Top of the plate	
———	———
Harpagoside: a green zone	A green zone (harpagoside)
———	———
	A yellow zone
	A light green zone
Fructose: a yellowish-grey zone	A yellowish-grey zone may be present (fructose)
	A brown zone
Reference solution	**Test solution**

TESTS

Starch. Examine the powdered drug (355) (*2.9.12*) under a microscope using *water R*. Add *iodine solution R1*. No blue colour develops.

Loss on drying (*2.2.32*): maximum 12.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C.

Total ash (*2.4.16*): maximum 10.0 per cent.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution. To 0.500 g of the powdered drug (355) (*2.9.12*) add 100.0 ml of *methanol R*. Shake for 4 h and filter through a membrane filter (nominal pore size: 0.45 µm).

Reference solution. Dissolve the contents of a vial of *harpagoside CRS* in *methanol R* and dilute to 10.0 ml with the same solvent.

Column:

– *size*: *l* = 0.10 m, Ø = 4.0 mm;

– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: *methanol R, water R* (50:50 *V/V*).

Flow rate: 1.5 ml/min.

Detection: spectrophotometer at 278 nm.

Injection: 10 µl.

Run time: 3 times the retention time of harpagoside.

Retention time: harpagoside = about 7 min.

Calculate the percentage content of harpagoside using the following expression:

$$\frac{m_2 \times A_1 \times 1000}{A_2 \times m_1}$$

A_1 = area of the peak due to harpagoside in the chromatogram obtained with the test solution;

A_2 = area of the peak due to harpagoside in the chromatogram obtained with the reference solution;

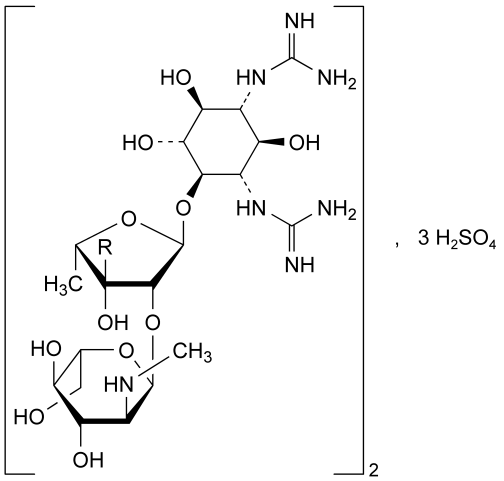
m_1 = mass of the drug to be examined used to prepare the test solution, in grams;

m_2 = mass of *harpagoside CRS* in the reference solution, in grams.

07/2008:0485

DIHYDROSTREPTOMYCIN SULPHATE FOR VETERINARY USE

Dihydrostreptomycini sulfas ad usum veterinarium



Compound	R	Molec. Formula	M_r
dihydrostreptomycin sulphate	CH_2OH	$C_{42}H_{88}N_{14}O_{36}S_3$	1461
streptomycin sulphate	CHO	$C_{42}H_{84}N_{14}O_{36}S_3$	1457

[5490-27-7]

DEFINITION

Main compound: bis[*N*,*N*‴-[(1*R*,2*R*,3*S*,4*R*,5*R*,6*S*)-4-[[5-deoxy-2-*O*-[2-deoxy-2-(methylamino)-α-L-glucopyranosyl]-3-*C*-(hydroxymethyl)-α-L-lyxofuranosyl]oxy]-2,5,6-trihydroxycyclohexane-1,3-diyl]diguanidine] trisulphate.

Sulphate of a substance obtained by catalytic hydrogenation of streptomycin or by any other means.

Semi-synthetic product derived from a fermentation product.

Stabilisers may be added.

Content:

– *sum of dihydrostreptomycin sulphate and streptomycin sulphate*: 95.0 per cent to 102.0 per cent (dried substance);

– *streptomycin sulphate*: maximum 2.0 per cent (dried substance).

PRODUCTION

The method of manufacture is validated to demonstrate that the product, if tested, would comply with the following test.

Abnormal toxicity (*2.6.9*). Inject into each mouse 1 mg dissolved in 0.5 ml of *water for injections R*.

CHARACTERS

Appearance: white or almost white, hygroscopic powder.

