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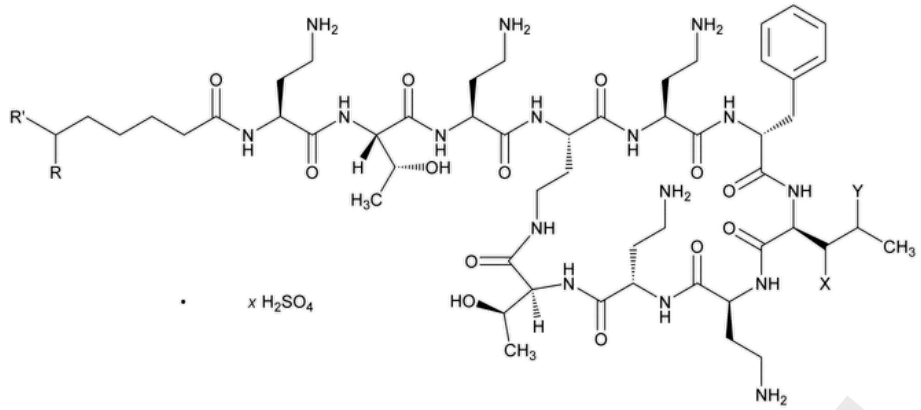
Polymyxin B Sulfate



Polymyxin	R	R'	X	Y
B1		—CH_3	—H	—CH_3
B2	—CH_3	—CH_3	—H	—CH_3
B3		—H	—H	—CH_3
B1-I		—CH_3	—CH_3	—H

$C_{56}H_{98}N_{16}O_{13}$ 1203.48

Polymyxin B1:

N-[(*S*)-4-Amino-1-{[(2*S*,3*R*)-1-{[(*S*)-4-amino-1-oxo-1-{{3*S*,6*S*,9*S*,12*S*,15*S*,18*S*,21*S*)-6,9,18-tris(2-aminoethyl)-15-benzyl-3-[(*R*)-1-hydroxyethyl]-12-isobutyl-2,5,8,11,14,17,20-heptaoxo-1,4,7,10,13,16,19-heptaazacyclotricosan-21-yl}amino)butan-2-yl]amino}-3-hydroxy-1-oxobutan-2-yl]amino}-1-oxobutan-2-yl]-6-methyloctanamide CAS RN®: 4135-11-9.

$C_{55}H_{96}N_{16}O_{13}$ 1189.45

Polymyxin B2:

N-[(*S*)-4-Amino-1-{[(2*S*,3*R*)-1-{[(*S*)-4-amino-1-oxo-1-{{3*S*,6*S*,9*S*,12*S*,15*S*,18*S*,21*S*)-6,9,18-tris(2-aminoethyl)-15-benzyl-3-[(*R*)-1-hydroxyethyl]-12-isobutyl-2,5,8,11,14,17,20-heptaoxo-1,4,7,10,13,16,19-heptaazacyclotricosan-21-yl}amino)butan-2-yl]amino}-3-hydroxy-1-oxobutan-2-yl]amino}-1-oxobutan-2-yl]-6-methylheptanamide CAS RN®: 34503-87-2.

$C_{55}H_{96}N_{16}O_{13}$ 1189.45

Polymyxin B3:

N-[(*S*)-4-Amino-1-{[(2*S*,3*R*)-1-{[(*S*)-4-amino-1-oxo-1-{{3*S*,6*S*,9*S*,12*S*,15*S*,18*S*,21*S*)-6,9,18-tris(2-aminoethyl)-15-benzyl-3-[(*R*)-1-hydroxyethyl]-12-isobutyl-2,5,8,11,14,17,20-heptaoxo-1,4,7,10,13,16,19-heptaazacyclotricosan-21-yl}amino)butan-2-yl]amino}-3-hydroxy-1-oxobutan-2-yl]amino}-1-oxobutan-2-yl]octanamide CAS RN®: 71140-58-4.

$C_{56}H_{98}N_{16}O_{13}$ 1203.48

Polymyxin B1-I:

N-[(*S*)-4-Amino-1-{[(2*S*,3*R*)-1-{[(*S*)-4-amino-1-oxo-1-{{3*S*,6*S*,9*S*,12*S*,15*S*,18*S*,21*S*)-6,9,18-tris(2-aminoethyl)-15-benzyl-3-[(*R*)-1-hydroxyethyl]-12-[(*S*)-sec-butyl]-2,5,8,11,14,17,20-heptaoxo-1,4,7,10,13,16,19-heptaazacyclotricosan-21-yl}amino)butan-2-yl]amino}-3-hydroxy-1-oxobutan-2-yl]amino}-1-oxobutan-2-yl]-6-methyloctanamide.

Polymyxin B, sulfate;

Polymyxin B sulfate CAS RN®: 1405-20-5; UNII: 19371312D4.

Polymyxin B CAS RN®: 1404-26-8; UNII: J2VZ07J96K.

DEFINITION

Polymyxin B Sulfate is the sulfate salt of a kind of polymyxin, a substance produced by the growth of *Bacillus polymyxa* (Prazmowski) Migula (Fam. Bacillaceae), or a mixture of two or more such salts. It has a potency of NLT 6000 Polymyxin B units/mg, calculated on the dried basis.

IDENTIFICATION

• **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the test for *Composition of Polymyxins*.

• **B.**

Solution A: 10 mg/mL of cupric sulfate

Sample solution: Dissolve 2 mg in 5 mL of water.

Analysis: To the *Sample solution* add 5 mL of 2.5 N sodium hydroxide, and mix. Add 5 drops of *Solution A*, shaking after the addition of each drop.

Acceptance criteria: A reddish-violet color is produced.

