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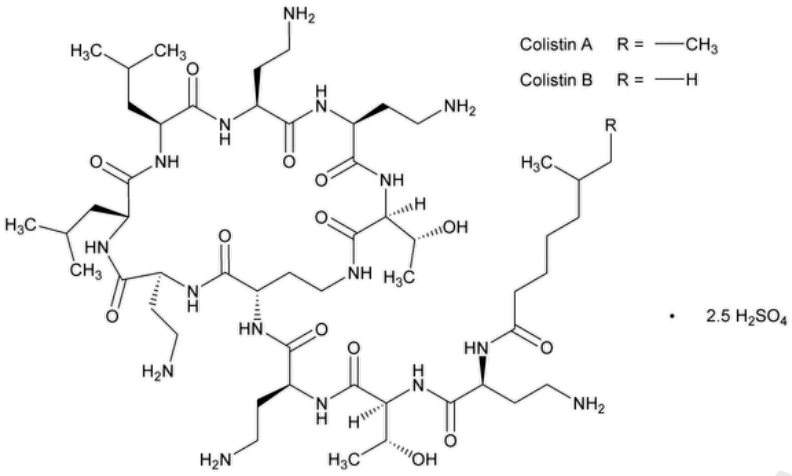
Colistin Sulfate



Colistin, sulfate;
Colistins sulfate

CAS RN®: 1264-72-8; UNII: WP15DXU577.

DEFINITION

Colistin Sulfate is the sulfate salt of an antibacterial substance produced by the growth of *Bacillus polymyxa* var. *colistinus*. It has a potency equivalent to NLT 500 µg of colistin/mg.

IDENTIFICATION

Delete the following:

▲• **A. Procedure**▲ (USP 1-Dec-2022)

Add the following:

▲• **A. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 197A or 197K▲ (USP 1-Dec-2022)

• **B. Identification Tests—General ⟨191⟩, *Chemical Identification Tests, Sulfate*:** Meets the requirements

Delete the following:

▲• **C. Liquid Chromatographic Identification Test**▲ (USP 1-Dec-2022)

Add the following:

▲• **C.** The retention times of the colistin A and colistin B peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the test for *Composition*.▲ (USP 1-Dec-2022)

ASSAY

Change to read:

• **Procedure**

Analysis: Proceed as directed for *Colistin* in *Antibiotics—Microbial Assays ⟨81⟩*.

Acceptance criteria: NLT 500 µg ▲of colistin▲ (USP 1-Dec-2022) /mg

Add the following:

▲**IMPURITIES**

• **Organic Impurities**

Solution A: 4.5 g of anhydrous sodium sulfate in 900 mL of water, adjusted with diluted phosphoric acid to a pH of 2.4 and diluted with water to 1000 mL

Mobile phase: *Solution A* and acetonitrile (78:22)

Diluent: Water and acetonitrile (80:20)

Sensitivity solution: 5 µg/mL of USP Colistin Sulfate RS in *Diluent*

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

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

Chromatographic system

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

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 3-µm packing L1

Column temperature: 50°

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Samples: *Sensitivity solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times. Use the reference chromatogram provided with the lot of USP Colistin Sulfate RS to identify the peaks.]

Suitability requirements

Peak-to-valley ratio: NLT 1.1 for the ratio of the height of the peak that immediately follows the colistin B peak to the height of the valley between the two peaks, *Standard solution*

Resolution: NLT 2.0 between polymyxin E6 and 7-L-isoleucinepolymyxin E2; NLT 3.0 between 2,3-dehydro colistin A and colistin A, *Standard solution*

Signal-to-noise ratio: NLT 10 for the colistin A peak, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each individual impurity (for peaks other than colistin A; colistin B; polymyxins E3, E4, E6, E7; 7-L-valinepolymyxin E2; 7-L-isoleucinepolymyxin E2; 7-L-norvalinepolymyxin E1; colistin III; and 2,3-dehydro colistin A) in the portion of Colistin Sulfate taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each individual impurity

r_T = sum of all the peak responses

F = relative response factor

Acceptance criteria: See *Table 1*. The reporting threshold is 0.35%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria (%)
7-L-Valinepolymyxin E2[a] and polymyxin E4[b]	0.28	1.0	—
Polymyxin E6[c]	0.39	1.0	NMT 4.5
7-L-Isoleucinepolymyxin E2[d]	0.42	1.0	NMT 2.5
Colistin B[e]	0.50	1.0	—

