Soluble Bacitracin Methylenedisalicylate

DEFINITION

Soluble Bacitracin Methylenedisalicylate is a mixture of Bacitracin Methylenedisalicylate and Sodium Bicarbonate. It has a potency of NLT 8 Bacitracin Units/mg, calculated on the dried basis.

ASSAY

- **ANTIBIOTICS—MICROBIAL ASSAYS ⟨81⟩**

  Diluent: 20 g/L of sodium bicarbonate

  Sample stock solution: Transfer a suitable amount of Soluble Bacitracin Methylenedisalicylate to a high-speed glass blender jar, add 99.0 mL of *Diluent* and 1.0 mL of polysorbate 80, and blend for 3 min.

  Test dilution: To a suitable aliquot of the *Sample stock solution*, add a suitable volume of 0.01 N hydrochloric acid, and dilute with *Buffer No. 1* to obtain a concentration of bacitracin assumed to be equal to the median dose level of the Standard. [NOTE—The amount of hydrochloric acid in the *Test dilution* should be the same as that in the median dose level of the Standard.]

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

  Acceptance criteria: NLT 8 Bacitracin Units/mg on the dried basis

SPECIFIC TESTS

- **pH ⟨791⟩:** 8.0–9.5, in a 25 mg/mL solution
- **LOSS ON DRYING ⟨731⟩:** Dry 100 mg in a capillar y-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h: it loses NMT 8.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label it to indicate that it is for veterinar y use only.
- **USP REFERENCE STANDARDS ⟨11⟩**

  USP Bacitracin Zinc RS

Bacitracin Methylenedisalicylate Soluble Powder

DEFINITION

Bacitracin Methylenedisalicylate Soluble Powder contains NL T 90.0% and NMT 120.0% of the labeled amount of bacitracin.

ASSAY

- **ANTIBIOTICS—MICROBIAL ASSAYS ⟨81⟩**

  Diluent: 20 g/L of sodium bicarbonate

  Sample stock solution: Transfer a suitable amount of Bacitracin Methylenedisalicylate Soluble Powder to a high-speed glass blender jar, add 99.0 mL of *Diluent* and 1.0 mL of polysorbate 80, and blend for 3 min.

  Test dilution: To a suitable aliquot of the *Sample stock solution*, add a suitable volume of 0.01 N hydrochloric acid and dilute with *Buffer No. 1* to obtain a concentration of bacitracin assumed to be equal to the median dose level of the Standard. [NOTE—The amount of hydrochloric acid in the *Test dilution* should be the same as that in the median dose level of the Standard.]

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

  Acceptance criteria: 90.0%—120.0%

SPECIFIC TESTS

- **pH ⟨791⟩:** 8.0–9.5 in a 50 mg/mL solution
- **LOSS ON DRYING ⟨731⟩:** Dry 100 mg in a capillar y-stoppered bottle in a vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h: it loses NMT 8.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label it to indicate that it is for veterinar y use only. Label it to state the content of bacitracin in terms of grams per pound, each gram of bacitracin being equivalent to 42,000 Bacitracin Units.
- **USP REFERENCE STANDARDS ⟨11⟩**

  USP Bacitracin Zinc RS

Bacitracin Zinc

Bacitracins, zinc complex.
Bacitracins zinc complex [1405-89-6].

» 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 not less than 65 Bacitracin Units per mg, calculated on the dried basis. It contains not less than 4.0 percent and not more than 6.0 per cent of zinc (Zn), calculated on the dried basis.

Packaging and storage—Preserve in tight containers, and store in a cool place.

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 per milligram. 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

Identification—

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

B: It meets the requirements of the liquid chromatographic procedure in the test for *Composition.*

Sterility ⟨71⟩**—**Where the label states that it is sterile, it meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, except to use *Fluid A* to each L of which has been added 20 g of edetate disodium.

pH ⟨791⟩: between 6.0 and 7.5, in a (saturated) solution containing approximately 100 mg per mL.

Loss on drying ⟨731⟩**—**Dry about 100 mg in a capillar y-stoppered bottle in vacuum at 60 ° for 3 hours: it loses not more than 5.0% of its weight.

Zinc content—[NOTE—The *Standard preparations* and the *Test preparation* may be quantitatively diluted with 0.001 N hydrochloric acid, if necessar y, to obtain solutions of suitable concentrations, adaptable to the linear or working range of the instrument.]

Standard preparations—Transfer 3.11 g of zinc oxide, accurately weighed, to a 250-mL volumetric flask, add 80 mL of 1 N hydrochloric acid, warm to dissolve, cool, dilute with water to volume, and mix. This solution contains 10 mg of zinc per mL. Further dilute this solution with 0.001 N hydrochloric acid to

obtain *Standard preparations* containing 0.5, 1.5, and 2.5 µg of zinc per mL, respectively.

Test preparation—Transfer about 200 mg of Bacitracin Zinc, accurately weighed, to a 100-mL volumetric flask. Dissolve in 0.01 N hydrochloric acid, dilute with the same solvent to volume, and mix. Pipet 2 mL of this solution into a 200-mL volumetric flask, dilute with 0.001 N hydrochloric acid to volume, and mix.

