Sulphated 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 sulphate CRS* in reference solution (a);

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

STORAGE

In an airtight container, protected from light.

01/2008:1862

COLOPHONY

Colophonium

DEFINITION

Residue remaining after distillation of the volatile oil from the oleoresin obtained from various species of *Pinus*.

IDENTIFICATION

A. Translucent, pale yellow to brownish-yellow, angular, irregularly-shaped, brittle, glassy pieces of different sizes the surfaces of which bear conchoidal markings.

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

Test solution. Dissolve 1 g in 10 ml of *methanol R* by gently warming.

Reference solution. Dissolve 10 mg of *thymol R* and 10 mg of *linalol R* in 10 ml of *methanol R*.

Plate: *TLC silica gel plate R*.

Mobile phase: *methylene chloride R*.

Application: 10 µl, as bands.

Development: over a path of 15 cm.

Drying: in air.

Detection: spray with *anisaldehyde solution R* and heat at 100-105 °C for 10 min; examine in daylight.

Results: see below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other coloured zones are present in the chromatogram obtained with the test solution.

Top of the plate	
	A purple band
	A purple band
———	———
	2 purple bands
Thymol: an orange band	
———	———
Linalol: a purple band	Sequence of narrow purple bands
	Purple extended baseline band
Reference solution	**Test solution**

TESTS

Acid value (*2.5.1*): 145 to 180, determined on 1.0 g.

Total ash (*2.4.16*): maximum 0.2 per cent.

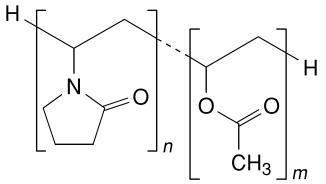
STORAGE

Do not reduce to a powder.

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 measure the absorbance (*2.2.25*) of each solution at 340 nm, using *water R* as the compensation liquid. To each cell, add 0.05 ml of *aldehyde dehydrogenase solution R*, mix and stopper tightly. Allow to stand at 22 ± 2 °C for 5 min. Measure the absorbance of each solution at 340 nm using *water R* as compensation liquid. Determine the content of aldehydes using the expression:

$$\frac{(A_{t2} - A_{t1}) - (A_{b2} - A_{b1})}{(A_{s2} - A_{s1}) - (A_{b2} - A_{b1})} \times \frac{100\,000 \times C}{m}$$

A_{t1} = absorbance of the test solution before the addition of aldehyde dehydrogenase,

A_{t2} = absorbance of the test solution after the addition of aldehyde dehydrogenase,

A_{s1} = absorbance of the reference solution before the addition of aldehyde dehydrogenase,

A_{s2} = absorbance of the reference solution after the addition of aldehyde dehydrogenase,

A_{b1} = absorbance of the blank before the addition of aldehyde dehydrogenase,

A_{b2} = absorbance of the blank after the addition of aldehyde dehydrogenase,

m = mass of povidone, in grams, calculated with reference to the dried substance,

C = concentration (mg/ml), of acetaldehyde in the reference solution, calculated from the weight of the acetaldehyde ammonia trimer trihydrate with the factor 0.72.

Peroxides: maximum 400 ppm, expressed as H_2O_2.

Dilute 10 ml of solution S to 25 ml with *water R*. Add 2 ml of *titanium trichloride-sulphuric acid reagent R* and allow to stand for 30 min. The absorbance (*2.2.25*) of the solution, measured at 405 nm using a mixture of 25 ml of a 40 g/l solution of the substance to be examined and 2 ml of a 13 per cent *V/V* solution of *sulphuric acid R* as the compensation liquid, is not greater than 0.35.

Hydrazine. Thin-layer chromatography (*2.2.27*). *Use freshly prepared solutions.*

Test solution. To 25 ml of solution S add 0.5 ml of a 50 g/l solution of *salicylaldehyde R* in *methanol R*, mix and heat in a water-bath at 60 °C for 15 min. Allow to cool, add 2.0 ml of *xylene R*, shake for 2 min and centrifuge. Use the clear supernatant layer.

Reference solution. Dissolve 9 mg of *salicylaldehyde azine R* in *xylene R* and dilute to 100 ml with the same solvent. Dilute 1 ml of this solution to 10 ml with *xylene R*.

Plate: *TLC silanised silica gel plate R*.

Mobile phase: *water R, methanol R* (20:80 *V/V*).

Application: 10 µl.

Development: over a path of 15 cm.

Drying: in air.

Detection: examine in ultraviolet light at 365 nm.

Limit:

– *hydrazine*: any spot corresponding to salicylaldehyde azine in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (1 ppm).

Monomers: maximum 0.1 per cent.

Dissolve 10.0 g in 30 ml of *methanol R* and add slowly 20.0 ml of *iodine bromide solution R*. Allow to stand for 30 min protected from light with repeated shaking. Add 10 ml of a 100 g/l solution of *potassium iodide R* and titrate with *0.1 M sodium thiosulphate* until a yellow colour is obtained. Continue titration dropwise until the solution becomes colourless. Carry out a blank titration. Not more than 1.8 ml of *0.1 M sodium thiosulphate* is used.

Impurity A. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 100 mg of the substance to be examined in *water R* and dilute to 50.0 ml with the same solvent.

Reference solution. Dissolve 100 mg of *2-pyrrolidone R* in *water R* and dilute to 100 ml with the same solvent. Dilute 1.0 ml to 100.0 ml with *water R*.