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria (%)
Polymyxin E3[f]	0.56	1.0	NMT 5.5
7-L-Norvalinepolymyxin E1[g]	0.59	1.0	NMT 4.5
Colistin III[h]	0.82	1.0	NMT 8.5
2,3-Dehydro colistin A[i]	0.90	3.3	NMT 1.5
Colistin A[j]	1.00	1.0	—
Polymyxin E7[k]	1.1	1.0	NMT 5.0
Sum of 7-L-valinepolymyxin E2 and polymyxin E4	—	—	NMT 3.0
Sum of colistin A, colistin B, polymyxin E3, polymyxin E4, polymyxin E6, polymyxin E7, 7-L-valinepolymyxin E2, 7-L-isoleucinepolymyxin E2, 7-L-norvalinepolymyxin E1, colistin III, and 2,3-dehydro colistin A	—	—	NLT 86.0
[N^4-Dab^5] Colistin A[l]	1.3	1.0	NMT 4.0
Any individual impurity other than [N^4-Dab^5] Colistin A	—	1.0	NMT 2.5; the total number of impurities exceeding 1.0 is NMT 4
Total impurities	—	—	NMT 11.0▲ (USP 1-Dec-2022)

[a] N^2-(6-Methyl-1-oxoheptyl-L-2,4-diaminobutanoyl-L-threonyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-D-leucyl-L-valyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-L-threonine, cyclic (10→4)-peptide.

[b] N^2-(1-Oxoheptyl)-L-2,4-diaminobutanoyl-L-threonyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-D-leucyl-L-leucyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-L-threonine, cyclic (10→4)-peptide.

[c] N^2-[(6*S*)-3-Hydroxyl-6-methyl-1-oxooctyl]-L-2,4-diaminobutanoyl-L-threonyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-D-leucyl-L-leucyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-L-threonine, cyclic (10→4)-peptide.

[d] N^2-(6-Methyl-1-oxoheptyl-L-2,4-diaminobutanoyl-L-threonyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-D-leucyl-L-isoleucyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-L-threonine, cyclic (10→4)-peptide.

[e] N^2-(6-Methyl-1-oxoheptyl)-L-2,4-diaminobutanoyl-L-threonyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-D-leucyl-L-leucyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-L-threonine, cyclic (10→4)-peptide.

[f] N^2-(1-Oxooctyl)-L-2,4-diaminobutanoyl-L-threonyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-D-leucyl-L-leucyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-L-threonine, cyclic (10→4)-peptide.

[g] N^2-[(*S*)-6-Methyl-1-oxooctyl]-L-2,4-diaminobutanoyl-L-threonyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-D-leucyl-L-norvalyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-L-threonine, cyclic (10→4)-peptide.

[h] N^2-[(*S*)-6-Methyl-1-oxooctyl]-L-2,4-diaminobutanoyl-L-threonyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-D-leucyl-L-isoleucyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-L-threonine, cyclic (10→4)-peptide.

[i] N^2-[(*S*,*E*)-6-Methyloct-2-enoyl]-L-2,4-diaminobutanoyl-L-threonyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-D-leucyl-L-leucyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-L-threonine, cyclic (10→4)-peptide.

[j] N^2-[(*S*)-6-Methyl-1-oxooctyl]-L-2,4-diaminobutanoyl-L-threonyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-D-leucyl-L-leucyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-L-threonine, cyclic (10→4)-peptide.

[k] N^2-(7-Methyl-1-oxooctyl)-L-2,4-diaminobutanoyl-L-threonyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-D-leucyl-L-leucyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-L-threonine, cyclic (10→4)-peptide.

[l] N^2-[(*S*)-6-Methyl-1-oxooctyl]-L-2,4-diaminobutanoyl-L-threonyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-4-[(*S*)-2-aminobutanoyl]-D-leucyl-L-leucyl-L-2,4-diaminobutanoyl-L-2,4-diaminobutanoyl-L-threonine, cyclic (10→4)-peptide.

SPECIFIC TESTS

- **pH** ⟨791⟩**:** 4.0–7.0, in 10 mg/mL solution
- **Loss on Drying** ⟨731⟩

Sample: 100 mg

Analysis: Dry in a capillary-stoppered bottle under vacuum at a pressure NMT 5 mm of mercury at 60° for 3 h.

Sample: It loses NMT 7.0% of its weight.

Add the following:

▲• **Composition**

Solution A, Mobile phase, Diluent, Sensitivity solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the test for *Organic Impurities*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each individual component (colistin A; colistin B; polymyxins E3, E4, E6, E7; 7-L-valinepolymyxin E2; 7-L-isoleucinepolymyxin E2; 7-L-norvalinepolymyxin E1; colistin III; and 2,3-dehydro colistin A) in the portion of Colistin Sulfate taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each individual component

r_T = sum of all the peak responses

F = relative response factor

Acceptance criteria: See *Table 1*.▲ (USP 1-Dec-2022)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.
- **USP Reference Standards** ⟨11⟩

USP Colistin Sulfate RS

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

Topic/Question	Contact	Expert Committee
COLISTIN SULFATE	Julie Zhang Associate Science & Standards Liaison	BIO42020 Biologics Monographs 4 - Antibiotics

Chromatographic Database Information: Chromatographic Database

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