Titrate with *0.05 M iodine* using 1 mL of *starch solution R* as indicator. The volume of *0.05 M iodine* used in the titration is 5.5 mL to 7.0 mL.

Loss on drying (*2.2.32*): maximum 5.0 per cent, determined on 1.000 g by drying at 60 °C over *diphosphorus pentoxide R* at a pressure not exceeding 670 Pa for 3 h.

Sulfated ash (*2.4.14*): 16 per cent to 21 per cent, determined on 0.50 g.

Pyrogens (*2.6.8*). If intended for use in the manufacture of parenteral preparations without a further appropriate procedure for removal of pyrogens, it complies with the test. Inject, per kilogram of the rabbit's mass, 1 mL of a solution in *water for injections R* containing 2.5 mg of the substance to be examined per millilitre.

ASSAY

Carry out the microbiological assay of antibiotics (*2.7.2*).

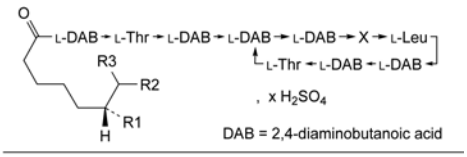
STORAGE

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

01/2008:0320

COLISTIN SULFATE

Colistini sulfas



polymyxin	X	R1	R2	R3	Mol. Formula	M_r
E1	D-Leu	CH_3	CH_3	H	$C_{53}H_{100}N_{16}O_{13}$	1170
E2	D-Leu	CH_3	H	H	$C_{52}H_{98}N_{16}O_{13}$	1155
E3	D-Leu	H	CH_3	H	$C_{52}H_{98}N_{16}O_{13}$	1155
E1-I	D-Ile	CH_3	CH_3	H	$C_{53}H_{100}N_{16}O_{13}$	1170
E1-7MOA	D-Leu	H	CH_3	CH_3	$C_{53}H_{100}N_{16}O_{13}$	1170

DEFINITION

A mixture of the sulfates of polypeptides produced by certain strains of *Bacillus polymyxa* var. *colistinus* or obtained by any other means.

Content:

– *sum of polymyxins E1, E2, E3, E1-I and E1-7MOA*: minimum 77.0 per cent (dried substance);
– *polymyxin E1-I*: maximum 10.0 per cent (dried substance);
– *polymyxin E1-7MOA*: maximum 10.0 per cent (dried substance);
– *polymyxin E3*: maximum 10.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, hygroscopic powder.

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

IDENTIFICATION

First identification: B, E.

Second identification: A, C, D, E.

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 moistened *blue litmus paper R* does not turn red. 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 10 mm bands, 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 a path of 12 cm.

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) and (b), but shows no zones corresponding to those in the chromatograms obtained with reference solutions (c) and (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 assay.

Results: the peaks due to polymyxin E1 and polymyxin E2 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 5 mg in 3 mL of *water R*. Add 3 mL of *dilute sodium hydroxide solution R*. Shake and add 0.5 mL of a 10 g/L solution of *copper sulfate R*. A violet colour is produced.

D. Dissolve about 50 mg in 1 mL of *1 M hydrochloric acid* and add 0.5 mL of *0.01 M iodine*. The solution remains coloured.

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

TESTS

pH (*2.2.3*): 4.0 to 6.0.

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*): – 63 to – 73 (dried substance).

Dissolve 1.25 g in *water R* and dilute to 25.0 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution. Dissolve 25.0 mg of the substance to be examined in 40 mL of *water R* and dilute to 50.0 mL with *acetonitrile R*.

Reference solution (a). Dissolve 25.0 mg of *colistin sulfate CRS* in 40 mL of *water R* and dilute to 50.0 mL with *acetonitrile R*.

Reference solution (b). Dilute 1.0 mL of reference solution (a) to 100.0 mL with a mixture of 20 volumes of *acetonitrile R* and 80 volumes of *water R*.

Column:

– *size*: *l* = 0.15 m, Ø = 4.6 mm;
– *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3.5 µm);
– *temperature*: 30 °C.

Mobile phase: mix 22 volumes of *acetonitrile R* and 78 volumes of a solution prepared as follows: dissolve 4.46 g of *anhydrous sodium sulfate R* in 900 mL of *water R*, adjust to pH 2.4 with *dilute phosphoric acid R* and dilute to 1000 mL with *water R*.

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 215 nm.

Injection: 20 µL.

Run time: 1.5 times the retention time of polymyxin E1.

Relative retention with reference to polymyxin E1 (retention time = about 16 min): polymyxin E2 = about 0.45; polymyxin E3 = about 0.5; polymyxin E1-I = about 0.8; polymyxin E1-7MOA = about 1.1.

System suitability: reference solution (a):

– *resolution*: minimum 8.0 between the peaks due to polymyxin E2 and polymyxin E1, minimum 6.0 between the peaks due to polymyxin E2 and polymyxin E1-I, minimum 2.5 between the peaks due to polymyxin E1-I and polymyxin E1, minimum 1.5 between the peaks due to polymyxin E1 and polymyxin E1-7MOA;

– the chromatogram obtained is similar to the chromatogram supplied with *colistin sulfate CRS*.

