

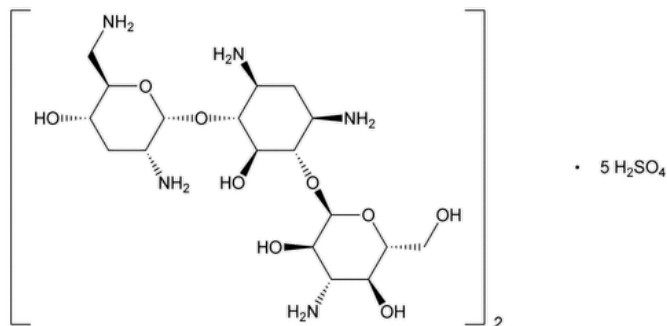
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Tobramycin Sulfate

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

D-Streptamine, 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-streptamine, 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 \(281\)](#).

Analysis: Moisten the charred residue with 2 mL of [nitric acid](#) and 5 drops of [sulfuric acid](#).

Acceptance criteria: NMT 1.0%

• **ORGANIC IMPURITIES**

Solution A: Dilute 20 mL of [sodium hypochlorite solution](#) with [water](#) to 100 mL.

Solution B: Dissolve 1.1 g of [potassium iodide](#) in 60 mL of [water](#), boil for 15 min, 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 min. Allow to cool, and dilute with [water](#) to 100 mL.

Solution C: 29.2 g of [sodium chloride](#) in 100 mL of [water](#)

Sample solution: Transfer 50 mg of Tobramycin Sulfate 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.

Standard solution: 0.05 mg/mL of tobramycin from *Sample solution* in [water](#)

Chromatographic system

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

Mode: TLC

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

Application volume: 1 μ L

Developing solvent system: [Alcohol](#), *Solution C*, and [water](#) (30:50:20)

Analysis

Samples: *Sample solution* and *Standard solution*

Apply the *Sample solution* and the *Standard solution* to the plate. Develop the chromatogram in a saturated chromatographic chamber containing the *Developing solvent system* 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 the plate at 110° for 10 min. Lightly spray the hot plate with *Solution A*. 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 *Solution B*. Then spray the plate with *Solution B*.

Acceptance criteria: Bluish-purple spots are immediately visible. Other than the principal tobramycin spot, no spot observed in the *Sample solution* is more intense than the principal spot from the *Standard solution* (1.0%).

SPECIFIC TESTS

• [pH \(791\)](#)

Sample solution: 40 mg/mL

Acceptance criteria: 6.0–8.0

Change to read:

• [WATER DETERMINATION \(921\)](#), *Method 1*: ▲NMT 7.0%▲ (RB 9-Nov-2022)

• [STERILITY TESTS \(71\)](#): Where the label states that Tobramycin Sulfate is sterile, it meets the requirements in [Test for Sterility of the Product to Be Examined, Membrane Filtration](#).

• [BACTERIAL ENDOTOXINS TEST \(85\)](#): Where the label states that Tobramycin Sulfate is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 2.00 USP Endotoxin Units/mg of tobramycin.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** Where Tobramycin Sulfate is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

• [USP REFERENCE STANDARDS \(11\)](#)

[USP Tobramycin RS](#)

Auxiliary Information - Please [check for your question in the FAQs](#) before contacting USP.

| Topic/Question | Contact | Expert Committee |
|----------------------------|---|---------------------------|
| TOBRAMYCIN SULFATE | Documentary Standards Support | SM12020 Small Molecules 1 |
| REFERENCE STANDARD SUPPORT | RS Technical Services RSTECH@usp.org | SM12020 Small Molecules 1 |

Chromatographic Database Information: [Chromatographic Database](#)

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