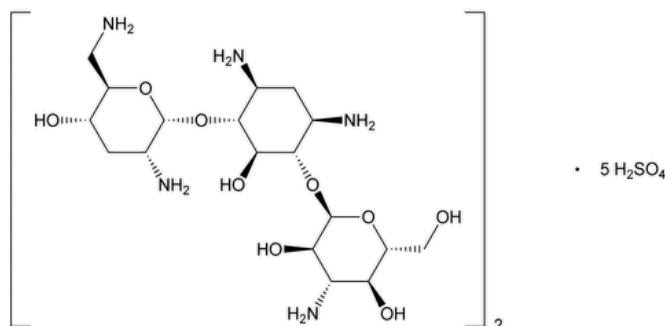


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Tobramycin Sulfate

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$(C_{18}H_{37}N_5O_9)_2 \cdot 5H_2SO_4$ 1425.42

D-Streptomine, O-3-amino-3-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 6)-O-[2,6-diamino-2,3,6-trideoxy- α -D-ribo- hexopyranosyl-(1 $\rightarrow$ 4)]-2-deoxy-, sulfate (2:5) (salt);

O-3-Amino-3-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-[2,6-diamino-2,3,6-trideoxy- α -D-ribo-hexopyranosyl-(1 $\rightarrow$ 6)]-2-deoxy-L-streptomine, sulfate (2:5) (salt) CAS RN[®]: 79645-27-5.

DEFINITION

Tobramycin Sulfate has a potency of NLT 634 μ g/mg and NMT 739 μ g/mg of tobramycin ($C_{18}H_{37}N_5O_9$).

IDENTIFICATION

• A. THIN-LAYER CHROMATOGRAPHY

Diluent: [Butyl alcohol](#) and [pyridine](#) (100:1)

Standard solution: 6 mg/mL of [USP Tobramycin RS](#) in [water](#)

Sample solution: 6 mg/mL of Tobramycin in [water](#)

Solution A: *Standard solution and Sample solution* (1:1)

Chromatographic system

(See [Chromatography \(621\)](#), [Thin-Layer Chromatography](#).)

Adsorbent: 0.25-mm layer of [chromatographic silica gel mixture](#)

Application volume: 3 μ L

Developing solvent system: [Methanol](#), [chloroform](#), and [ammonium hydroxide](#) (60:25:30)

Spray reagent: 10 mg/mL of [ninhydrin](#) in *Diluent*

Analysis

Samples: *Standard solution, Sample solution, and Solution A*

Apply the *Standard solution*, the *Sample solution*, and *Solution A* to the plate. Place the plate in a suitable chromatographic chamber, and develop the chromatogram in the *Developing solvent system* 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 min. Immediately locate the spots on the plate by spraying it with *Spray reagent*.

Acceptance criteria: Tobramycin appears as a pink spot, and the R_f values of the spots of the *Sample solution* and of *Solution A*, respectively, correspond to those of the *Standard solution*.

• **B.** The retention time of the major peak of the *Derivatized sample solution* corresponds to that of the *Derivatized standard solution* obtained as directed in the Assay.

• **C.** [IDENTIFICATION TESTS—GENERAL, Sulfate\(191\)](#): Meets the requirements

ASSAY**• PROCEDURE**

Mobile phase: Dissolve 2.0 g of [tris\(hydroxymethyl\)aminomethane](#) in 800 mL of [water](#). Add 20 mL of [1 N sulfuric acid](#), and dilute with [acetonitrile](#) to obtain 2000 mL of solution. Cool, and pass through a filter of 0.2-μm or finer pore size.

Solution A: 10 mg/mL of [2,4-dinitrofluorobenzene](#) in [alcohol](#). This solution may be used for 5 days if refrigerated when not in use.

Solution B: 15 mg/mL of [tris\(hydroxymethyl\)aminomethane](#) in [water](#). This solution may be used for 1 month if refrigerated when not in use.

Solution C: 3 mg/mL of [tris\(hydroxymethyl\)aminomethane](#) prepared as follows. Transfer 40 mL of *Solution B* to a 200-mL volumetric flask.

Add [dimethyl sulfoxide](#) while mixing, and dilute with [dimethyl sulfoxide](#) to volume. Use this reagent within 4 h. If kept immersed in an ice-water bath below 10°, the reagent may be used for up to 8 h.

Standard stock solution: 1.1 mg of [USP Tobramycin RS](#) prepared as follows. Transfer 55 mg of [USP Tobramycin RS](#) into a 50-mL volumetric flask. Add 1 mL of [1 N sulfuric acid](#) and enough [water](#) to dissolve it, and dilute with [water](#) to volume.

Standard solution: 0.22 mg/mL of [USP Tobramycin RS](#) from *Standard stock solution* in [water](#)

Sample solution: Nominally 0.2 mg/mL of tobramycin from Tobramycin Sulfate in [water](#)

Derivatized standard solution, Derivatized sample solution, and Blank solution: Proceed as follows. 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 solution*, 4.0 mL of the *Sample solution*, and 4.0 mL of [water](#). To each flask add 10 mL of *Solution A* and 10 mL of *Solution C*, shake, and insert the stopper. Place the flasks in a constant temperature bath at 60 ± 2°, and heat for 50 ± 5 min. Remove the flasks from the bath, and allow to stand for 10 min. Add [acetonitrile](#) to about 2 mL below the 50-mL mark, allow to cool to room temperature, then dilute with [acetonitrile](#) to volume. The solutions thus obtained are the *Derivatized standard solution*, the *Derivatized sample solution*, and the *Blank solution*, respectively.

System suitability stock solution: 0.24 mg/mL of *p*-naphtholbenzein in [acetonitrile](#). Prepare freshly.

System suitability solution: Transfer 2 mL of the *System suitability stock solution* to a 10-mL volumetric flask, dilute with *Derivatized standard solution* to volume, and use promptly.

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: LC

Detector: UV 365 nm

Column: 3.9-mm × 30-cm; packing [L1](#)

Flow rate: 1.2 mL/min

Injection volume: 20 μL

System suitability

Samples: *Derivatized standard solution* and *System suitability solution*

[NOTE—The relative retention times for *p*-naphtholbenzein and tobramycin are about 0.6 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4.0 between *p*-naphtholbenzein and tobramycin, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Derivatized standard solution*

Analysis

Samples: *Derivatized standard solution*, *Derivatized sample solution*, and *Blank solution*

Use the *Blank solution* to identify the solvent and reagent peaks.

Calculate the quantity, in μg/mg, of tobramycin (C₁₈H₃₇N₅O₉) in the portion of Tobramycin Sulfate taken:

$$\text{Result} = (r_u / r_s) \times (C_s / C_u) \times P$$

r_u = peak area of tobramycin from the *Derivatized sample solution*

r_s = peak area of tobramycin from the *Derivatized standard solution*

C_s = concentration of [USP Tobramycin RS](#) in the *Standard solution* (mg/mL)

C_u = concentration of Tobramycin Sulfate in the *Sample solution* (mg/mL)

P = potency of tobramycin in [USP Tobramycin RS](#) (μg/mg)

Acceptance criteria: 634–739 μg/mg

IMPURITIES

• RESIDUE ON IGNITION (281).