Limits:

– *any impurity*: maximum 4.0 per cent;

– *total*: maximum 23.0 per cent;

– *disregard limit*: the area of the peak due to polymyxin E1 in the chromatogram obtained with reference solution (b); disregard the peaks due to polymyxins E2, E3, E1-I, E1 and E1-7MOA.

Sulfate: 16.0 per cent to 18.0 per cent (dried substance).

Dissolve 0.250 g in 100 mL of *water R* and adjust 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 3.5 per cent, determined on 1.000 g by drying at 60 °C over *diphosphorus pentoxide R* at a pressure not exceeding 670 Pa for 3 h.

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

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 polymyxin E3, of polymyxin E1-I, of polymyxin E1-7MOA, and of the sum of polymyxins E1, E2, E3, E1-I and E1-7MOA, using the following expression:

$$C_{Ei} = \frac{A_{Ei} \times m_2 \times D_{Ei}}{m_1 \times B_{Ei}}$$

C_{Ei} = percentage content of polymyxin E*i*;

A_{Ei} = area of the peak due to polymyxin E*i* in the chromatogram obtained with the test solution;

m_1 = mass in milligrams of the substance to be examined (dried substance) in the test solution;

B_{Ei} = area of the peak due to polymyxin E*i* in the chromatogram obtained with reference solution (a);

m_2 = mass in milligrams of *colistin sulfate CRS* in reference solution (a);

D_{Ei} = declared percentage content for polymyxin E*i* in *colistin sulfate CRS*.

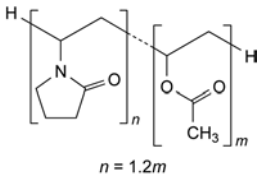
STORAGE

In an airtight container, protected from light.

01/2008:0891
corrected 6.0

COPOVIDONE

Copovidonum



$n = 1.2m$

$(C_6H_9NO)_n$, $(C_4H_6O_2)_m$ M_r $(111.1)_n + (86.1)_m$

DEFINITION

Copovidone is a copolymer of 1-ethenylpyrrolidin-2-one and ethenyl acetate in the mass proportion 3:2.

Content:

– nitrogen (N; A_r 14.01): 7.0 per cent to 8.0 per cent (dried substance),

– ethenyl acetate $C_4H_6O_2$; M_r 86.10): 35.3 per cent to 42.0 per cent (dried substance).

K-value: 90.0 per cent to 110.0 per cent of the value stated on the label.

CHARACTERS

Aspect: white or yellowish-white powder or flakes, hygroscopic.

Solubility: freely soluble in water, in alcohol and in methylene chloride.

IDENTIFICATION

First identification: A.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *Ph. Eur. reference spectrum of copovidone.*

B. To 1 mL of solution S (see Tests) add 5 mL of *water R* and 0.2 mL of *0.05 M iodine*. A red colour appears.

C. Dissolve 0.7 g of *hydroxylamine hydrochloride R* in 10 mL of *methanol R*, add 20 mL of a 40 g/L solution of *sodium hydroxide R* and filter if necessary. To 5 mL of the solution add 0.1 g of the substance to be examined and boil for 2 min. Transfer 50 µL to a filter paper and add 0.1 mL of a mixture of equal volumes of *ferric chloride solution R1* and *hydrochloric acid R*. A violet colour appears.

TESTS

Solution S. Dissolve 10 g in *water R* and dilute to 100 mL with the same solvent. Add the substance to be examined to the *water R* in small portions with constant stirring.

Appearance of solution. Solution S is not more opalescent than reference suspension III (*2.2.1*) and not more intensely coloured than reference solution B_5, R_5 or BY_5 (*2.2.2, Method II*).

Aldehydes: maximum 500 ppm, expressed as acetaldehyde.

Test solution. Dissolve 1.0 g of the substance to be examined in *phosphate buffer solution pH 9.0 R* and dilute to 100.0 mL with the same solvent. Stopper the flask and heat at 60 °C for 1 h. Allow to cool.

Reference solution. Dissolve 0.140 g of *acetaldehyde ammonia trimer trihydrate R* in *water R* and dilute to 200.0 mL with the same solvent. Dilute 1.0 mL of this solution to 100.0 mL with *phosphate buffer solution pH 9.0 R*.

Into 3 identical spectrophotometric cells with a path length of 1 cm, introduce separately 0.5 mL of the test solution, 0.5 mL of the reference solution and 0.5 mL of *water R* (blank). To each cell, add 2.5 mL of *phosphate buffer solution pH 9.0 R* and 0.2 mL of *nicotinamide-adenine dinucleotide solution R*. Mix and stopper tightly. Allow to stand at 22 ± 2 °C for 2-3 min and