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Kanamycin Acid Sulfate

General Notices

Kanamycin Acid Sulphate

(Ph. Eur. monograph 0033)

Action and use

Aminoglycoside antibacterial.

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DEFINITION

Kanamycin acid sulfate is a form of kanamycin sulfate prepared by adding sulfuric acid to a solution of kanamycin monosulfate and drying by a suitable method. The potency is not less than 670 IU/mg, calculated with reference to the dried substance.

Fermentation product.

PRODUCTION

It is produced by methods of manufacture designed to eliminate or minimise substances lowering blood pressure.

CHARACTERS

A white or almost white powder, hygroscopic, soluble in about 1 part of water, practically insoluble in acetone and in ethanol (96 per cent).

IDENTIFICATION

A. Examine by thin-layer chromatography (2.2.27), using a plate coated with a 0.75 mm layer of the following mixture: mix 0.3 g of *carbomer R* with 240 mL of *water R* and allow to stand, with moderate shaking, for 1 h; adjust to pH 7 by the gradual addition, with continuous shaking, of *dilute sodium hydroxide solution R* and add 30 g of *silica gel H R*.

Heat the plate at 110 °C for 1 h, allow to cool and use immediately.

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 10 mg of *kanamycin monosulfate CRS* in *water R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 10 mg of *kanamycin monosulfate CRS*, 10 mg of *neomycin sulfate CRS* and 10 mg of *streptomycin sulfate for identification CRS* in *water R* and dilute to 10 mL with the same solvent.

Apply separately to the plate 10 µL of each solution. Develop over a path of 12 cm using a 70 g/L solution of *potassium dihydrogen phosphate R*. Dry the plate in a current of warm air and 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 *sulfuric acid R*. Heat at 150 °C for 5 min to 10 min. 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). The test is not valid unless the chromatogram obtained with reference solution (b) shows 3 clearly separated spots.

B. Dissolve 0.5 g in 10 mL of *water R*. Add 10 mL of *picric acid solution R*. Initiate crystallisation if necessary by scratching the wall of the tube with a glass rod and allow to stand. Collect the crystals, wash with 20 mL of *water R* and filter. Dry at 100 °C. The crystals melt (*2.2.14*) at about 235 °C, with decomposition.
C. Dissolve about 50 mg in 2 mL of *water R*. Add 1 mL of a 10 g/L solution of *ninhydrin R* and heat for a few minutes on a water-bath. A violet colour develops.
D. It gives the reactions of sulfates (*2.3.1*).

TESTS

Solution S

Dissolve 0.20 g in *carbon dioxide-free water R* and dilute to 20.0 mL with the same solvent.

pH (*2.2.3*)

The pH of solution S is 5.5 to 7.5.

Specific optical rotation (*2.2.7*)

+ 103 to + 115, determined on solution S and calculated with reference to the dried substance.

Kanamycin B

Examine by thin-layer chromatography (*2.2.27*), using a plate prepared as prescribed under identification test A.

Heat the plate at 110 °C for 1 h, allow to cool and use immediately.

Test solution Dissolve 0.11 g of the substance to be examined in *water R* and dilute to 20 mL with the same solvent.

Reference solution Dissolve 4 mg of *kanamycin B sulfate CRS* in *water R* and dilute to 20 mL with the same solvent.

Apply separately to the plate 4 µL of each solution. Develop over a path of 12 cm using a 70 g/L solution of *potassium dihydrogen phosphate R*. Dry the plate in a current of warm air and spray with *ninhydrin and*

stannous chloride reagent R. Heat the plate at 110 °C for 15 min. Any spot corresponding to kanamycin B in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (4.0 per cent).

Loss on drying (*2.2.32*)

Not more than 5.0 per cent, determined on 1.00 g by drying at 60 °C at a pressure not exceeding 670 Pa for 3 h.

Sulfated ash (*2.4.14*)

Not more than 0.5 per cent, determined on 1.0 g.

Sulfate

23.0 per cent to 26.0 per cent of sulfate (SO_4), calculated with reference to the dried substance. Dissolve 0.175 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 *ethanol (96 per cent) R* when the colour of the solution begins to change and continue the titration until the violet-blue colour disappears.

1 mL of *0.1 M barium chloride* is equivalent to 9.606 mg of sulfate (SO_4).

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 10 mg per millilitre of the substance to be examined.

ASSAY

Carry out the microbiological assay of antibiotics (*2.7.2*). Use *kanamycin monosulfate CRS* as the reference substance.

STORAGE

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

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