Precolumn:

– *size*: *l* = 0.025 m, Ø = 4 mm,

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

Column:

– *size*: *l* = 0.25 m, Ø = 4 mm,

– *stationary phase*: spherical *aminohexadecylsilyl silica gel for chromatography R* (5 µm),

– *temperature*: 30 °C.

Mobile phase: *water R*, adjusted to pH 2.4 with *phosphoric acid R*.

Flow rate: 1 ml/min.

Detection: spectrophotometer at 205 nm. A detector is placed between the precolumn and the analytical column. A second detector is placed after the analytical column.

Injection: 10 µl. When impurity A has left the precolumn (after about 1.2 min) switch the flow directly from the pump to the analytical column. Before the next chromatogram is run, wash the precolumn by reversed flow.

Limit:

– *impurity A*: not more than the area of the principal peak obtained with the reference solution (0.5 per cent).

Heavy metals (*2.4.8*): maximum 20 ppm.

12 ml of solution S complies with limit test A. Prepare the standard using *lead standard solution (2 ppm Pb) R*.

Loss on drying (*2.2.32*): maximum 5.0 per cent, determined on 0.500 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

Viscosity, expressed as *K*-value. Dilute 5.0 ml of solution S to 50.0 ml with *water R*. Allow to stand for 1 h and determine the viscosity (*2.2.9*) of the solution at 25 ± 0.1 °C using viscometer No. 1 with a minimum flow time of 100 s. Calculate the *K*-value from the expression:

$$\frac{1.5log\eta - 1}{0.15 + 0.003c} + \frac{\sqrt{300c\,log\eta + (c + 1.5c\,log\eta)^2}}{0.15c + 0.003c^2}$$

c = percentage concentration (g/100 ml) of the substance to be examined, calculated with reference to the dried substance,

η = viscosity of the solution relative to that of water.

ASSAY

Ethenyl acetate. Determine the saponification value (*2.5.6*) on 2.00 g of the substance to be examined. Multiply the result obtained by 0.1534 to obtain the percentage content of the ethenyl acetate component.

Nitrogen. Carry out the determination of nitrogen (*2.5.9*) using 30.0 mg of the substance to be examined and 1 g of a mixture of 3 parts of *copper sulphate R* and 997 parts of *dipotassium sulphate R*, heating until a clear, light green solution is obtained and then for a further 45 min.

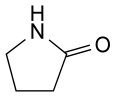
STORAGE

In an airtight container.

LABELLING

The label states the *K*-value.

IMPURITIES



A. pyrrolidin-2-one (2-pyrrolidone).

01/2008:0893

COPPER SULPHATE, ANHYDROUS

Cupri sulfas anhydricus

$CuSO_4$ M_r 159.6

[7758-98-7]

DEFINITION

Content: 99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: greenish-grey powder, very hygroscopic.

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

IDENTIFICATION

A. Add several drops of *dilute ammonia R2* to 1 ml of solution S (see Tests). A blue precipitate is formed. On further addition of *dilute ammonia R2* the precipitate dissolves and a dark blue colour is produced.

B. Loss on drying (see Tests).

C. Dilute 1 ml of solution S to 5 ml with *water R*. The solution gives reaction (a) of sulphates (*2.3.1*).

TESTS

Solution S. Dissolve 1.6 g in *water R* and dilute to 50 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*).

Chlorides (*2.4.4*): maximum 150 ppm.

Dilute 10 ml of solution S to 15 ml with *water R*.

Iron: maximum 1.50 × 10^2 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution. Dissolve 0.32 g in 10 ml of *water R*, add 2.5 ml of *lead-free nitric acid R* and dilute to 25.0 ml with *water R*.

Reference solutions. Prepare the reference solutions using *iron standard solution (20 ppm Fe) R*, adding 2.5 ml of *lead-free nitric acid R* and diluting to 25.0 ml with *water R*.

Source: iron hollow-cathode lamp.

Wavelength: 248.3 nm.

Atomisation device: air-acetylene flame.

Copper may form explosive acetylides with acetylene. Therefore, clean the burner thoroughly before any residues become dry.

Lead: maximum 80.0 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution. Dissolve 1.6 g in 10 ml of *water R*, add 2.5 ml of *lead-free nitric acid R* and dilute to 25.0 ml with *water R*.

Reference solutions. Prepare the reference solutions using *lead standard solution (100 ppm Pb) R*, adding 2.5 ml of *lead-free nitric acid R* and diluting to 25.0 ml with *water R*.

Source: lead hollow-cathode lamp.

Wavelength: 217.0 nm.

Atomisation device: air-acetylene flame.

Copper may form explosive acetylides with acetylene. Therefore, clean the burner thoroughly before any residues become dry.

Loss on drying (*2.2.32*): maximum 1.0 per cent, determined on 0.500 g by drying in an oven at 250 ± 10 °C.

ASSAY

Dissolve 0.125 g in 50 ml of *water R*. Add 2 ml of *sulphuric acid R* and 3 g of *potassium iodide R*. Titrate with *0.1 M sodium thiosulphate*, using 1 ml of *starch solution R*, added towards the end of the titration.

1 ml of *0.1 M sodium thiosulphate* is equivalent to 15.96 mg of $CuSO_4$.

STORAGE

In an airtight container.