EAG/Panel/Working Party	Antibiotics
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Notes: New monograph	

Tobramycin Inhalation Powder, hard capsule

Tobramycin Preparations

Action and use
Antibacterial.

DEFINITION

Tobramycin Inhalation Powder, hard capsule consists of Tobramycin, in *microfine powder* or aerodynamically equivalent, either alone or combined with a suitable carrier. The capsule is loaded into a dry-powder inhaler to generate an aerosol.

The inhalation powder, hard capsule complies with the requirements stated under Preparations for Inhalation and with the following requirements.

PRODUCTION

The size of aerosol particles to be inhaled is controlled so that a consistent portion is deposited in the lungs. The fine-particle characteristics of Preparations for Inhalation are determined using the method described in Appendix XII C7. Preparations for inhalation: Aerodynamic Assessment of Fine Particles. The test and limits should be agreed with the competent authority.

The water content is controlled to ensure the performance of the product as justified and authorised by the competent authority.

CONTENT

Content of tobramycin, 93.0–105.0% of the stated amount

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dissolve a quantity of the capsule contents in sufficient *water* to produce a solution containing 0.4% w/v of Tobramycin.

(2) 0.4% w/v of *tobramycin BPCRS* in *water*.

(3) 0.4% w/v each of *kanamycin monosulfate BPCRS*, *neomycin sulfate EPCRS* and *tobramycin BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel*.

(b) Use the mobile phase as described below.

(c) Apply 5 µL of each solution.

(d) Develop the plate to 15 cm.

(e) After removal of the plate, dry it in a current of warm air, spray with a mixture of equal volumes of a 0.2% w/v solution of *naphthalene-1,3-diol* in *ethanol* (*96%*) and a 46% w/v solution of *sulfuric acid* and heat at 105°.

MOBILE PHASE

17 volumes of *dichloromethane*, 33 volumes of 13.5M *ammonia* and 50 volumes of *methanol*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated principal spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position, colour and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is the same as that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Uniformity of delivered dose
Complies with the requirements stated under Inhalation Powders using the following method of analysis.

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 1% w/v solution of *1-fluoro-2,4-dinitrobenzene* in *ethanol (96%)*

Solution B Dilute 20 volumes of a 1.5% w/v solution of *tris(hydroxymethyl)methylamine* to 100 mL with *dimethyl sulfoxide*.

(1) Collect single doses of the preparation being examined using the procedure described under Non-Pressurised Pre-metered Inhalation Powders, *Uniformity of delivered dose* and dissolve the collected dose in sufficient ***water*** to produce a solution containing 0.014% w/v of Tobramycin.

(2) 0.014% w/v of Tobramycin BPCRS in Water.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions under Assay may be used.

DETERMINATION OF CONTENT

Calculate the content of tobramycin $C_{18}H_{37}N_5O_9$, per delivered dose using the declared content of $C_{18}H_{37}N_5O_9$, in tobramycin BPCRS.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 1% w/v solution of *1-fluoro-2,4-dinitrobenzene* in *ethanol (96%)*

Solution B Dilute 20 volumes of a 1.5% w/v solution of *tris(hydroxymethyl)methylamine* to 100 mL with *dimethyl sulfoxide*.

(1) Dissolve a quantity of the capsule contents in sufficient *water* to produce a solution containing 0.02% w/v of Tobramycin.

(2) Dilute 1 volume of solution (1) to 200 volumes with *water*.

(3) Dilute 1 volume of solution (2) to 5 volumes with *water*.

(4) Add 1 mL of 0.5M *sulfuric acid* to 50 mg of *tobramycin BPCRS*, dissolve in *water*, add sufficient *water* to produce 50 mL and mix. Dilute 1 volume of this solution to 5 volumes with *water*.

(5) Heat a 50-mL portion of solution (4) at 100° for 8 to 9 hours, allow to cool and dilute to 50 mL with *water* (generation of impurity B).

(6) *water* (blank solution).

Derivatise the solutions using the following method. Transfer 3.75 mL of each of the 6 solutions separately into 15-mL glass tubes. To each solution add 2.5 mL *solution A* and 2.5 mL of *solution B*. Heat in a water bath at 60° for 50 minutes. Remove the tubes, allow to cool to room temperature, add 3.75 mL of *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *phenyl silica gel for chromatography* (5 µm) (Waters XBridge Phenyl is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use a column temperature of 25°.

