Tobramycin Sulfate

Type of Posting	Notice of Intent to Revise
Posting Date	28-Oct-2022
Targeted Official Date	To Be Determined, Revision Bulletin
Expert Committee	Small Molecules 1

In accordance with the Rules and Procedures of the Council of Experts and the Pending Monograph Guideline, this is to provide notice that the Small Molecules 1 Expert Committee intends to revise the Tobramycin Sulfate monograph.

The purpose of this revision is to widen the *Acceptance criteria* for the *Water Determination* test from NMT 2.0% to NMT 7.0%.

The proposed revision is contingent on FDA approval of a product that meets the proposed monograph specifications. The proposed revision will be published as a Revision Bulletin and an official date will be assigned to coincide as closely as possible with the FDA approval of the associated product.

See below for additional information about the proposed text.[1]

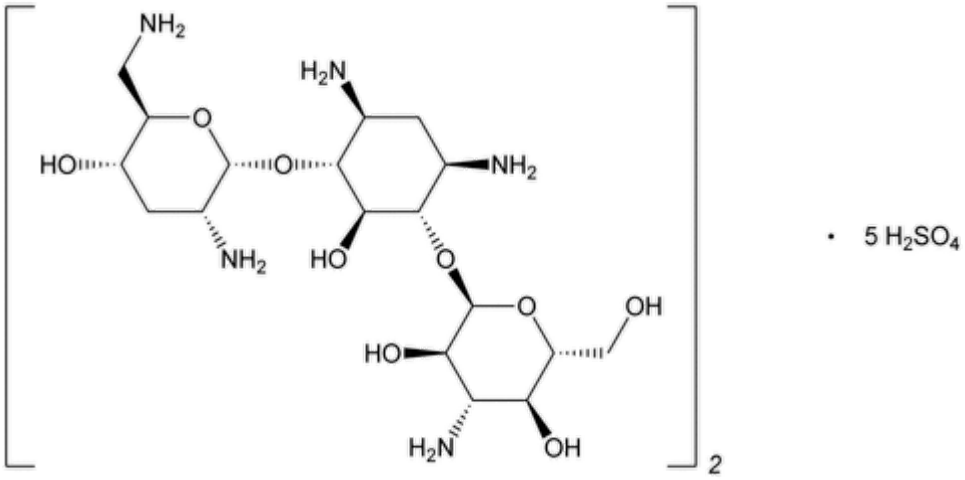
Should you have any questions, please contact Claire Chisolm, Senior Scientist II (301-230-3215 or cnc@usp.org).

[1] This text is not the official version of a *USP–NF* monograph and may not reflect the full and accurate contents of the currently official monograph. Please refer to the current edition of the *USP–NF* for official text.

USP provides this text to indicate changes that we anticipate will be made official once the product subject to this proposed revision under the Pending Monograph Program receives FDA approval. Once FDA approval is granted for the associated revision request, a Revision Bulletin will be posted that will include the changes indicated herein, as well as any changes indicated in the product's final approval, combined with the text of the monograph as effective on the date of approval. Any revisions made to a monograph under the Pending Monograph Program that are posted without prior publication for comment in the *Pharmacopeial Forum* must also meet the requirements outlined in the USP Guideline on Use of Accelerated Processes for Revisions to the *USP–NF*.

Notice of Intent to Revise
Official: To Be Determined

Tobramycin Sulfate



Click image to enlarge

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

D-Streptamine, *O*-3-amino-3-deoxy-α-D-glucopyranosyl-(1→6)-*O*-[2,6-diamino-2,3,6-tridoexy-α-D-*ribo*-hexopyranosyl-(1→4)]-2-deoxy-, sulfate (2:5) (salt);
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, sulfate (2:5) (salt) CAS RN®: 79645-27-5.

DEFINITION

Tobramycin Sulfate has a potency of NLT 634 µg/mg and NMT 739 µg/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 µL

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 ($C_{18}H_{37}N_5O_9$) 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 µL

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,** *Method I*⟨921⟩**:** ▲NMT 7.0%▲ (TBD)
- **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

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Not Applicable

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