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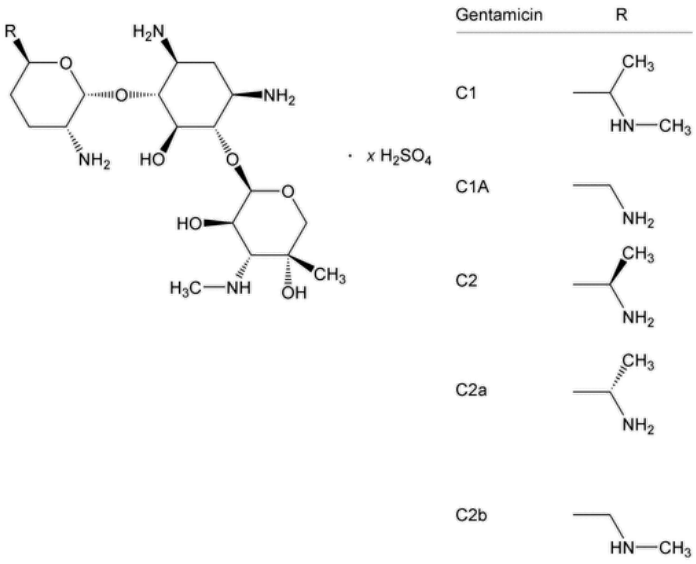
Gentamicin Sulfate



Gentamicin sulfate (salt);
Gentamycin sulfate
CAS RN®: 1405-41-0; UNII: 8X7386QRLV.

DEFINITION

Gentamicin Sulfate is the sulfate salt, or a mixture of such salts, of the antibiotic substances produced by the growth of *Micromonospora purpurea*. It has a potency equivalent to NLT 590 µg/mg of gentamicin, calculated on the dried basis.

IDENTIFICATION

Change to read:

• **A.** ▲Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

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

ASSAY

• **Procedure**

Analysis: Proceed as directed for Gentamicin under *Antibiotics—Microbial Assays* ⟨81⟩.

Acceptance criteria: NLT 590 µg/mg of gentamicin, calculated on the dried basis

IMPURITIES

• **Residue on Ignition** ⟨281⟩**:** NMT 1.0%

• **Limit of Methanol** (if present)

Internal standard solution: 0.50% v/v of *n*-propyl alcohol

Standard solution: 0.25% v/v each of methanol and *n*-propyl alcohol

Control solution: 250 mg/mL of Gentamicin Sulfate

Sample solution: 250 mg/mL of Gentamicin Sulfate in a mixture of *Internal standard solution* and water (1:1)

Chromatographic system

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

Mode: GC

Detector: Flame ionization

Column: 4-mm × 1.5-m; packed with support S3

Temperature

Column: Constant temperature between 120° and 140°

Injector: Constant temperature at least 50° higher than the column temperature

Detector: Constant temperature at least 50° higher than the column temperature

Carrier gas: Nitrogen

Flow rate: Constant flow rate between 30 and 40 mL/min

Injection type: Syringe with a polytef-tipped plunger

Injection volume: 2 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.0 between *n*-propyl alcohol and methanol

Analysis

Samples: *Standard solution*, *Sample solution*, and *Control solution*

Chromatograph the *Control solution*, and examine the chromatogram: if any peak is observed at a retention time corresponding to that of *n*-propyl alcohol, use the response of that peak to correct the *n*-propyl alcohol peak response of the *Sample solution*.

Calculate the percentage of methanol in the Gentamicin Sulfate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times D \times F$$

R_U = peak area response of methanol to *n*-propyl alcohol (corrected for any peak at the locus of the *n*-propyl alcohol peak in the *Control solution*) from the *Sample solution*

R_S = peak area response of methanol to *n*-propyl alcohol from the *Standard solution*

C_S = percentage of methanol in the *Standard solution*

C_U = concentration of Gentamicin Sulfate in the *Sample solution* (mg/mL)

D = density of methanol (g/mL)

F = conversion factor, 1000 mg/g

Acceptance criteria: NMT 1.0%

SPECIFIC TESTS

• **Content of Gentamicins**

Mobile phase: To 900 mL of carbonate-free water, add 7 mL of trifluoroacetic acid, 250 µL of pentafluoropropanoic acid, and 4 mL of 12.5 M sodium hydroxide (carbonate-free). Allow to equilibrate, and adjust with 0.5 M sodium hydroxide (carbonate-free) to a pH of 2.6. Add 15 mL of acetonitrile, and dilute with carbonate-free water to 1 L. If necessary, adjust the volume of acetonitrile in the *Mobile phase*. A total volume of up to 50 mL can be added per L of *Mobile phase*.