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

IDENTIFICATION

First identification: A, E.

Second identification: B, C, D, E.

A. Examine the chromatograms obtained in the assay.

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

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

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

Reference solution (a). Dissolve the contents of a vial of *dihydrostreptomycin sulphate CRS* in 5.0 ml of *water R*. Dilute 1.0 ml of this solution to 5.0 ml with *water R*.

Reference solution (b). Dissolve the contents of a vial of *dihydrostreptomycin sulphate CRS* in 5.0 ml of *water R*.

Reference solution (c). Dissolve 10 mg of *kanamycin monosulphate CRS* and 10 mg of *neomycin sulphate CRS* in *water R*, add 2.0 ml of reference solution (b), mix thoroughly and dilute to 10 ml with *water R*.

Plate: *TLC silica gel plate R*.

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

Application: 10 µl.

Development: over 2/3 of the plate.

Drying: in a current of warm air.

Detection: spray with a mixture of equal volumes of a 2 g/l solution of *1,3-dihydroxynaphthalene R* in *ethanol (96 per cent) R* and a 460 g/l solution of *sulphuric acid R*; heat at 150 °C for 5-10 min.

System suitability: reference solution (c):

– the chromatogram shows 3 clearly separated spots.

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

C. Dissolve 0.1 g in 2 ml of *water R* and add 1 ml of *α-naphthol solution R* and 2 ml of a mixture of equal volumes of *strong sodium hypochlorite solution R* and *water R*. A red colour develops.

D. Dissolve 10 mg in 5 ml of *water R* and add 1 ml of *1 M hydrochloric acid*. Heat in a water-bath for 2 min. Add 2 ml of a 5 g/l solution of *α-naphthol R* in *1 M sodium hydroxide* and heat in a water-bath for 1 min. A violet-pink colour is produced.

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

TESTS

Solution S. Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Appearance of solution. Solution S is not more intensely coloured than intensity 5 of the range of reference solutions of the most appropriate colour (*2.2.2, Method II*). Allow to stand protected from light at about 20 °C for 24 h; solution S is not more opalescent than reference suspension II (*2.2.1*).

pH (*2.2.3*): 5.0 to 7.0 for solution S.

Specific optical rotation (*2.2.7*): – 83.0 to – 91.0 (dried substance).

Dissolve 0.200 g in *water R* and dilute to 10.0 ml with the same solvent.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (a). Dissolve the contents of a vial of *dihydrostreptomycin sulphate CRS* (containing impurities A, B and C) in 5.0 ml of *water R*.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with *water R*.

Reference solution (c). Dilute 5.0 ml of reference solution (b) to 50.0 ml with *water R*.

Reference solution (d). Dissolve 10 mg of *streptomycin sulphate CRS* in *water R* and dilute to 20 ml with the same solvent. Mix 0.1 ml of this solution with 1.0 ml of reference solution (a).

Column:

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

Mobile phase: solution in *water R* containing 4.6 g/l of *anhydrous sodium sulphate R*, 1.5 g/l of *sodium octanesulphonate R*, 120 ml/l of *acetonitrile R1* and 50 ml/l of a 27.2 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 3.0 with a 22.5 g/l solution of *phosphoric acid R*.

Flow rate: 1.0 ml/min.

Detection: spectrophotometer at 205 nm.

Injection: 20 µl.

Run time: 1.5 times the retention time of dihydrostreptomycin.

Identification of impurities: use the chromatogram supplied with *dihydrostreptomycin sulphate CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to streptomycin and impurities A, B and C.

Relative retention with reference to dihydrostreptomycin (retention time = about 57 min): impurity A = about 0.2; impurity B = about 0.8; streptomycin = about 0.9; impurity C = about 0.95.

System suitability:

– peak-to-valley ratio (a): minimum 1.1, where H_p = height above the baseline of the peak due to streptomycin and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity C in the chromatogram obtained with reference solution (d);
– peak-to-valley ratio (b): minimum 5, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to dihydrostreptomycin in the chromatogram obtained with reference solution (d);
– the chromatogram obtained with reference solution (a) is similar to the chromatogram supplied with *dihydrostreptomycin sulphate CRS*.

