

Status: Currently Official on 13-Feb-2025

Official Date: Official as of 01-Mar-2019

Document Type: USP Monographs

DocId: GUID-7075FBB3-B0D7-49E2-89FA-91F1B5A075F6_5_en-US

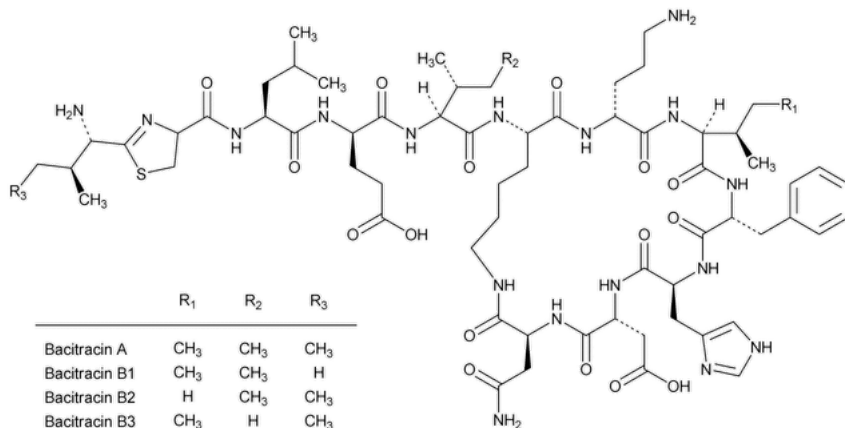
DOI: https://doi.org/10.31003/USPNF_M6830_05_01

DOI Ref: 0in5p

© 2025 USPC

Do not distribute

Bacitracin



Bacitracin

CAS RN[®]: 1405-87-4; UNII: 58H6RW052I.

DEFINITION

Bacitracin is a mixture of polypeptides produced by the growth of an organism of the *licheniformis* group of *Bacillus subtilis* (Fam. Bacillaceae), 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*

Change to read:

- **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 TS 3](#). ▲_{2S} (USP41)

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 A* and *Solution B* (2:9). 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 D* and *Solution E* (37:63)

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 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 times 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, *System suitability solution*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***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*