System suitability solution: 100 µg/mL of USP Gentamicin Sulfate RS and 20 µg/mL of USP Sisomicin Sulfate RS in *Mobile phase*

Sample solution: 0.2 mg/mL of Gentamicin Sulfate in *Mobile phase*

Chromatographic system

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

Mode: LC

Detector: Pulsed amperometric electrochemical detector

Indicator electrode: Gold

Reference electrode: Silver–silver chloride

Auxiliary electrode: Stainless steel. [Note—If the cell body is made of stainless steel, it can be used as the auxiliary electrode.]

Waveform: See *Table 1*.

Table 1

Time (s)	Potential (V)	Integration
0.00	+0.05	—
0.10	+0.05	Begin

Time (s)	Potential (V)	Integration
0.40	+0.05	End
0.41	+0.75	—
0.55	+0.75	—
0.56	−0.15	—
1.00	−0.15	—

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

Column temperature: 35°

Flow rate: 1 mL/min

Post-column reagent: 20 g/L sodium hydroxide (carbonate-free), degassed and introduced pulselessly using a 375-µL polymeric mixing coil. [NOTE—A suitable mixing coil is the knitted reaction coil, part number 043700, available from Dionex Corporation (www.dionex.com).]

Flow rate of post-column reagent: 0.3 mL/min

Injection volume: 20 µL

Run time: 1.2 times the retention time of gentamicin C_1

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.5 between gentamicin C_2 and gentamicin C_{2b}

Analysis

Sample: *Sample solution*

Calculate the percentage of each gentamicin in the portion of Gentamicin Sulfate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area response corresponding to the particular gentamicin

r_T = sum of the area responses of the gentamicin C_{1a}, gentamicin C_2, gentamicin C_{2a}, gentamicin C_{2b}, and gentamicin C_1 peaks

Acceptance criteria: Identify peaks by the relative retention times in Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Garamine[a,b]	0.35	—
Sisomicin[a,c]	1.0	—
Gentamicin C_{1a}	1.1	10%–35%
Gentamicin C_2	1.8	25%–55%[d]
Gentamicin C_{2a}	2.3	
Gentamicin C_{2b}	2.0	25%–50%[e]
Gentamicin C_1	2.9	

[a] These compounds are listed for information only and are not to be reported in this test.

[b] 4-*O*-[3-Deoxy-4-*C*-methyl-3-(methylamino)-β-L-arabinopyranosyl]-2-deoxy-L-streptamine.

[c] *O*-3-Deoxy-4-*C*-methyl-3-(methylamino)-β-L-arabinopyranosyl-(1→4)-*O*-[2,6-diamino-2,3,4,6-tetradeoxy-α-D-glycero-hex-4-enopyranosyl-(1→6)]-2-deoxy-D-streptamine.

[d] The limit is for the sum of gentamicin C_2 and gentamicin C_{2a}.

[e] The limit is for the sum of gentamicin C_{2b} and gentamicin C_1.

• **Optical Rotation,** *Specific Rotation*⟨781S⟩
Sample solution: 10 mg/mL
Acceptance criteria: +107° to +121°

• **pH** ⟨791⟩
Sample solution: 40 mg/mL
Acceptance criteria: 3.5–5.5

• **Loss on Drying** ⟨731⟩
Analysis: Dry it in vacuum at a pressure NMT 5 mm of mercury at 110° for 3 h.
Acceptance criteria: NMT 18.0%

• **Sterility Tests** ⟨71⟩**:** Where the label states that Gentamicin Sulfate is sterile, it meets the requirements.

• **Bacterial Endotoxins Test** ⟨85⟩**:** Where the label states that Gentamicin Sulfate is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.71 USP Endotoxin Unit/mg of gentamicin.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.

• **Labeling:** Where it 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 Gentamicin Sulfate RS
USP Sisomicin Sulfate RS

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

Topic/Question	Contact	Expert Committee
GENTAMICIN SULFATE	Ying Han Associate Science & Standards Liaison	BIO42020 Biologics Monographs 4 - Antibiotics

Chromatographic Database Information: Chromatographic Database

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