is not more intense that that in a standard prepared at the same time in the same manner, using 5 ml of *phosphate standard solution (5 ppm PO_4) R* (500 ppm).

Heavy metals (*2.4.8*). Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with limit test A for heavy metals (10 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Water (*2.5.12*). Not more than 0.5 per cent, determined on 0.500 g by the semi-micro determination of water.

ASSAY

Examine the substance by liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution. Dissolve 0.60 g of the substance to be examined in the mobile phase and dilute to 5.0 ml with the mobile phase.

Reference solution (a). Dissolve 0.60 g of *fosfomycin trometamol CRS* in the mobile phase and dilute to 5.0 ml with the mobile phase.

Reference solution (b). Dissolve 8.7 mg of *fosfomycin trometamol impurity A CRS* (disodium salt) in the mobile phase and dilute to 20.0 ml with the mobile phase.

Reference solution (c). Dissolve 5 mg of *fosfomycin trometamol impurity A CRS* (disodium salt) and 10 mg of *fosfomycin trometamol CRS* in the mobile phase and dilute to 5 ml with the mobile phase.

Blank solution. A 0.3 g/l solution of *anhydrous disodium hydrogen phosphate R* in the mobile phase.

The chromatographic procedure may be carried out using:

- a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with *aminopropylsilyl silica gel for chromatography R* (5 μm),
- as mobile phase at a flow rate of 1 ml/min a 10.89 g/l solution of *potassium dihydrogen phosphate R,*
- as detector a differential refractometer maintaining the temperature at 35 °C.

When the chromatograms are recorded in the prescribed conditions, the relative retention times (to fosfomycin) are about 0.3 for the two peaks corresponding to trometamol and 0.8 for impurity A.

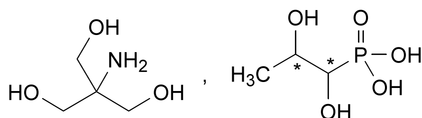
Inject 5 μl of reference solution (c). The test is not valid unless the resolution between the peaks corresponding to fosfomycin and impurity A is at least 1.5. Inject reference solution (a) six times. The test is not valid unless the relative standard deviation of the peak area for fosfomycin is at most 1.0 per cent. Inject alternately the test solution and reference solution (a).

Calculate the percentage content of fosfomycin trometamol.

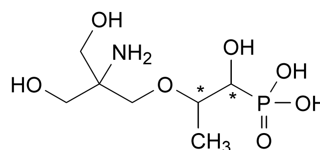
STORAGE

Store in an airtight container.

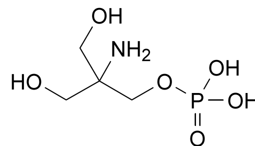
IMPURITIES



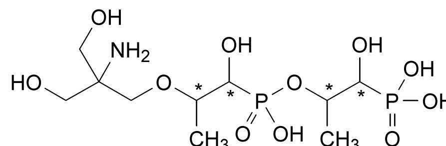
A. 1,3-dihydroxy-2-(hydroxymethyl)propan-2-aminium (1,2-dihydroxypropyl)phosphonate,



B. [2-[2-amino-3-hydroxy-2-(hydroxymethyl)propoxy]-1-hydroxypropyl]phosphonic acid,



C. 2-amino-3-hydroxy-2-(hydroxymethyl)propyl dihydrogenphosphate (trometamol phosphoric ester),



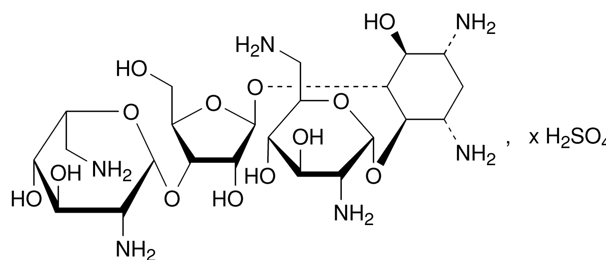
D. [2-[[[2-[2-amino-3-hydroxy-2-(hydroxymethyl)propoxy]-1-hydroxypropyl]hydroxyphosphoryl]oxy]-1-hydroxypropyl]phosphonic acid (trometamoyloxy fosfomycin dimer).

Monographs F-H

01/2005:0180
corrected

FRAMYCETIN SULPHATE

Framycetini sulfas



$C_{23}H_{46}N_6O_{13},xH_2SO_4$ M_r 615 (base)

DEFINITION

Sulphate of 2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-D-streptamine (neomycin B), a substance produced by the growth of selected strains of *Streptomyces fradiae* or *Streptomyces decaris* or obtained by any other means.

Content: minimum of 630 IU/mg (dried substance).

CHARACTERS

Appearance: white or yellowish-white powder, hygroscopic.