Procedure—Concomitantly determine the absorbances of the *Standard preparations* and the *Test preparation* at the zinc resonance line of 213.8 nm, with a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light-scattering* ⟨851⟩), equipped with a zinc hollow-cathode lamp and an air–acetylene flame, using 0.001 N hydrochloric acid as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in µg per mL, of zinc, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, in µg per mL, of zinc in the *Test preparation*. Calculate the content of zinc, in per cent, in the portion of Bacitracin Zinc taken by the formula:

$$1000C / W$$

in which C is the concentration in µg per mL, of zinc in the *Test preparation;* and W is the weight, in mg, of the portion of Bacitracin Zinc taken.

Composition—

Buffer—Dissolve 34.8 g of potassium phosphate, dibasic, in 1 L of water. Adjust with 27.2 g of potassium phosphate, monobasic, dissolved in 1 L of water, to a pH of 6.0.

Mobile Phase—Prepare a mixture of methanol, water, *Buffer*, and acetonitrile (26:15:5:2). Mix well, and degas.

Diluent—Dissolve 40 g of edetate disodium in 1 L of water. Adjust with dilute sodium hydroxide to a pH of 7.0.

System suitability solution—Dissolve an accurately weighed quantity of USP Bacitracin Zinc RS in *Diluent* to obtain a solution with a nominal concentration of about 2.0 mg per mL.

Reporting threshold solution—Dilute quantitatively, with water, a suitable volume of *System suitability solution* to obtain a solution with a known concentration of 0.01 mg per mL.

Peak identification solution—Dissolve a weighed quantity of USP Bacitracin Zinc RS in a suitable volume of *Diluent* to obtain a solution with a nominal concentration of about 2.0 mg per mL. Heat in boiling water bath for 30 minutes. Cool to room temperature.

Test solution—Dissolve an accurately weighed quantity of Bacitracin Zinc in *Diluent* to obtain a solution with a nominal concentration of about 2.0 mg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with an absorbance detector and an end-capped 4.6- × 250-mm column that contains 5- µm packing L1. The flow rate is 1.0 mL per minute. Set the wavelength of the detector at 300 nm. Inject about 100 µL of the *Peak identification solution*, and identify the location of bacitracin F, which is a known impurity, using the relative retention time shown in *Table 1*.

Table 1

Component Name	Relative Retention Time (approximate)
Bacitracin C1	0.5
Bacitracin C2	0.6
Bacitracin C3	0.6
Bacitracin B1	0.7
Bacitracin B2	0.7
Bacitracin B3	0.8
Bacitracin A	1.0
Bacitracin F	2.4

Change the wavelength of the detector and set it to 254 nm. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* 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 given in *Table 1*. Calculate the peak-to-valley ratio using the formula:

$$H_p / H_V$$

in which H_p is the height above the baseline of the peak due to bacitracin B1; and H_V is the height above the baseline of the lowest point of the cur ve separating the bacitracin B1 peak from the peak due to bacitracin B2. The peak-to-valley ratio is not less than 1.2.

Procedure—Separately inject equal volumes (100 µL) of *Diluent, Test solution,* and *Reporting threshold solution*. Record the chromatograms for about three times the retention time of bacitracin A. Identify the peaks using the relative retention times shown in *Table 1*. Measure the peak areas of all peaks in the *Test solution*. [NOTE—Disregard any peak in the *Test solution* having an area less than the area of the bacitracin A peak in the *Reporting threshold solution;* disregard any peak obser ved in the *Diluent*.]

NOTE—*Total area* in the following calculations is defined as the area of all peaks except the reporting threshold.

CONTENT OF BACITRACIN A—Calculate the per centage of bacitracin A using the formula:

$$(r_A / \textit{Total area}) \times 100$$

in which r_A is the area response from bacitracin A. Bacitracin A content is not less than 40.0% of the *Total area*.

CONTENT OF ACTIVE BACITRACIN—Calculate the per centage of active bacitracin (bacitracin A, B1, B2, and B3) using the formula:

$$(r_A + r_{B1} + r_{B2} + r_{B3} / \textit{Total area}) \times 100$$

in which r_A, r_{B1}, r_{B2}, and r_{B3} are the area responses from bacitracin A, B1, B2, and B3, respectively. The sum of bacitracin A, B1, B2, and B3 is not less than 70.0% of the *Total area*.

LIMIT OF EARLY ELUTING PEPTIDES—Calculate the per centage of all peaks eluting before the peak due to bacitracin B1 using the formula:

$$(r_{PreB1} / \textit{Total area}) \times 100$$

in which r_{PreB1} is the sum of the responses of all peaks eluting before the peak for bacitracin B1. The limit of early eluting peptides (those eluting before the peak due to bacitracin B1) is not more than 20.0%.

LIMIT OF BACITRACIN F—Calculate the per centage of bacitracin F using the formula:

$$100 \times (r_F / r_A)$$

in which r_F is the response of bacitracin F from the *Test solution*; and r_A is the response of bacitracin A from the *Test solution*. The limit of bacitracin F, a known impurity, is not more than 6.0%.

Assay—Proceed with Bacitracin Zinc as directed under *Antibiotics—Microbial Assays* ⟨81⟩.

Bacitracin Zinc Ointment

» Bacitracin Zinc Ointment is Bacitracin Zinc in an anhydrous ointment base. It contains not less than 90.0 per cent and not more than 140.0 per cent of the labeled amount of bacitracin.