident container.

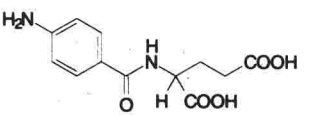
Labelling The label on the container states (1) where applicable, that the substance is sterile; (2) where applicable, that the substance is free from bacterial endotoxins.

Action and use Antidote to folic acid antagonists.

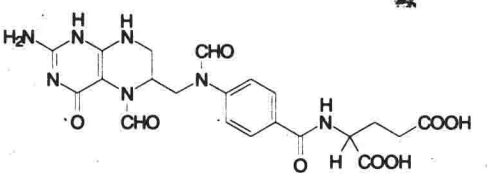
Calcium Folinate intended for use in the manufacture of parenteral dosage forms without a further appropriate

The impurities limited by the requirements of this monograph include:

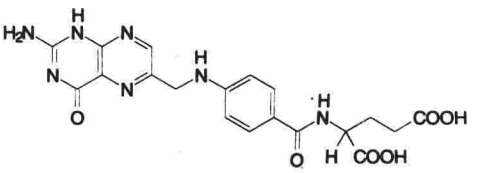
4-aminobenzoylglutamic acid



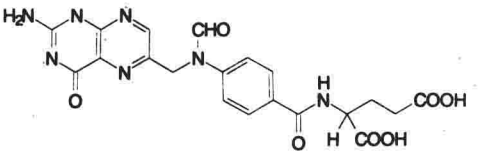
5,10-diformyltetrahydrofolic acid



folic acid



10-formylfolic acid



5-formyltetrahydropteroic acid