Solubility: freely soluble in water, very slightly soluble in alcohol, practically insoluble in acetone.

IDENTIFICATION

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

Results:

- the retention time of the principal peak in the chromatogram obtained with the test solution is approximately the same as that of the principal peak in the chromatogram obtained with reference solution (a),
- it complies with the limit given for impurity C.

B. It gives reaction (a) of sulphates (*2.3.1*).

TESTS

pH (*2.2.3*): 6.0 to 7.0.

Dissolve 0.1 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Specific optical rotation (*2.2.7*): + 52.5 to + 55.5 (dried substance).

Dissolve 1.00 g in *water R* and dilute to 10.0 ml with the same solvent

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a). Dissolve the contents of a vial of *framycetin sulphate CRS* in the mobile phase and dilute with the mobile phase to obtain a solution containing 0.5 mg/ml.

Reference solution (b). Dilute 3.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Reference solution (c). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Reference solution (d). Dissolve the contents of a vial of *neamine CRS* (corresponding to 0.5 mg) in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (e). Dissolve 10 mg of *neomycin sulphate CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm,
- *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (5 μm),
- *temperature*: 25 °C.

Mobile phase: mix 20.0 ml of *trifluoroacetic acid R*, 6.0 ml of *carbonate-free sodium hydroxide solution R* and 500 ml of *water R*, allow to equilibrate, dilute to 1000 ml with *water R* and degas.

Flow rate: 0.7 ml/min.

Post-column solution: *carbonate-free sodium hydroxide solution R* diluted 1 in 25 previously degassed, which is added pulse-less to the column effluent using a 375 μl polymeric mixing coil.

Flow rate: 0.5 ml/min.

Detection: pulsed amperometric detector with a gold working electrode, a silver-silver chloride reference electrode and a stainless steel auxiliary electrode which is the cell body, held at respectively 0.00 V detection, + 0.80 V oxidation and − 0.60 V reduction potentials, with pulse durations according to the instrument used.

Injection: 10 μl.

Run time: 1.5 times the retention time of neomycin B.

Relative retention with reference to neomycin B (retention time = about 10 min): impurity A = about 0.65; impurity C = about 0.9; impurity G = about 1.1.

System suitability:

- *resolution*: minimum 2.0 between the peaks due to impurity C and to neomycin B in the chromatogram obtained with reference solution (e); if necessary, adjust the volume of the carbonate-free sodium hydroxide solution in the mobile phase,
- *signal-to-noise ratio*: minimum 10 for the principal peak in the chromatogram obtained with reference solution (c).

Limits:

- *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) and taking into account the declared content of *neamine CRS* (1.0 per cent),
- *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent),
- *total of other impurities*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent),
- *disregard limit*: area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent).

Sulphate: 27.0 per cent to 31.0 per cent (dried substance).

Dissolve 0.250 g in 100 ml of *water R* and adjust the solution to pH 11 using *concentrated ammonia R*. Add 10.0 ml of *0.1 M barium chloride* and about 0.5 mg of *phthalein purple R*. Titrate with *0.1 M sodium edetate* adding 50 ml of *alcohol R* when the colour of the solution begins to change and continuing the titration until the violet-blue colour disappears.

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

Loss on drying (*2.2.32*): maximum 8.0 per cent, determined on 1.000 g by drying at 60 °C over *diphosphorus pentoxide R* at a pressure not exceeding 0.7 kPa for 3 h.

Sulphated ash (*2.4.14*): maximum 1.0 per cent, determined on 1.0 g.

Sterility (*2.6.1*). If intended for introduction into body cavities without a further appropriate sterilisation procedure, it complies with the test for sterility.

Bacterial endotoxins (*2.6.14, Method D*): less than 1.3 IU/mg if intended for introduction into body cavities without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

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

STORAGE

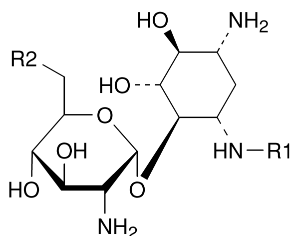
In an airtight container, protected from light. If the substance is intended for introduction into body cavities, store in a sterile, tamper-proof container.

LABELLING

The label states:

- where applicable, that the substance is sterile,
- where applicable, that the substance is free from bacterial endotoxins.

