D. It gives reaction (a) of sulphates.

B. Examine the chromatograms obtained in the assay.

C. Dissolve about 2 mg in 5 ml of water R and dilute to 10 ml with the same solvent.

D. It gives reaction (a) of sulphates (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.

Specific optical rotation (2.2.7): −78 to −90 (dried substance).

Dissolve 0.50 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 50.0 mg of the substance to be examined in a mixture of 20 volumes of acetonitrile R and 80 volumes of water R and dilute to 100.0 ml with the same mixture of solvents.

Reference solution (a). Dissolve 50.0 mg of polymyxin B sulphonate CRS in a mixture of 20 volumes of acetonitrile R and 80 volumes of water R and dilute to 100.0 ml with the same mixture of solvents.

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 = 0.25 m, Ø = 4.6 mm,
- stationary phase: base-deactivated end-capped octadecylsilyl silica gel for chromatography R (5 µm),
- temperature: 30 °C.

Mobile phase: mix 20 volumes of acetonitrile R and 80 volumes of a solution prepared as follows: dissolve 4.46 g of anhydrous sodium sulphate R in 900 ml of water R, adjust to pH 2.3 using 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: 1.4 times the retention time of polymyxin B1.

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: reference solution (a):
- resolution: minimum of 3.0 between the peaks due to polymyxin B2 and polymyxin B3.

Limits:
- any impurity: maximum 3.0 per cent,
- total: maximum 17.0 per cent,
- disregard limit: 0.7 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Sulphate: 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 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 SO₄.<sup>2-</sup>

Loss on drying (2.2.32): maximum 6.0 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.

Sulphated 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 dosage forms 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

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 B3 and B1-I, and the sum of polymyxins B1, B2, B3 and B1-I using the corresponding declared contents of polymyxin sulphate CRS.

STORAGE

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

LABELLING

The label states, where applicable, that the substance is pyrogenic.

01/2005:0426
(corrected)

POLYSORBATE 20

Polysorbatum 20

DEFINITION

Mixture of partial esters of fatty acids, mainly lauric acid, with sorbitol and its hydrates ethoxylated with approximately 20 moles of ethylene oxide for each mole of sorbitol and sorbitol anhydrides.

CHARACTERS

Appearance: oily, yellow to brownish-yellow, clear or slightly opalescent liquid.
**Polysorbate 40**

**DEFINITION**
Mixture of partial esters of fatty acids, mainly *Palmitic acid* (1904), with sorbitol and its anhydrides ethoxylated with approximately 20 moles of ethylene oxide for each mole of sorbitol and sorbitol anhydrides.

**CHARACTERS**
- **Appearance**: oily, viscous, yellowish or brownish-yellow liquid.
- **Solubility**: miscible with water, with ethanol, with ethyl acetate and with methanol, practically insoluble in fatty oils and in liquid paraffin.
- **Relative density**: about 1.10.
- **Viscosity**: about 400 mPas at 30 °C.

**TESTS**

**Acid value** (2.5.1): maximum 2.0.

**Hydroxyl value** (2.5.3, Method A): 96 to 108.

**Peroxide value**: maximum 10.0.

**Saponification value** (2.5.6): 40 to 50, determined on 4.0 g.

**Ethylene oxide and dioxan** (2.4.25, Method A): maximum 1 ppm of ethylene oxide and 10 ppm of dioxan.

**Heavy metals** (2.4.8): maximum 10 ppm.

**Water** (2.5.12): maximum 0.25 per cent, determined on 2.0 g.

**Total ash** (2.4.16): maximum 0.25 per cent, determined on 2.0 g.

**STORAGE**
In an airtight container, protected from light.

**01/2005:1914**

**POLYSORBATE 40**

**Polysorbitum 40**

**DEFINITION**
Mixture of partial esters of fatty acids, mainly *Palmitic acid* (1904), with sorbitol and its anhydrides ethoxylated with approximately 20 moles of ethylene oxide for each mole of sorbitol and sorbitol anhydrides.

