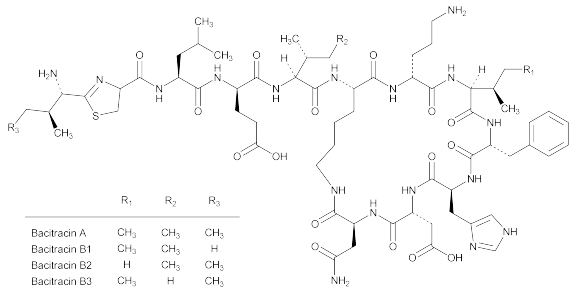
Bacitracin



	R_1	R_2	R_3
Bacitracin A	CH_3	CH_3	CH_3
Bacitracin B1	CH_3	CH_3	H
Bacitracin B2	H	CH_3	CH_3
Bacitracin B3	CH_3	H	CH_3

Bacitracin [1405-87-4].

DEFINITION

Bacitracin is a mixture of polypeptides produced by the growth of an organism of the *licheniformis* group of *Bacillus subtilis* (Fam. Bacillacaea), the main components being bacitracins A, B1, B2, and B3. It has a potency of NLT 65 Bacitracin Units/mg, calculated on the dried basis.

IDENTIFICATION

- **A.** Meets the requirements of the test for *Composition of Bacitracin*
- **B.**

Sample: 0.2 g

Analysis: Ignite the *Sample*. Allow to cool. Dissolve the residue in 0.1 mL of dilute hydrochloric acid. Add 5 mL of water and 0.2 mL of sodium hydroxide.

Acceptance criteria: No white precipitate is formed.

ASSAY

- **PROCEDURE**

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

Analysis: Proceed as directed in the chapter.

Acceptance criteria: NLT 65 Bacitracin Units/mg on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.5%

SPECIFIC TESTS

- **COMPOSITION OF BACITRACIN**

Diluent: 40 g/L of edetate disodium in water adjusted with 8 N sodium hydroxide to a pH of 7.0

Solution A: 34.8 g/L of dibasic potassium phosphate in water

Solution B: 27.2 g/L of monobasic potassium phosphate in water

Solution C: *Solution B* and *Solution A* (9:2). The pH of the mixture is about 6.

Solution D: 0.1 mM edetate disodium in a mixture of *Solution C* and water (1:3)

Solution E: Methanol and acetonitrile (27:2)

Mobile phase: *Solution E* and *Solution D* (63:37)

System suitability solution: 2 mg/mL of USP Bacitracin Zinc RS in *Diluent*

Reporting threshold solution: 0.01 mg/mL of USP Bacitracin Zinc RS from *System suitability solution* in water

Peak identification solution: 2 mg/mL of USP Bacitracin Zinc RS in *Diluent*. Heat in a boiling water bath for 30 min, and cool to room temperature.

Sample solution: 2 mg/mL of Bacitracin in *Mobile phase*

Chromatographic system

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

Mode: LC

Detector: UV 254 nm and 300 nm. Quantitative analysis is performed at 254 nm; 300 nm is only used to identify the location of bacitracin F.

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

Flow rate: 1 mL/min

Injection volume: 100 µL

System suitability

Samples: *System suitability solution* and *Peak identification solution*

Analyze the *Peak identification solution* at 300 nm. Identify bacitracin F, a known impurity, using the relative retention time provided in *Table 1*. Analyze the *System suitability solution* at 254 nm. Identify the peaks of the most active components of bacitracin (bacitracins A, B1, B2, and B3), early eluting peptides (those eluting before the bacitracin B1 peak), and the impurity (bacitracin F) using the relative retention time values in *Table 1*.

Table 1

Name	Nature of Component	Relative Retention Time
Bacitracin C1	Early eluting peptides	0.5
Bacitracin C2		0.6
Bacitracin C3		0.6
Bacitracin B1	Active bacitracin	0.7
Bacitracin B2		0.7
Bacitracin B3		0.8
Bacitracin A		1.0
Bacitracin F	Impurity	2.4

Suitability requirements

Peak-to-valley ratio: NLT 1.2

The *Peak-to-valley ratio* is calculated as follows:

$$\text{Result} = H_P/H_V$$

H_P = height above the baseline of the peak due to bacitracin B1

H_V = height above the baseline of the lowest point of the curve separating the bacitracin B1 peak from the bacitracin B2 peak

Analysis

Samples: *Diluent*, *Reporting threshold solution*, and *Sample solution*

Quantitative analysis is performed at 254 nm.

