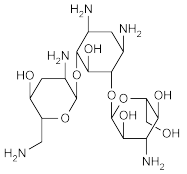
Tobramycin



$C_{18}H_{37}N_5O_9$ 467.52

D-Streptamine, *O*-3-amino-3-deoxy-α-D-glucopyranosyl-(1→6)-*O*-[2,6-diamino-2,3,6-trideoxy-α-D-*ribo*-hexopyranosyl-(1→4)]-2-deoxy-.

O-3-Amino-3-deoxy-α-D-glucopyranosyl-(1→4)- *O*-[2,6-diamino-2,3,6-trideoxy-α-D-*ribo*-hexopyranosyl-(1→6)]-2-deoxy-L-streptamine [32986-56-4].

» Tobramycin has a potency of not less than 900 µg of $C_{18}H_{37}N_5O_9$ per mg, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.

Labeling—Where it is intended for use in preparing injectable or ophthalmic dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable or ophthalmic dosage forms.

USP Reference standards ⟨11⟩**—**
USP Endotoxin RS
USP Tobramycin RS

Identification—

A: Prepare a solution of it in water containing 6 mg per mL. Apply 3 µL of this test solution, 3 µL of a Standard solution of USP Tobramycin RS containing 6 mg per mL, and 3 µL of a mixture of equal volumes of the two solutions to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Place the plate in a suitable chromatographic chamber, and develop the chromatogram in a solvent system consisting of a mixture of methanol, ammonium hydroxide, and chloroform (60:30:25) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, allow the solvent to evaporate, and heat the plate at 110° for 15 minutes. Immediately locate the spots on the plate by spraying with a 1 in 100 solution of ninhydrin in a mixture of butyl alcohol and pyridine (100:1): tobramycin appears as a pink spot, and the spots obtained from the test solution and from the mixture of test solution and Standard solution, respectively, correspond in distance from the origin to that obtained from the Standard solution.

B: The chromatogram of the *Derivatized assay preparation* obtained as directed in the *Assay* exhibits a major peak for tobramycin, the retention time of which corresponds to that exhibited in the chromatogram of the *Derivatized standard preparation* obtained as directed in the *Assay*.

pH ⟨791⟩: between 9 and 11, in a solution (1 in 10).

Water, *Method I* ⟨921⟩: not more than 8.0%.

Residue on ignition ⟨281⟩: not more than 1.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.

Heavy metals, *Method II* ⟨231⟩: 0.003%.

Chromatographic purity—

Diluted sodium hypochlorite solution—Dilute 20 mL of sodium hypochlorite solution with water to obtain 100 mL of solution.

Starch-potassium iodide 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, dilute with water to 100 mL, and mix.

Procedure—Transfer 50 mg of Tobramycin to a 10-mL volumetric flask, add 7 mL of water to dissolve it, and adjust with 1 N sulfuric acid to a pH of 5.5 ± 0.4. Dilute with water to volume, and mix to obtain the test solution. Prepare a standard solution by diluting the test solution quantitatively with water to obtain a solution containing 0.05 mg per mL. Separately apply 1 µL of these solutions to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel, and allow to dry. Develop the chromatogram in a saturated chromatographic chamber containing a mixture of sodium chloride solution (29.2 in 100), alcohol, and water (50:30:20) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chromatographic chamber, evaporate the solvent in a current of hot air, then heat it at 110° for 10 minutes. Lightly spray the hot plate with *Diluted sodium hypochlorite solution.* Dry the plate in a current of cold air until a sprayed area of the plate below the origin gives at most a faint blue color with a drop of *Starch-potassium iodide reagent*. Then spray the plate with *Starch-potassium iodide reagent:* bluish-purple spots are immediately visible. Other than the principal tobramycin spot, no spot observed in the chromatogram obtained from the test solution is more intense than the principal spot obtained from the standard solution (1.0%).