• **C. Identification Tests—General ⟨191⟩, *Sulfate*:** A 50-mg/mL solution of Polymyxin B Sulfate meets the requirements.

ASSAY

• **Procedure**

Analysis: Proceed with Polymyxin B Sulfate as directed for *Antibiotics—Microbial Assays* ⟨81⟩.

Acceptance criteria: NLT 6000 Polymyxin B units/mg on the dried basis

IMPURITIES

• **Residue on Ignition ⟨281⟩**

Analysis: Proceed as directed in the chapter, moistening the charred residue with 2 mL of nitric acid and 5 drops of sulfuric acid.

Acceptance criteria: NMT 5.0% where used for prescription compounding

• **Organic Impurities**

Buffer, Mobile phase, Diluent, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the test for *Composition of Polymyxins*.

Analysis

Samples: *Standard solution* (the *Standard solution* is used to determine the disregard limit) and *Sample solution*

Calculate the percentage of each impurity in the portion of Polymyxin B Sulfate taken:

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

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

r_T = sum of the responses of all peaks from the *Sample solution*

Acceptance criteria: Disregard any peak less than 0.007 times the peak response of the polymyxin B1 peak from the *Standard solution.*

Any individual impurity: NMT 3.0%

Total impurities: NMT 17.0%

SPECIFIC TESTS

• **pH ⟨791⟩**

Sample solution: 5 mg/mL

Acceptance criteria: 5.0–7.5

• **Loss on Drying ⟨731⟩**

Analysis: Dry 100 mg in a capillary-stoppered bottle under vacuum at 60° for 3 h.

Acceptance criteria: NMT 7.0%

• **Sterility Tests ⟨71⟩:** Meets the requirements where the label states that Polymyxin B Sulfate is sterile

• **Pyrogen Test ⟨151⟩**

Sample solution: Nominally 20×10^3 Polymyxin B units/mL in pyrogen-free saline TS

Analysis: Proceed as directed in the chapter using a test dose of 1.0 mL/kg.

Acceptance criteria: Meets the requirements where intended for injectable dosage forms or where the label states that Polymyxin B Sulfate must be subjected to further processing during the preparation of injectable dosage forms

• **Composition of Polymyxins**

Buffer: 4.5 g/L of sodium sulfate anhydrous. The pH is adjusted to 2.3 with dilute phosphoric acid (10% w/w) prior to final dilution.

Mobile phase: Acetonitrile and *Buffer* (20:80)

Diluent: Acetonitrile and water (20:80)

Standard solution: 0.5 mg/mL of USP Polymyxin B Sulfate RS in *Diluent*

Sample solution: 0.5 mg/mL of Polymyxin B Sulfate in *Diluent*

Chromatographic system

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

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L1. [NOTE—A 4.6-mm × 25-cm; 5-µm packing L1 column was also found to be suitable.]

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: 1.4 times the retention time of polymyxin B1

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between polymyxin B2 and polymyxin B3

Relative standard deviation: NMT 2.0% for the polymyxin B1 peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of polymyxin B1 in the portion of Polymyxin B Sulfate taken:

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

r_U = peak response of polymyxin B1 from the *Sample solution*

r_S = peak response of polymyxin B1 from the *Standard solution*

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

C_U = concentration of Polymyxin B Sulfate in the *Sample solution* corrected for loss on drying (mg/mL)

P = potency of polymyxin B1 in USP Polymyxin B Sulfate RS (mg/mg)

Calculate the percentage of polymyxin B2 in the portion of Polymyxin B Sulfate taken:

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

r_U = peak response of polymyxin B2 from the *Sample solution*

r_S = peak response of polymyxin B2 from the *Standard solution*

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

C_U = concentration of Polymyxin B Sulfate in the *Sample solution* corrected for loss on drying (mg/mL)

P = potency of polymyxin B2 in USP Polymyxin B Sulfate RS (mg/mg)

Calculate the percentage of polymyxin B3 in the portion of Polymyxin B Sulfate taken:

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

r_U = peak response of polymyxin B3 from the *Sample solution*

r_S = peak response of polymyxin B3 from the *Standard solution*

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

C_U = concentration of Polymyxin B Sulfate in the *Sample solution* corrected for loss on drying (mg/mL)

P = potency of polymyxin B3 in USP Polymyxin B Sulfate RS (mg/mg)

Calculate the percentage of polymyxin B1-I in the portion of Polymyxin B Sulfate taken:

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

r_U = peak response of polymyxin B1-I from the *Sample solution*

r_S = peak response of polymyxin B1-I from the *Standard solution*

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

C_U = concentration of Polymyxin B Sulfate in the *Sample solution* corrected for loss on drying (mg/mL)

P = potency of polymyxin B1-I in USP Polymyxin B Sulfate RS (mg/mg)

Acceptance criteria: See *Table 1*. Report the components on the dried basis.

Table 1

Name	Relative Retention Time	Acceptance Criteria, (%)
Polymyxin B2[a]	0.5	—
Polymyxin B3	0.6	NMT 6.0
Polymyxin B1-I	0.8	NMT 15.0
Polymyxin B1[a]	1.0	—
Sum of polymyxins B1, B2, B3, and B1-I	—	NLT 80.0

[a] These components are not reported individually. They are only reported in the sum of polymyxins.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers.

• **Labeling:** Where packaged for prescription compounding, the label states the number of Polymyxin B units in the container and per mg, that it is not intended for manufacturing use, that it is not sterile, and that its potency cannot be assured for longer than 60 days after opening. 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.

• **USP Reference Standards** ⟨11⟩

USP Polymyxin B Sulfate RS

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

Topic/Question	Contact	Expert Committee
POLYMYXIN B SULFATE	Ying Han 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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