Amikacin for injection (Amikacini ad injectionem)

Description. A white or almost white powder.

Category. Antibacterial.

Storage. Amikacin for injection should be kept in a hermetically closed container.

Labelling. The designation on the container of amikacin for injection should state that the active ingredient is in the sulfate form, and the quantity should be indicated in mg/mL in terms of the equivalent amount of amikacin.

Additional information. Strengths in the current WHO Model list of essential medicines: 100 mg, 500 mg, 1 g in vial. Strengths in the current WHO Model list of essential medicines for children: 100 mg, 500 mg, 1 g in vial.

The injection is reconstituted by dilution of Amikacin sulfate in Water for injections or by dilution of Amikacin in Water for injections with the aid of sulfuric acid.

The reconstituted injection should be used immediately after preparation.

Requirements

The powder for injection and the reconstituted injection comply with the monograph for "Parenteral preparations".

Definition. Amikacin for injection is a sterile powder containing Amikacin sulfate.

The powder is sterilized by a suitable method (see 5.8 Methods of sterilization).

Amikacin for injection contains not less than 90.0% and not more than 120.0% of the amount of amikacin ($C_{22}H_{43}N_5O_{13}$) stated on the label.

Identity tests

-Either tests A and D or tests B, C and D may be applied.

A. Carry out the test as described under *1.14.1 Chromatography*, Thin-layer chromatography, using silica gel R5 as the coating substance and a mixture of 40 volumes of methanol R, 30 volumes of ammonia (~260 g/l) TS and 25 volumes of dichloromethane R as the mobile phase. Apply separately to the plate 2 µl of each of the following solutions in water R. For solution (A) dissolve a quantity of the powder to obtain a solution containing the equivalent of 5 mg of Amikacin per mL. For solution (B) use 5 mg of amikacin RS per mL. For solution (C) use a mixture of 5 mg of amikacin RS and 5 mg of kanamycin monosulfate RS per mL. After removing the plate from the chromatographic chamber heat it at 110 °C for 5 minutes, spray it with triketohydrindene/methanol reagent TS and heat further at 110 °C for 15 minutes.

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B. The test is not valid unless the chromatogram obtained with solution C shows two clearly separated spots.

B. Dissolve a quantity of the powder to obtain a solution containing the equivalent of 10 mg of Amikacin per mL. Use 1 mL of this solution and add 1 mL of sodium hydroxide (~80 g/l) TS and mix, then add 2 mL of cobalt(II) nitrate (10 g/l) TS; a violet colour with precipitate is produced.

C. Dissolve a quantity of the powder to obtain a solution containing the equivalent of 0.02 g of Amikacin per mL. Use 3 mL of this solution and add 4 mL of anthrone TS; a bluish violet colour is produced.

D. A solution prepared in dissolving the powder to obtain the equivalent of 20 mg of Amikacin per mL yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

pH value (*1.13*). pH of the reconstituted injection, 3.5–5.5.

Kanamycin A. Carry out the test as described under *1.14.1 Chromatography*, Thin-layer chromatography, using silica gel R5 as the coating substance and a mixture of 40 volumes of methanol R, 30 volumes of ammonia (~260 g/l) TS and 25 volumes of dichloromethane R as the mobile phase. Apply separately to the plate 5 µl of each of the following solutions in water R. For solution (A) dissolve a quantity of the powder to obtain a solution containing the equivalent of 40.0 mg of Amikacin per mL. For solution (B) use 1.6 mg of kanamycin monosulfate RS per mL. For solution (C) use a mixture of 2 mg of amikacin RS and 2 mg of kanamycin monosulfate RS per mL. After removing the plate from the chromatographic chamber, heat it at 110 °C for 5 minutes, spray it with triketohydrindene/methanol reagent TS and heat further at 110 °C for 15 minutes.

In the chromatogram obtained with solution A any spot corresponding to kanamycin A is not more intense than that obtained with solution B (4.0%). The test is not valid unless the chromatogram obtained with solution C shows two clearly separated spots.

Related substances

Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography, using a stainless steel column (25 cm x 4.6 mm) packed with base-deactivated particles of silica gel the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm).

The mobile phases for gradient elution consist of a mixture of Mobile phase A and Mobile phase B, using the following conditions:

Mobile phase A: dissolve 1.8 g of sodium octanesulfonate R and 20.0 g of anhydrous sodium sulfate R with 50.0 mL of 0.2 M phosphate buffer pH 3.0 and 50.0 mL of acetonitrile R. Dilute to 1000 mL with water R.

Mobile phase B: dissolve 1.8 g of sodium octanesulfonate R and 20.0 g of anhydrous sodium sulfate R with 50.0 mL of 0.2 M phosphate buffer pH 3.0 and 100.0 mL of acetonitrile R. Dilute to 1000 mL with water R.

Prepare the 0.2 M phosphate buffer pH 3.0 by mixing 0.2 M potassium dihydrogen phosphate R with 0.2 M phosphoric acid R until pH 3.0 is reached.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0-30	100	0	Isocratic
30-75	100 to 0	0 to100	Linear gradient
75-77	0 to 100	100 to 0	Return to initial composition
77-87	100	0	Re-equilibration

Prepare the following solutions in the mobile phase A. For solution (1) dissolve a quantity of the powder to obtain a solution containing the equivalent of 5.0 mg of Amikacin per mL. For solution (2) dilute 1.0 mL of solution (1) to 100.0 mL.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 200 nm. Maintain the temperature of the column at 40 °C.

Inject alternatively 20 µl each of solutions (1) and (2). The test is not valid unless in the chromatogram obtained with solution (2) the retention time of the principal peak is between 25 and 30 minutes and the signal-to-noise ratio at least 20. When the retention time of the principal peak is not reached, adjust the content of acetonitrile R in mobile phase A.

In the chromatogram obtained with solution (1), the area of any peak, other than the principal peak, is not greater than five times the area of the principal peak obtained with solution (2) (5.0%). The sum of the areas of all peaks, other than the principal peak, is not greater than eight times the area of the principal peak obtained with solution (2) (8.0%). Disregard any peak with an area less than 0.5 times the area of the principal peak obtained with solution (2) (0.5%).

Assay

Carry out the assay as described under *1.14.1 Chromatography*, High-performance liquid chromatography, using a stainless steel column (25 cm x 4.6 mm) packed with base-deactivated particles of silica gel the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm).

As the mobile phase use a solution prepared as follows: dissolve 1.8 g of sodium octanesulfonate R and 20.0 g of anhydrous sodium sulfate R with 50.0 mL of 0.2 M phosphate buffer pH 3.0 and 50.0 mL of acetonitrile R. Dilute to 1000 mL with water R.

Prepare the 0.2 M phosphate buffer pH 3.0 by mixing 0.2 M potassium dihydrogen phosphate R with 0.2 M phosphoric acid R until pH 3.0 is reached.

Prepare the following solutions in the mobile phase. For solution (1) dissolve a quantity of the powder to obtain a solution containing the equivalent of 2.0 mg of Amikacin per mL. For solution (2) dissolve 50.0 mg of amikacin RS in 25.0 mL.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 200 nm. Maintain the temperature of the column at 40 °C.

Inject alternatively 20 µl each of solutions (1) and (2). The assay is not valid unless the symmetry factor of the principal peak is less than 1.5.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2) and calculate the content of amikacin ($C_{22}H_{43}N_5O_{13}$).

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.33 IU of endotoxin per mg of amikacin.

Impurities

The impurities limited by the requirements of this monograph include those listed in the monograph for Amikacin sulfate.