(e) Use a detection wavelength of 365 nm.

(f) Inject 45 µL of each solution.

MOBILE PHASE

Mobile phase A 0.8 volumes of *orthophosphoric acid*, 50 volumes of *acetonitrile* and 950 volumes of *water*.

Mobile phase B 0.8 volumes of *orthophosphoric acid*, 750 volumes of *acetonitrile* and 250 volumes of *water*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-2	79	21	isocratic
2-16	79→66	21→34	linear gradient
16-27	66→30	34→70	linear gradient
27-37	30	70	isocratic
37-42	30→20	70→80	linear gradient
42-52	20→5	80→95	linear gradient
52-62	5	95	isocratic
62-67	5→79	95→21	linear gradient
67-72	79	21	re-equilibration

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When the chromatograms are recorded under the prescribed conditions the retention times relative to tobramycin (retention time about 49 minutes) are: apramycin, about 0.59; deoxystreptamine kanosamide, about 0.62; impurity C, about 0.9; impurity B, about 0.96 and impurity A, about 0.96. Identify impurities A and B using the chromatograms obtained with solutions (4) and (5). The area of the peak due to impurity B obtained in solution (4) is observed to increase in solution (5).

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (5), the *resolution* between the peaks due to impurity A and impurity B is at least 1.0.

LIMITS

Identify the peak in the chromatogram obtained with solution (1) corresponding to apramycin and multiply the area of this peak by a correction factor of 2.1.

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0 %);

not more than one such peak has an area greater than the area of the peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of all the *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%).

Disregard any peaks in the chromatogram obtained with solution (6) and any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (3) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 1% w/v solution of *1-fluoro-2,4-dinitrobenzene* in *ethanol (96%)*

Solution B Dilute 20 volumes of a 1.5% w/v solution of *tris(hydroxymethyl)methylamine* to 100 mL with *dimethyl sulfoxide*.

(1) Dissolve a quantity of the capsule contents in sufficient *water* to produce a solution containing 0.02% w/v of Tobramycin.

(2) Add 1 mL of 0.5M *sulfuric acid* to 50 mg of *tobramycin BPCRS*, dissolve in *water*, add sufficient *water* to produce 50 mL and mix. Dilute 1 volume of this solution to 5 volumes with *water*.

(3) Dilute 1 volume of a 0.024% w/v solution of *1-naphtholbenzein* in *acetonitrile* to 5 volumes with derivatised solution (2).

Derivatise solutions (1) and (2) using the following method. Transfer 4 mL of each solution separately into 50-mL volumetric flasks. To each solution add 10 mL of *solution A* and 10 ml of *solution B* and mixing. Heat in a water bath at 60° for 50 minutes. Remove the flasks, allow to stand for 10 minutes and add *acetonitrile* to about 2 mL below the meniscus. Allow to cool to room temperature and add sufficient *acetonitrile* to produce 50 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Symmetry C18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.2 mL per minute.

(d) Use an ambient column temperature.

(e) Use a detection wavelength of 365 nm.

(f) Inject 20 µL of each solution.

MOBILE PHASE

Dissolve 2.0 g of *tris(hydroxymethyl)methylamine* in 800 mL of *water*, add 20 mL of 0.5M *sulfuric acid* and sufficient *acetonitrile* to produce 2000 mL.

SYSTEM SUITABILITY

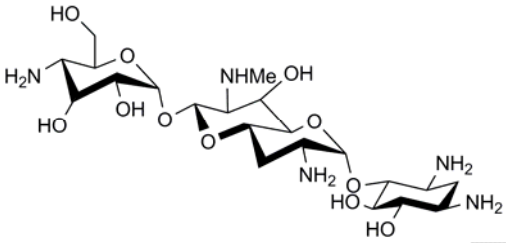
The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to 1-naphtholbenzein and tobramycin is at least 4.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{18}H_{37}N_5O_9$ in the capsules using the declared content of $C_{18}H_{37}N_5O_9$ in *tobramycin BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Tobramycin and the following.



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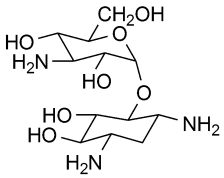
1. Apramycin,



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2. Deoxystreptamine kanosamide.