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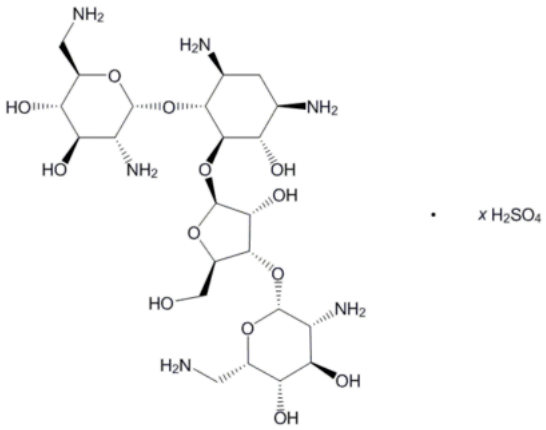
Neomycin Sulfate

Change to read:



▲$C_{23}H_{46}N_6O_{13}$ (free base) 614.65 (free base)

Neomycin B

2-Deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-D-streptamine (free base) CAS RN®: 119-04-0.▲ (USP 1-Aug-2023)

Neomycin sulfate CAS RN®: 1405-10-3; UNII: 057Y626693.

Change to read:

DEFINITION

Neomycin Sulfate is the sulfate salt of a kind of neomycin, an antibacterial substance produced by the growth of *Streptomyces fradiae* Waksman (Fam. Streptomycetaceae), or a mixture of two or more such salts▲, the main component being neomycin B sulfate.▲ (USP 1-Aug-2023) It has a potency equivalent to NLT 600 µg of neomycin/mg, calculated on the dried basis.

IDENTIFICATION

• **A.** It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* ⟨201BNP⟩.

Change to read:

• **B.**

▲**Mobile phase, System suitability solution, Sensitivity solution, Standard solution, Chromatographic system,** and **System suitability:** Proceed as directed in the test for *Composition of Neomycin Sulfate*.

Sample identification solution: 0.025 mg/mL of Neomycin Sulfate in *Mobile phase*

Acceptance criteria: The retention time of the neomycin B peak of the *Sample identification solution* corresponds to that of the *Standard solution*, as obtained in the test for *Composition of Neomycin Sulfate*.▲ (USP 1-Aug-2023)

• **C.** *Identification Tests—General* ⟨191⟩, *Chemical Identification Tests, Sulfate*

Sample solution: 50 mg/mL

Acceptance criteria: Meets the requirements

ASSAY

• **Procedure**

(See *Antibiotics—Microbial Assays* ⟨81⟩.)

Analysis: Proceed as directed in the chapter.

Acceptance criteria: NLT 600 µg of neomycin/mg on the dried basis

Add the following:

▲**IMPURITIES**

• **Organic Impurities**

Mobile phase, System suitability solution, Sensitivity solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the test for *Composition of Neomycin Sulfate*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Neomycin Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response of neomycin B from the *Standard solution*

C_S = concentration of USP Neomycin B RS in the *Standard solution* (mg/mL)

C_U = concentration of neomycin sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. The reporting threshold is 1.0%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Neomycin B-LP[a]	1.1	—
Any individual unspecified impurity	—	5.0
Total unspecified impurities	—	15.0

[a] 3-*N*-Acetyl-2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-D-streptamine.

▲ (USP 1-Aug-2023)

SPECIFIC TESTS

Add the following:

▲• **Composition of Neomycin Sulfate**

Mobile phase: 20 mL of trifluoroacetic acid, 6 mL of 50% sodium hydroxide TS, and 500 mL of water, diluted with water to 1000 mL

System suitability solution: 0.1 mg/mL of USP Neomycin Sulfate System Suitability Mixture RS (contains neomycin B and neomycin C) in *Mobile phase*

Sensitivity solution: 0.005 mg/mL of USP Neomycin B RS in *Mobile phase*

Standard solution: 0.025 mg/mL of USP Neomycin B RS and 0.01 mg/mL of USP Neomycin A RS in *Mobile phase*

Sample solution: 0.5 mg/mL of Neomycin Sulfate in *Mobile phase*

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: Pulsed amperometric electrochemical

Electrodes

Indicator: Gold

Reference: Silver–silver chloride

Auxiliary: Stainless steel (cell body)

Waveform: See *Table 2*.

