Tobramycin Sulphate

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Manufacturers, regulatory authorities, health authorities, researchers, and other stakeholders are invited to provide their feedback and comments on this draft proposal. Manufacturers are also invited to submit samples of their products to the IPC to ensure that the proposed monograph adequately controls the quality of the product(s) they manufacture. Comments and samples received after the last date will not be considered by the IPC before finalizing the monograph.

Please send any comments you may have on this draft document to lab.ipc@gov.in, with a copy to Dr. Gaurav Pratap Singh (email: gpsingh.ipc@gov.in) before the last date for comments.

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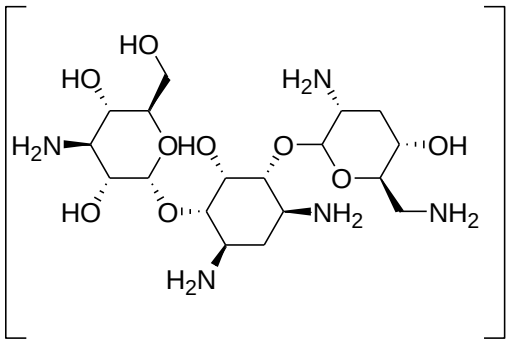
Tobramycin Sulphate



. $5H_2SO_4$

$(C_{18}H_{37}N_5O_9)_2,5H_2SO_4$ Mol. Wt. 1425.4

Tobramycin Sulphate is 4-O-(3-Amino-3-deoxy-α-D-g1ucopyranosyl)-2-deoxy-6-O-(2,6-diamino-2,3,6-trideoxy-α-D-ribo-hexopyranosyl)-L-streptaminesulphate.

Tobramycin Sulphate contains not less than 97.0 per cent and not more than 102.0 per cent of the stated amount of $(C_{18}H_{37}N_5O_9)_2,5H_2SO_4$, calculated on the anhydrous basis.

Category. Aminoglycoside antibacterial.

Description. Awhite to off-white powder.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel* (such as Merck silica gel 60).

Mobile phase. A mixture of 17 volumes of *dichloromethane*, 33 volumes of *13.5 M ammonia* and 50 volumes of *methanol.*

Test solution. Dissolve 90 mg of the substance under examination in *water* and dilute to 10.0 ml with *water*.

Reference solution (a). A 0.6 per cent w/v solution of *tobramycin IPRS* in *water*.

Reference solution (b). A solution containing 0.4 per cent w/v, each of, *kanamycin monosulphate IPRS*, *neomycin sulphate IPRS* and *tobramycin IPRS* in *water.*

Apply to the plate 5µl of each solution. Develop the plate and allow the mobile phase to rise 15 cm. Dry the plate in warm air, spray with a mixture of equal volumes of 0.2 per cent w/v solution of *naphthalene-1,3-diol* in *ethanol (95 per cent)* and 46 per cent w/v solution of *sulphuric acid* and heat at 105º for 5 to 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated principal spots.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

C. It gives the reactions of sulphates (2.3.1).

Tests

pH (2.4.24). 6.0 to 8.0, determined in a 0.4 percent w/v solution in *water*.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel* (such as Merck silica gel 60).

Potassium iodide-starch reagent. Dissolve 1.1 g of *potassium iodide* in 60 ml of *water*, boil for 15 minutes, and slowly add a suspension of 1.5 g of *soluble starch* in 10 ml of *water*. Add 25 ml of *water*, and boil for 10 minutes. Allow to cool, and dilute to 100 ml with *water*.

Mobile phase. A mixture of 30 volumes of *ethanol*, 50 volumes of 29.2 per cent w/v solution of *sodium chloride* in *water* and 20 volumes of *water.*

Test solution. Dissolve 50 mg of the substance under examination in 7 ml of *water*, adjusted to pH of 5.5 with *0.5 M sulphuric acid* and dilute to 10.0 ml with *water*.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 with *water*.

Apply to the plate 1 µl of each solution. Develop the plate and allow the mobile phase rise to 15 cm. Dry the plate in warm air, heat at 110º for 10 minutes and spray the hot plate with a 20 per cent v/v solution of *sodium hypochlorite* in *water*. Dry the plate in air and then spray with *potassium iodide-starch solution*. Any secondary spot in the

chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Heavy metals (2.3.13). 1 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 1.0 per cent, determined by moisten the charred residue with 2 ml of *nitric acid* and 5 drops of *sulphuric acid*.

Water (2.3.43). Not more than 2.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14)

Tris (hydroxymethyl) aminomethane reagent. A 1.5 per cent w/v solution of *tris (hydroxymethyl) aminomethane* in *water*. Dilute 20.0 ml of the solution to 100.0 ml with *dimethyl sulphoxide* and mix.

Test solution. Dissolve 30 mg of the substance under examination in *water* and dilute to 100.0 ml with *water*.

Reference solution (a). Dissolve 50 mg of *tobramycin IPRS* in 1 ml of *0.5 M sulphuric acid* and *water*,dilute to 50.0 ml with *water*. Dilute 10.0 ml of the solution to 50.0 ml with *water*.

Reference solution (b). A 0.024 per cent w/v solution of *p-naptholbenzein* in *acetonitrile*. Dilute 2.0 ml of the solution to 10.0 ml with reference solution (a).

Blank solution. *Water*

NOTE - Derivatise test solution, reference solution (a), reference solution (b) and blank solution. Heat all solutions at the same temperature and for the same duration of time as indicated.

Transfer 4.0 ml of each solution separately into 50-ml volumetric flasks. To each solution, add 10 ml of a 1 per cent w/v solution of *1-fluoro-2-4-dinitrobenzene* in *ethanol (95 per cent)* and 10 ml of *tris(hydroxymethyl)methylamine* reagent. Heat in a water-bath at 60° for 50 minutes. Remove the flasks and allow to stand for 10 minutes and add *acetonitrile* to about 2 ml below the meniscus. Allow to cool to room temperature and dilute to volume with *acetonitrile*.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5µm) (Such as Nucleosil 5 C18),
- mobile phase:dissolve 2.0 g of *tris(hydroxymethyl)aminomethane* in about 800 ml of *water*, add 20 ml of *0.5 M sulphuric acid*, dilute to 2000 ml with *acetonitrile*. Cool and pass through 0.2 µm filter or finer pore size.
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 365 nm,
- injection volume: 20 µl.

The relative retention time with reference to tobramycin for p-naphtholbenzein is about 0.6.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to p-naphtholbenzein and tobramycin is not less than 4.0.

Inject reference solution (a)andthe test solution.

Calculate the content of $(C_{18}H_{37}N_5O_9)_2,5H_2SO_4$.

1 mg of $C_{18}H_{37}N_5O_9$ is equivalent to 1.5245 mg of $(C_{18}H_{37}N_5O_9)_2,5H_2SO_4$.

Storage. Store protected from moisture.