Content of bacitracin A

Calculate the percentage of bacitracin A in the portion of Bacitracin taken:

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

r_A = peak area of bacitracin A from the *Sample solution*

r_T = sum of all peak areas above the reporting threshold from the *Sample solution*

Content of active bacitracin

Calculate the percentage of active bacitracin (bacitracin A, B1, B2, and B3) in the portion of Bacitracin taken:

$$\text{Result} = [(r_A + r_{B1} + r_{B2} + r_{B3})/r_T] \times 100$$

r_A = peak area of bacitracin A from the *Sample solution*

r_{B1} = peak area of bacitracin B1 from the *Sample solution*

r_{B2} = peak area of bacitracin B2 from the *Sample solution*

r_{B3} = peak area of bacitracin B3 from the *Sample solution*

r_T = sum of all peak areas above the reporting threshold from the *Sample solution*

Limit of early eluting peptides
Calculate the percentage of early eluting peptides (peaks eluting before bacitracin B1) in the portion of Bacitracin taken:

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

r_P = sum of peak areas for all peaks before bacitracin B1 from the *Sample solution*
r_T = sum of all peak areas above the reporting threshold from the *Sample solution*

Limit of bacitracin F
Calculate the percentage of bacitracin F in the portion of Bacitracin taken:

$$\text{Result} = (r_F/r_A) \times 100$$

r_F = peak area for bacitracin F from the *Sample solution*
r_A = peak area for bacitracin A from the *Sample solution*

Acceptance criteria: See *Table 2*. Disregard any peaks from the *Sample solution* that are observed in the *Diluent* chromatogram. Disregard any peaks from the *Sample solution* having a peak area less than bacitracin A in the *Reporting threshold solution*.

Table 2

	Acceptance Criteria (%)
Content of bacitracin A	NLT 40.0
Content of active bacitracin	NLT 70.0
Limit of early eluting peptides	NMT 20.0
Limit of bacitracin F	NMT 6.0

- **pH** ⟨**791**⟩
  Sample solution: 10,000 Bacitracin Units/mL in water
  Acceptance criteria: 5.5–7.5
- **Loss on Drying** ⟨**731**⟩
  Sample: 100 mg
  Analysis: Dry the *Sample* in a capillary-stoppered bottle under vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h.
  Acceptance criteria: NMT 5.0%
- **Sterility Tests** ⟨**71**⟩**:** Where the label states that the Bacitracin is sterile, it meets the requirements.
- **Bacterial Endotoxins Test** ⟨**85**⟩**:** Where the label states that the Bacitracin is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.01 USP Endotoxin Units/Bacitracin Unit.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and store below 8°.
- **Labeling:** Where it is packaged for prescription compounding, label it to indicate that it is not sterile and that the potency cannot be assured for longer than 60 days after opening, and to state the number of Bacitracin Units/mg. 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 Bacitracin Zinc RS
  USP Endotoxin RS

Bacitracin for Injection

DEFINITION

Bacitracin for Injection has a potency of NLT 50 Bacitracin Units/mg. It contains NLT 90.0% and NMT 115.0% of the labeled amount of bacitracin.

IDENTIFICATION

- **A. Thin-Layer Chromatographic Identification Test** ⟨**201BNP**⟩**:** Meets the requirements

ASSAY

- **Procedure**
  (See *Antibiotics—Microbial Assays* ⟨81⟩.)
  Sample solution 1: Nominally 100 Bacitracin Units/mL, prepared as follows. Constitute one container of Bacitracin for Injection as directed in the labeling. Using a suitable hypodermic needle and syringe, withdraw the contents of the container, and dilute with *Buffer B.1* (see the chapter) to a suitable volume.
  Sample solution 2 (where the label states the number of Bacitracin Units in a given volume of constituted solution)**:** Nominally 100 Bacitracin Units/mL, prepared as follows. Constitute one container of Bacitracin for Injection as directed in the labeling. Dilute a suitable aliquot of the constituted solution with *Buffer B.1* (see the chapter) to a suitable final volume.
  Analysis
  Samples: *Sample solution 1* or *Sample solution 2*
  Proceed as directed in the chapter. Add sufficient 0.01 N hydrochloric acid to the *Sample solution* so that the amount of hydrochloric acid in the *Test Dilution* is the same as in the median level of the standard. Dilute with *Buffer B.1* to obtain a *Test Dilution* having a bacitracin concentration that is nominally equivalent to the median level of the standard.
  Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS

- **Uniformity of Dosage Units** ⟨**905**⟩**:** Meets the requirements

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 3.0%

Delete the following:

- **Heavy Metals,** *Method II* ⟨**231**⟩**:** NMT 30 ppm (Official 1-Jan-2018)

SPECIFIC TESTS

- **Constituted Solution:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩*, Specific Tests, Completeness and clarity of solutions*.
- **pH** ⟨**791**⟩
  Sample solution: A solution containing 10,000 Bacitracin Units/mL
  Acceptance criteria: 5.5–7.5
- **Loss on Drying** ⟨**731**⟩
  Analysis: Dry 100 mg in a capillary-stoppered bottle under vacuum at a pressure of NMT 5 mm of mercury at 60° for 3 h.