Other requirements—Where the label states that Tobramycin is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Tobramycin for Injection.* Where the label states that Tobramycin must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Tobramycin for Injection.* Where it is intended for use in preparing ophthalmic dosage forms, it is exempt from the requirements for *Bacterial endotoxins*.

Assay—

Mobile phase—Dissolve 2.0 g of tris(hydroxymethyl)aminomethane in about 800 mL of water. To this solution add 20 mL of 1 N sulfuric acid, dilute with acetonitrile to obtain 2000 mL of solution, and mix. Allow to cool, and pass through a filter of 0.2-µm or finer porosity. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

2,4-Dinitrofluorobenzene reagent—Prepare a solution of 2,4-dinitrofluorobenzene in alcohol containing 10 mg per mL. This solution may be used for 5 days if refrigerated when not in use.

Tris(*hydroxymethyl*)*aminomethane reagent*—Prepare a stock solution of tris(hydroxymethyl)aminomethane in water containing 15 mg per mL. This stock solution may be used for 1 month if refrigerated when not in use. Transfer 40 mL of this stock solution to a 200-mL volumetric flask, add dimethyl sulfoxide with mixing, dilute with dimethyl sulfoxide to volume, and mix. Use this reagent within 4 hours. [NOTE—If kept immersed in an ice-water bath below 10°, the reagent may be used for up to 8 hours.]

Standard preparation—Transfer about 55 mg of USP Tobramycin RS, accurately weighed, to a 50-mL volumetric flask, add 1 mL of 1 N sulfuric acid and enough water to dissolve it, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a second 50-mL volumetric flask, dilute with water to volume, and mix. This solution contains about 0.22 mg of USP Tobramycin RS per mL.

Assay preparation—Transfer about 55 mg of Tobramycin, accurately weighed, to a 50-mL volumetric flask, add 1 mL of 1 N sulfuric acid and enough water to dissolve it, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a second 50-mL volumetric flask, dilute with water to volume, and mix.

Derivatization procedure—[NOTE—Heat all solutions at the same temperature and for the same duration of time as indicated. Move all flasks to and from the 60° constant temperature bath at the same time.] To separate 50-mL volumetric flasks transfer 4.0 mL of the *Standard preparation*, 4.0 mL of the *Assay preparation*, and 4.0 mL of water. To each flask add 10 mL of *2,4-Dinitrofluorobenzene reagent* and 10 mL of *Tris*(*hydroxymethyl*)*aminomethane reagent*, shake, and insert the stopper.

Place the flasks in a constant temperature bath at 60 ± 2°, and heat for 50 ± 5 minutes. Remove the flasks from the bath, and allow to stand for 10 minutes. Add acetonitrile to about 2 mL below the 50-mL mark, allow to cool to room temperature, then dilute with acetonitrile to volume, and mix. The solutions thus obtained are the *Derivatized standard preparation*, the *Derivatized assay preparation*, and the *Blank preparation*, respectively.

Resolution solution—Prepare a fresh solution of *p*-naphtholbenzein in acetonitrile containing about 0.24 mg per mL. Transfer 2 mL of this solution to a 10-mL volumetric flask, dilute with *Derivatized standard preparation* to volume, and use promptly.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 365-nm detector and a 3.9-mm × 30-cm column containing packing L1. The flow rate is about 1.2 mL per minute. Chromatograph the *Blank preparation*, and record the responses as directed for *Procedure*. Identify the solvent and reagent peaks. Chromatograph the *Resolution solution*, and record the responses as directed for *Procedure:* the relative retention times are about 0.6 for *p*-naphtholbenzein and 1.0 for tobramycin, and the resolution, *R,* between the two peaks is not less than 4.0. Chromatograph the *Derivatized standard preparation*, and record the responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Derivatized standard preparation* and the *Derivatized assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in µg, of $C_{18}H_{37}N_5O_9$ in each mg of the Tobramycin taken by the formula:

$$250(CE / W)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Tobramycin RS in the *Standard preparation; E* is the tobramycin equivalent, in µg per mg, of USP Tobramycin RS; *W* is the weight, in mg, of the portion of Tobramycin taken; and r_U and r_S are the tobramycin peak area responses obtained from the *Derivatized assay preparation* and the *Derivatized standard preparation*, respectively.