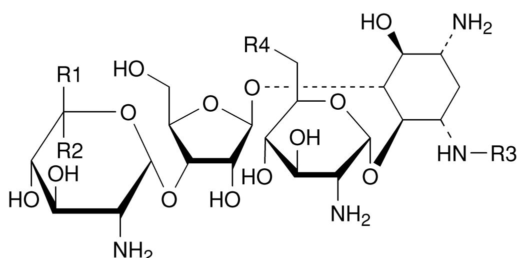
IMPURITIES



A. R1 = H, R2 = NH_2: 2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-D-streptamine (neamine or neomycin A-LP),

B. R1 = CO-CH_3, R2 = NH_2: 3-*N*-acetyl-2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-D-streptamine (3-acetylneamine),

D. R1 = H, R2 = OH: 4-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-2-deoxy-D-streptamine (paromamine or neomycin D),



C. R1 = CH_2-NH_2, R2 = R3 = H, R4 = NH_2: 2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-β-D-ribofuranosyl]-D-streptamine (neomycin C),

E. R1 = R3 = H, R2 = CH_2-NH_2, R4 = OH: 4-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-2-deoxy-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-D-streptamine (paromomycin I or neomycin E),

F. R1 = CH_2-NH_2, R2 = R3 = H, R4 = OH: 4-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-2-deoxy-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-β-D-ribofuranosyl]-D-streptamine (paromomycin II or neomycin F),

G. R1 = H, R2 = CH_2-NH_2, R3 = CO-CH_3, R4 = NH_2: 3-*N*-acetyl-2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-D-streptamine (neomycin B-LP).

01/2005:0025

FRANGULA BARK

Frangulae cortex

DEFINITION

Frangula bark consists of the dried, whole or fragmented bark of the stems and branches of *Rhamnus frangula* L. (*Frangula alnus* Miller). It contains not less than 7.0 per cent of glucofrangulins, expressed as glucofrangulin A ($C_{27}H_{30}O_{14}$; M_r 578.5) and calculated with reference to the dried drug.

CHARACTERS

It has the macroscopic and microscopic characters described under Identification tests A and B.

IDENTIFICATION

A. The bark occurs in curved, almost flat or rolled fragments or in single or double quilled pieces usually 0.5 mm to 2 mm thick and variable in length and width. The greyish-brown or dark brown outer surface is wrinkled longitudinally and covered with numerous greyish, transversely elongated lenticels; when the outer layers are removed, a dark red layer is exposed. The orange-brown to reddish-brown inner surface is smooth and bears fine longitudinal striations; it becomes red when treated with alkali. The fracture is short, fibrous in the inner part.

B. Reduce to a powder (355). The powder is yellowish or reddish-brown. Examine under a microscope using *chloral hydrate solution R*. The powdered drug shows: numerous phloem fibres, partially lignified, in groups with crystal sheaths containing calcium oxalate prisms; reddish-brown fragments of cork; fragments of parenchyma containing calcium oxalate cluster crystals. Sclereids are absent.

C. Examine the chromatogram obtained in the test for "Other species of *Rhamnus*; anthrones" in daylight. The chromatogram obtained with the test solution shows two orange brown zones (glucofrangulins) in the lower third and two to four red zones (frangulins, not always clearly separated, and above them frangula-emodin) in the upper third.

D. To about 50 mg of the powdered drug (180) add 25 ml of *dilute hydrochloric acid R* and heat the mixture on a water-bath for 15 min. Allow to cool, shake with 20 ml of *ether R* and discard the aqueous layer. Shake the ether layer with 10 ml of *dilute ammonia R1*. The aqueous layer becomes reddish-violet.

TESTS

Other species of *Rhamnus*; anthrones. Examine by thin-layer chromatography (*2.2.27*), using a suitable silica gel as the coating substance.

Test solution. To 0.5 g of the powdered drug (180) add 5 ml of *alcohol (70 per cent V/V) R* and heat to boiling. Cool and centrifuge. Decant the supernatant solution immediately and use within 30 min.

Reference solution. Dissolve 20 mg of *barbaloin R* in *alcohol (70 per cent V/V) R* and dilute to 10 ml with the same solvent.

Apply separately to the plate, as bands, 10 µl of each solution. Develop over a path of 10 cm using a mixture of 13 volumes of *water R*, 17 volumes of *methanol R* and 100 volumes of *ethyl acetate R*. Allow the plate to dry for 5 min, spray with a 50 g/l solution of *potassium hydroxide R* in *alcohol (50 per cent V/V) R*, and heat at 100-105 °C for 15 min. Examine in ultraviolet light at 365 nm. The chromatogram obtained with the reference solution shows a brownish-yellow zone corresponding to barbaloin in the central part. The chromatogram obtained with the test solution shows no zones of intense yellow fluorescence and no zone of orange to reddish fluorescence similar in position to the zone of barbaloin in the chromatogram obtained with the reference solution.

Apply to another plate, as a band, 10 µl of the test solution and develop as described above. Allow the plate to dry for not longer than 5 min and spray immediately with a 5 g/l solution of *nitrotetrazolium blue R* in *methanol R*. Examine the chromatogram immediately. No violet or greyish-blue zones appear.

Foreign matter (*2.8.2*). Not more than 1 per cent.