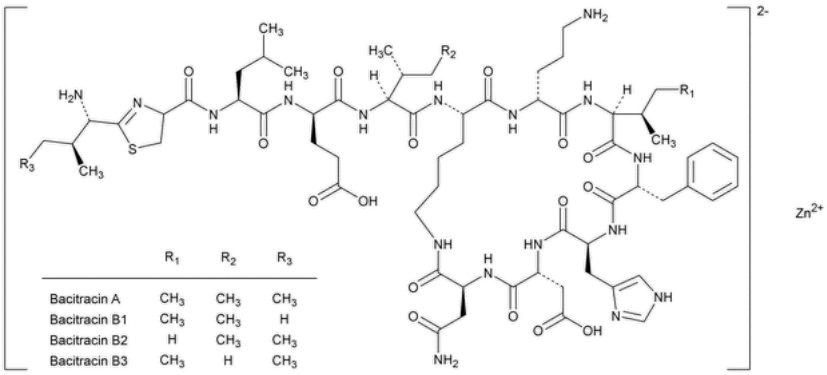
Status: Currently Official on 13-Feb-2025
Official Date: Official as of 01-May-2016
Document Type: USP Monographs
DocId: GUID-3AE5D6B9-7165-4B38-8DD4-867D6814A9FA_1_en-US
DOI: https://doi.org/10.31003/USPNF_M6910_01_01
DOI Ref: isr73

Bacitracin Zinc



Bacitracins, zinc complex;
Bacitracin zinc complex
CAS RN®: 1405-89-6; UNII: 89Y4M234ES.

DEFINITION

Bacitracin Zinc is the zinc complex of bacitracin, which consists of a mixture of antimicrobial polypeptides, 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. It contains NLT 4.0% and NMT 6.0% of zinc (Zn), calculated on the dried basis.

IDENTIFICATION

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

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

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 Zinc in *Diluent*

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

Run time: NLT 3 times the retention time of bacitracin A

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 peak due to bacitracin B1), 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 Zinc 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 Zinc 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 Zinc 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 Zinc taken:

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

r_F = peak area of bacitracin F from the *Sample solution*

r_A = peak area of 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

• **Zinc Content**

[Note—The *Standard solutions* and the *Sample solution* may be quantitatively diluted with 1 mM hydrochloric acid, if necessary, to obtain solutions of suitable concentrations, adaptable to the linear or working range of the instrument.]

Standard stock solution: 10 mg/mL of zinc from zinc oxide in 1 N hydrochloric acid. Prepare as follows. Transfer a suitable amount of zinc oxide to a suitable volumetric flask, add 1 N hydrochloric acid using 32% of the final volume, warm to dissolve, cool and dilute with water to volume.

Standard solutions: 0.5, 1.5, and 2.5 µg/mL of zinc from *Standard stock solution* in 0.001 N hydrochloric acid

Sample stock solution: 2 mg/mL of Bacitracin Zinc in 0.01 N hydrochloric acid

Sample solution: 0.02 mg/mL of Bacitracin Zinc from *Sample stock solution* in 0.001 N hydrochloric acid

Instrumental conditions

(See *Atomic Absorption Spectroscopy (852)*.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 213.8 nm

Lamp: Zinc hollow-cathode

Flame: Air–acetylene

Blank: 0.001 N hydrochloric acid

Analysis

Samples: *Standard solutions*, *Sample solution*, and *Blank*

Plot the absorbances of the *Standard solutions* versus concentration, in µg/mL, of zinc, and draw the straight line best fitting the three plotted points. From the graph, determine the concentration, in µg/mL, of zinc in the *Sample solution*.

Calculate the percentage of zinc in the portion of Bacitracin Zinc taken:

$$\text{Result} = C \times D \times (V/W) \times F \times 100$$

C = concentration of zinc in the *Sample solution* obtained from the curve (µg/mL)

D = dilution factor for the *Sample solution*, 100 mL/mL

V = volume of *Sample stock solution* (mL)

W = weight of Bacitracin Zinc used to prepare the *Sample stock solution* (mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: 4.0%–6.0% on the dried basis

• **pH** ⟨791⟩

Sample solution: A saturated solution in water containing about 100 mg/mL

Acceptance criteria: 6.0–7.5

• **Loss on Drying** ⟨731⟩

Sample: 100 mg

Analysis: Dry the *Sample* in a capillary-stoppered bottle under vacuum at 60° for 3 h.

Acceptance criteria: NMT 5.0%

• **Sterility Tests** ⟨71⟩**:** Where the label states that it is sterile, it meets the requirements of the chapter. If the membrane filtration test is used, add 20 g of edetate disodium to each L of *Fluid A*.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, and store below 25°.

• **Labeling:** Label it to indicate that it is to be used in the manufacture of nonparenteral drugs only. 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 sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of sterile dosage forms.

• **USP Reference Standards** ⟨11⟩

USP Bacitracin Zinc RS

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

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

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. PF 40(6)

Current DocID: GUID-3AE5D6B9-7165-4B38-8DD4-867D6814A9FA_1_en-US

DOI: https://doi.org/10.31003/USPNF_M6910_01_01

DOI ref: isr73