Tobramycin Injection

» Tobramycin Injection is a sterile solution of Tobramycin Sulfate in Water for Injection, or of Tobramycin in Water for Injection prepared with the aid of Sulfuric Acid. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$).

Packaging and storage—Preserve in single-dose or multiple-dose glass or plastic containers. Glass containers are preferably of Type I glass.

USP Reference standards ⟨11⟩**—**
USP Endotoxin RS
USP Tobramycin RS

Identification—

A: Dilute the Injection with water to obtain a solution containing 6 mg of tobramycin per mL, and proceed as directed for *Identification* test *A* under *Tobramycin*, beginning with "Apply 3 µL of this test solution".

B: The retention time of the major peak for tobramycin in the chromatogram of the *Derivatized assay preparation* corresponds to that in the chromatogram of the *Derivatized standard preparation*, as obtained in the *Assay*.

Bacterial endotoxins ⟨85⟩**—**It contains not more than 2.00 USP Endotoxin Units per mg of tobramycin.

Sterility ⟨71⟩**—**It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

pH ⟨791⟩: between 3.0 and 6.5.

Particulate matter ⟨788⟩: meets the requirements for small-volume injections.

Other requirements—It meets the requirements under *Injections* ⟨1⟩.

Assay—

Mobile phase, 2,4-Dinitrofluorobenzene reagent, Tris(hydroxymethyl)aminomethane reagent, Standard preparation, Derivatization procedure, Resolution solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Tobramycin.*

Assay preparation—Dilute an accurately measured volume of Injection quantitatively, and stepwise if necessary, with water to obtain a solution containing the equivalent of about 0.2 mg of tobramycin per mL.

Procedure—Proceed as directed in the *Assay* under *Tobramycin*. Calculate the quantity, in mg, of tobramycin ($C_{18}H_{37}N_5O_9$) in each mL of the Injection taken by the formula:

$$(L / D)(CE / 1000)(r_U / r_S)$$

in which *L* is the labeled quantity, in mg per mL, of tobramycin ($C_{18}H_{37}N_5O_9$) in the Injection; *D* is the concentration, in mg per mL, of tobramycin in the *Assay preparation*, on the basis of the labeled quantity, the volume taken, and the extent of dilution; and the other terms are as defined therein.

Tobramycin for Injection

» Tobramycin for Injection contains an amount of Tobramycin Sulfate equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$).

Packaging and storage—Preserve in *Containers for Sterile Solids* as described under *Injections* ⟨1⟩.

USP Reference standards ⟨11⟩**—**
USP Endotoxin RS
USP Tobramycin RS

Constituted solution—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* ⟨1⟩.

Identification—

A: It responds to the *Identification* tests under *Tobramycin*.

B: It responds to the tests for *Sulfate* ⟨191⟩.

Bacterial endotoxins ⟨85⟩**—**It contains not more than 2.00 USP Endotoxin Units per mg of tobramycin.

Sterility ⟨71⟩**—**It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, 6 g being used if it is not packaged for dispensing.

pH ⟨791⟩: between 6.0 and 8.0, in a solution containing 40 mg per mL (or, where packaged for dispensing, in the solution constituted as directed in the labeling).

Water, *Method I* ⟨921⟩: not more than 2.0%.

Particulate matter ⟨788⟩: meets the requirements for small-volume injections.

Other requirements—It meets the requirements for *Residue on ignition* and *Heavy metals* under *Tobramycin.* It meets also the requirements for *Uniformity of Dosage Units* ⟨905⟩ and *Labeling* under *Injections* ⟨1⟩.