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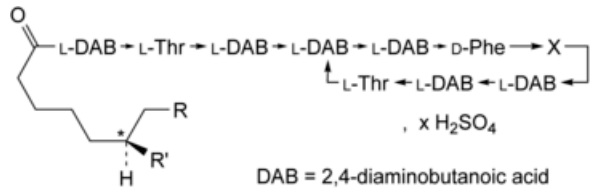
Polymyxin B Sulfate

General Notices

Polymyxin B Sulphate

(Ph. Eur. monograph 0203)



Polymyxin	R	R′	X	Molecular formula	M_r
B1	CH_3	CH_3	L-Leu	$C_{56}H_{98}N_{16}O_{13}$	1203
B2	H	CH_3	L-Leu	$C_{55}H_{96}N_{16}O_{13}$	1189
B3	CH_3	H	L-Leu	$C_{55}H_{96}N_{16}O_{13}$	1189
B1-I	CH_3	CH_3	L-Ile	$C_{56}H_{98}N_{16}O_{13}$	1203

Action and use

Antibacterial.

Preparation

Polymyxin and Bacitracin Ointment

Ph Eur

DEFINITION

Mixture of the sulfates of polypeptides produced by the growth of certain strains of *Paenibacillus polymyxa*, the main component being polymyxin B1.

Potency

Minimum 6500 IU/mg (dried substance).

CHARACTERS

Appearance

White or almost white, hygroscopic powder.

Solubility

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

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Thin-layer chromatography (2.2.27).

Test solution Dissolve 5 mg of the substance to be examined in 1 mL of a mixture of equal volumes of *hydrochloric acid R* and *water R*. Heat at 135 °C in a sealed tube for 5 h. Evaporate to dryness on a water-bath and continue the heating until the hydrochloric acid has evaporated. Dissolve the residue in 0.5 mL of *water R*.

Reference solution (a) Dissolve 20 mg of *leucine R* in *water R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 20 mg of *threonine R* in *water R* and dilute to 10 mL with the same solvent.

Reference solution (c) Dissolve 20 mg of *phenylalanine R* in *water R* and dilute to 10 mL with the same solvent.

Reference solution (d) Dissolve 20 mg of *serine R* in *water R* and dilute to 10 mL with the same solvent.

Plate *TLC silica gel G plate R*.

Carry out the following procedures protected from light.

Mobile phase *water R*, *phenol R* (25:75 *V/V*).

Application 5 µL as bands of 10 mm, then place the plate in the chromatographic tank so that it is not in contact with the mobile phase, and allow it to become impregnated with the vapour of the mobile phase for at least 12 h.

Development Over 2/3 of the plate using the same mobile phase.

Drying At 100-105 °C.

Detection Spray with *ninhydrin solution R1* and heat at 110 °C for 5 min.

Results The chromatogram obtained with the test solution shows zones corresponding to those in the chromatograms obtained with reference solutions (a), (b) and (c), but shows no zone corresponding to that in the chromatogram obtained with reference solution (d); the chromatogram obtained with the test solution also shows a zone with a very low R_F value (2,4-diaminobutyric acid).

B. Examine the chromatograms obtained in the test for composition.

Results The peaks due to polymyxins B1, B2, B3 and B1-I in the chromatogram obtained with the test solution are similar in retention time to the corresponding peaks in the chromatogram obtained with reference solution (a).

C. Dissolve about 2 mg in 5 mL of *water R* and add 5 mL of a 100 g/L solution of *sodium hydroxide R*. Shake and add dropwise 0.25 mL of a 10 g/L solution of *copper sulfate pentahydrate R*, shaking after each addition. A reddish-violet colour develops.

D. It gives reaction (a) of sulfates (2.3.1).

TESTS

pH (2.2.3)

5.0 to 7.0.

Dissolve 0.2 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Composition

Liquid chromatography (2.2.29): use the normalisation procedure.

Solvent mixture acetonitrile R, water R (20:80 *V/V*).

Test solution Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Reference solution (a) Dissolve the contents of a vial of polymyxin B sulfate CRS in the solvent mixture and dilute to 50 mL with the solvent mixture.

Reference solution (b) Dilute 1 mL of reference solution (a) to 100 mL with the solvent mixture.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: end-capped octadecylsilyl silica gel for chromatography R (5 µm);

— *temperature*: 30 °C.

Mobile phase Mix 20 volumes of acetonitrile R1 and 80 volumes of a solution prepared as follows: dissolve 4.46 g of anhydrous sodium sulfate R in 900 mL of water for chromatography R, adjust to pH 2.3 with dilute phosphoric acid R and dilute to 1000 mL with water for chromatography R.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µL.

Run time 1.4 times the retention time of polymyxin B1.

Identification of peaks Use the chromatogram supplied with polymyxin B sulfate CRS and the chromatogram obtained with reference solution (a) to identify the peaks due to polymyxins B1, B2, B3 and B1-I.

Relative retention With reference to polymyxin B1 (retention time = about 35 min): polymyxin B2 = about 0.5; polymyxin B3 = about 0.6; polymyxin B1-I = about 0.8.

System suitability:

— resolution: minimum 3.0 between the peaks due to polymyxin B2 and polymyxin B3 in the chromatogram obtained with reference solution (a);

— signal-to-noise ratio: minimum 20 for the peak due to polymyxin B1 in the chromatogram obtained with reference solution (b).

Limits:

— *polymyxin B1-I*: maximum 15.0 per cent;

— *polymyxin B3*: maximum 6.0 per cent;

— *sum of polymyxins B1, B2, B3 and B1-I*: minimum 80.0 per cent;

— *reporting threshold*: 0.40 per cent.

Related substances

Liquid chromatography (2.2.29) as described in the test for composition.

Limits:

— *any impurity*: for each impurity, maximum 3.0 per cent;

— *total*: maximum 17.0 per cent;

— *reporting threshold*: 0.40 per cent.

Sulfate

15.5 per cent to 17.5 per cent (dried substance).

Dissolve 0.250 g in 100 mL of *water R* and adjust the solution to pH 11 with *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 *ethanol (96 per cent) 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 6.0 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C at a pressure not exceeding 0,7 kPa for 3 h.

Sulfated ash (*2.4.14*)

Maximum 0.75 per cent, determined on 1.0 g.

Pyrogens (*2.6.8*)

If intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of pyrogens, it complies with the test for pyrogens. Inject, per kilogram of the rabbit's mass, 1 mL of a solution in *water for injections R* containing 1.5 mg of the substance to be examined per millilitre.

ASSAY

Carry out the microbiological assay of antibiotics (*2.7.2*). Use *polymyxin B sulfate for microbiological assay CRS* as the chemical reference substance.

STORAGE

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

LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.