Limits:

– *correction factor*: for the calculation of content, multiply the peak area of impurity A by 0.5;
– *impurities A, B*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
– *impurity C*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);
– *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

– *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (5.0 per cent);

– *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent); disregard the peak due to streptomycin.

Heavy metals (*2.4.8*): 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*): maximum 5.0 per cent, determined on 1.000 g by drying under high vacuum at 60 °C for 4 h.

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

Bacterial endotoxins (*2.6.14*): less than 0.50 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for removal of bacterial endotoxins.

ASSAY

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

Injection: test solution and reference solution (a).

Calculate the percentage content of $C_{42}H_{88}N_{14}O_{36}S_3$ and of $C_{42}H_{84}N_{14}O_{36}S_3$ using the chromatogram obtained with reference solution (a) and the declared contents of *dihydrostreptomycin sulphate CRS*. Calculate the sum of these percentage contents.

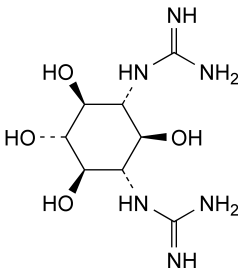
STORAGE

In an airtight container, protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

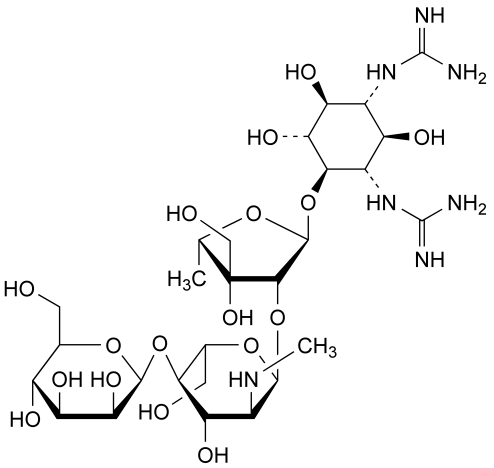
IMPURITIES

Specified impurities: A, B, C.

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

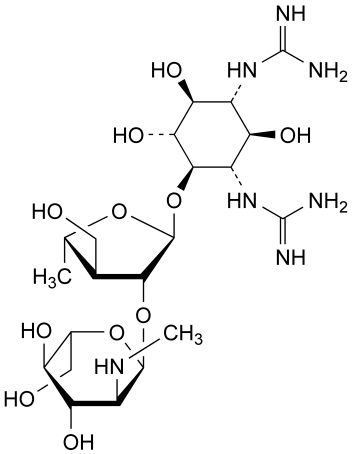


A. *N,N'''*-[(1*R*,2*s*,3*S*,4*R*,5*r*,6*S*)-2,4,5,6-tetrahydroxycyclohexane-1,3-diyl]diguanidine (streptidine),



B. *N,N'''*-[(1*S*,2*R*,3*R*,4*S*,5*R*,6*R*)-2,4,5-trihydroxy-6-[[β-D-mannopyranosyl-(1→4)-2-deoxy-2-(methylamino)-α-L-glucopyranosyl-(1→2)-5-deoxy-3-*C*-(hydroxymethyl)-α-L-lyxofuranosyl]oxy]cyclohexane-1,3-diyl]diguanidine (dihydrostreptomycin B),

C. unknown structure,

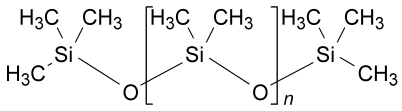


D. *N,N'''*-[(1*R*,2*R*,3*S*,4*R*,5*R*,6*S*)-4-[[3,5-dideoxy-2-*O*-[2-deoxy-2-(methylamino)-α-L-glucopyranosyl]-3-(hydroxymethyl)-α-L-arabinofuranosyl]oxy]-2,5,6-trihydroxycyclohexane-1,3-diyl]diguanidine (deoxydihydrostreptomycin).

01/2008:0138
corrected 6.2

DIMETICONE

Dimeticonum



[9006-65-9]

DEFINITION

Poly(dimethylsiloxane) obtained by hydrolysis and polycondensation of dichlorodimethylsilane and chlorotrimethylsilane. Different grades of dimeticone exist which are distinguished by a number indicating the nominal kinematic viscosity placed after the name.