Table 2

Time (s)	Potential (V)	Integration
0.00	+0.00	—
0.10	+0.00	Begin
0.40	+0.00	End
0.41	+0.80	—

Time (s)	Potential (V)	Integration
0.55	+0.80	—
0.56	−0.60	—
1.00	−0.60	—

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 0.7 mL/min

Post-column reagent: 20 g/L of sodium hydroxide (carbonate-free), prepared from 50% sodium hydroxide TS in water, carbon dioxide-free.

This solution is added pulselessly to the column effluent using a 375-µL polymeric mixing coil.[1]

Flow rate of post-column reagent: 0.5 mL/min

[NOTE—Adjust accordingly to meet the *System suitability* requirements.]

Injection volume: 10 µL

Run time: NLT 1.5 times the retention time of neomycin B

System suitability

Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between the neomycin C and neomycin B peaks, *System suitability solution*

Relative standard deviation: NMT 5.0% each for neomycin A and neomycin B, *Standard solution*

Signal-to-noise ratio: NLT 10 for the neomycin B peak, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of neomycin A in the portion of Neomycin Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of neomycin A from the *Sample solution*

r_S = peak response of neomycin A from the *Standard solution*

C_S = concentration of USP Neomycin A RS in the *Standard solution* (mg/mL)

C_U = concentration of neomycin sulfate in the *Sample solution* (mg/mL)

Calculate the percentage of neomycin C in the portion of Neomycin Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of neomycin C from the *Sample solution*

r_S = peak response of neomycin B from the *Standard solution*

C_S = concentration of USP Neomycin B RS in the *Standard solution* (mg/mL)

C_U = concentration of neomycin sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Neomycin A	0.7	2.0
Neomycin C[a]	0.9	3.0–15.0
Neomycin B	1.0	—

[a] 2-Deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-β-D-ribofuranosyl]-D-streptamine.

▲ (USP 1-Aug-2023)

• **Loss on Drying ⟨731⟩**

Sample: About 100 mg

Analysis: Dry the *Sample* under vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h.

Acceptance criteria: NMT 8.0%

• **pH ⟨791⟩**

Sample solution: 33 mg/mL of neomycin

Acceptance criteria: 5.0–7.5

Change to read:

• **▲Sterility Tests ⟨71⟩:▲** (USP 1-Aug-2023) Where the label states that Neomycin Sulfate is sterile, it meets the requirements under the relevant ▲▲ (USP 1-Aug-2023) dosage form. ▲▲ (USP 1-Aug-2023)

Change to read:

• **▲Bacterial Endotoxins Test ⟨85⟩:▲** (USP 1-Aug-2023) Where the label states that Neomycin Sulfate is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms, the level of bacterial endotoxins is such that the requirement under the relevant dosage form monograph(s) in which neomycin is used can be met. ▲▲ (USP 1-Aug-2023)

ADDITIONAL REQUIREMENTS

Change to read:

• **Packaging and Storage:** Preserve in tight, light-resistant containers. ▲Store at controlled room temperature.▲ (USP 1-Aug-2023)

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

Change to read:

• **USP Reference Standards ⟨11⟩**

USP Neomycin Sulfate RS

▲ USP Neomycin A RS

2-Deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-D-streptamine (free base).

$C_{12}H_{26}N_4O_6$ (free base) 322.36 (free base)

USP Neomycin B RS

USP Neomycin Sulfate System Suitability Mixture RS

A mixture of neomycin B sulfate and neomycin C sulfate.▲ (USP 1-Aug-2023)

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[1] A suitable mixing coil is the knitted reaction coil, part #043700, available from Dionex Corporation (www.thermofisher.com).

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
NEOMYCIN SULFATE	Julie Zhang Associate Science & Standards Liaison	BIO42020 Biologics Monographs 4 - Antibiotics
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	BIO42020 Biologics Monographs 4 - Antibiotics

Chromatographic Database Information: Chromatographic Database

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