**CHARACTERS**
- **Appearance**: oily, viscous, yellowish or brownish-yellow liquid.
- **Solubility**: miscible with water, with ethanol, with ethyl acetate and with methanol, practically insoluble in fatty oils and in liquid paraffin.
- **Relative density**: about 1.10.
- **Viscosity**: about 400 mPas at 30 °C.

**IDENTIFICATION**

**First identification**: A, D.

**Second identification**: B, C, D, E.

A. **Infrared absorption spectrophotometry** (2.2.24).


B. It complies with the test for hydroxyl value (see Tests).

C. It complies with the test for saponification value (see Tests).

D. It complies with the test for composition of fatty acids (see Tests).

E. Dissolve 0.1 g in 5 ml of methylene chloride R. Add 0.01 M sodium thiosulphate solution and allow to stand for 1 min. Add 50 ml of saturated potassium iodide solution R and 0.1 g of potassium thiocyanate R. Stir with a glass rod. The solution becomes blue.

**Detection**: flame ionisation.

**Injection**: 1 µl.

**Composition of the fatty acid fraction of the substance**:
- **caproic acid**: maximum 1.0 per cent,
- **caprylic acid**: maximum 10.0 per cent,
- **capric acid**: maximum 10.0 per cent,
- **lauric acid**: 40.0 per cent to 60.0 per cent,
- **myristic acid**: 14.0 per cent to 25.0 per cent,
- **palmitic acid**: 7.0 per cent to 15.0 per cent,
- **stearic acid**: maximum 7.0 per cent,
- **oleic acid**: maximum 11.0 per cent,
- **linoleic acid**: maximum 3.0 per cent.

**Ethylene oxide and dioxan** (2.4.25, Method A): maximum 1 ppm of ethylene oxide and 10 ppm of dioxan.

**Heavy metals** (2.4.8): maximum 10 ppm.

**Water** (2.5.12): maximum 0.25 per cent, determined on 2.0 g.

**Total ash** (2.4.16): maximum 0.25 per cent, determined on 2.0 g.

**STORAGE**
In an airtight container, protected from light.

**01/2005:1914**

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**Solubility**: soluble in water, in ethanol, in ethyl acetate and in methanol, practically insoluble in fatty oils and in liquid paraffin.

**Relative density**: about 1.10.

**Viscosity**: about 400 mPas at 25 °C.

**IDENTIFICATION**

**First identification**: A, D.

**Second identification**: B, C, D, E.

A. **Infrared absorption spectrophotometry** (2.2.24).


B. It complies with the test for hydroxyl value (see Tests).

C. It complies with the test for saponification value (see Tests).

D. It complies with the test for composition of fatty acids (see Tests).

E. Dissolve 0.1 g in 5 ml of methylene chloride R. Add 0.01 M sodium thiosulphate solution and allow to stand for 1 min. Add 50 ml of saturated potassium iodide solution R and 0.1 g of potassium thiocyanate R. Stir with a glass rod. The solution becomes blue.

**Detection**: flame ionisation.

**Injection**: 1 µl.

**Composition of the fatty acid fraction of the substance**:
- **caproic acid**: maximum 1.0 per cent,
- **caprylic acid**: maximum 10.0 per cent,
- **capric acid**: maximum 10.0 per cent,
- **lauric acid**: 40.0 per cent to 60.0 per cent,
- **myristic acid**: 14.0 per cent to 25.0 per cent,
- **palmitic acid**: 7.0 per cent to 15.0 per cent,
- **stearic acid**: maximum 7.0 per cent,
- **oleic acid**: maximum 11.0 per cent,
- **linoleic acid**: maximum 3.0 per cent.

**Ethylene oxide and dioxan** (2.4.25, Method A): maximum 1 ppm of ethylene oxide and 10 ppm of dioxan.

**Heavy metals** (2.4.8): maximum 10 ppm.

**Water** (2.5.12): maximum 0.25 per cent, determined on 2.0 g.

**Total ash** (2.4.16): maximum 0.25 per cent, determined on 2.0 g.

**STORAGE**
In an airtight container, protected from light.

**01/2005:1914**