Assay for ampicillin—
Not more than 5.0%.

**Method I**

Water, a 125-mL separator, and add 8.0 mL of 1.0 N hydrochloric acid containing 0.5 and 1.0, respectively, pH 7.91; deliverable volume: meets the requirements.

Calculate the quantity, in mg, of probenecid (C_{13}H_{19}NO_{4}S) in the Ampicillin and Probenecid sodium solution (1 in 100) as the blank. Calculate the quantity of this stock solution with water to obtain an equivalent to 1 mg of ampicillin per mL.

**Assay preparation**—Constitute Ampicillin and Probenecid for Oral Suspension as directed in the labeling, and mix. Transfer the resulting suspension to a high-speed glass blender jar containing sufficient water to make 500.0 mL, and blend for about 10 minutes. Quantitatively dilute an accurately measured volume of this stock solution with water to obtain an Assay preparation containing about 1.25 mg of ampicillin per mL.

Procedure—Proceed as directed for Procedure under Iodometric Assay—Antibiotics (425), using USP Ampicillin RS.

**Assay preparation**—Constitute Ampicillin and Probenecid for Oral Suspension as directed in the labeling, and mix. Transfer the resulting suspension to a high-speed glass blender jar containing sufficient water to make 500.0 mL, and blend for about 10 minutes. Quantitatively dilute an accurately measured volume of this stock solution with water to obtain an Assay preparation containing about 1.25 mg of ampicillin per mL.

**Assay for probenecid—**

**Standard preparation**—Prepare as directed for Standard Preparation under Iodometric Assay—Antibiotics (425), using USP Ampicillin RS.

**Assay preparation**—Constitute Ampicillin and Probenecid for Oral Suspension as directed in the labeling, and mix. Quantitatively dilute the resulting suspension with sodium carbonate solution (1 in 100) to obtain a solution containing about 1 mg of ampicillin per mL.

**Procedure**—Proceed as directed for Procedure under Iodometric Assay—Antibiotics (425). Calculate the quantity, in mg, of ampicillin (C_{16}H_{19}N_{3}O_{4}S) in the Ampicillin and Probenecid for Oral Suspension taken by the formula:

\[(L / D)(F / 2000)(B - I)\]

in which \(L\) is the labeled quantity, in mg, of ampicillin in the Ampicillin and Probenecid for Oral Suspension; and \(D\) is the concentration, in mg per mL, of ampicillin in the Assay preparation on the basis of the labeled quantity in the Ampicillin and Probenecid for Oral Suspension and the extent of dilution.

**System suitability**

Samples: Standard solution and System suitability solution. Calculate the quantity in \(\mu g\), of C_{16}H_{19}N_{3}O_{4}S in each mg of Ampicillin Sodium taken:

\[\text{Result} = (r_U / r_S) \times (C_S / C_U) \times P\]

where \(r_U, r_S\) are the absorbances of the solutions from the Assay preparation and the Standard preparation, respectively.

### Ampicillin Sodium

C_{16}H_{19}N_{3}O_{4}S 371.39

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, [6-(aminonaphenylacetyl)amino]-3,3-dimethyl-7-oxo-, monosodium salt, [2S-[2α,3α,6(3′S)]]:

Monosodium D-(-)-6-(2-amino-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [69-52-3].

**Definition**

Ampicillin Sodium has a potency equivalent to NL T 845 μg and NMT 988 μg of ampicillin (C_{16}H_{19}N_{3}O_{4}S) per mg, calculated on the anhydrous basis.

**Identification**

- A. Infrared Absorption (197M)
- B. Identification Tests—General, Sodium (191)

### Assay

**Diluent**: Water, 1 M monobasic potassium phosphate, and 1 N acetic acid (989:10:1)

**Mobile phase**: Acetonitrile, water, 1 M monobasic potassium phosphate, and 1 N acetic acid (80:900:10:1)

**Standard solution**: 1 mg/mL of USP Ampicillin RS in Diluent using shaking and sonication, if necessary, to dissolve. Use this solution promptly after preparation.

**System suitability solution**: 0.12 mg/mL of caffeine in Standard solution

**Sample solution**:

- **NOTE**: Ampicillin Sodium is hygroscopic. Minimize exposure to the atmosphere, and weigh promptly. Equivalent to 1 mg/mL of anhydrous ampicillin in Diluent. **NOTE**: Use this solution promptly after preparation.

**Chromatographic system**

(See Chromatography (621), System Suitability.)

- **Mode**: LC
- **Detector**: UV 254 nm
- **Column**: Pre-column: 4-mm × 5-cm; 5- to 10-μm packing L1
  - **Analytical column**: 4-mm × 30-cm; 5- to 10-μm packing L1
  - **Flow rate**: 2 mL/min
  - **Injection size**: 20 μL

**System suitability**

**Samples**: Standard solution and System suitability solution

**NOTE**: The relative retention times for ampicillin and cafefine are 0.5 and 1.0, respectively, System suitability solution.

**Suitability requirements**

**Resolution**: NLT 2.0 between the caffeine and the ampicillin peaks, System suitability solution

**Tailing factor**: NMT 1.4, Standard solution

**Capacity factor**: NMT 2.5, Standard solution

**Relative standard deviation**: NMT 2.0%, Standard solution

**Analysis**

**Samples**: Standard solution and Sample solution

Calculate the quantity, in \(\mu g\), of C_{16}H_{19}N_{3}O_{4}S in each mg of Ampicillin Sodium taken:

\[\text{Result} = (r_U / r_S) \times (C_S / C_U) \times P\]

where \(r_U, r_S\) are the absorbances of the solutions from the Assay preparation and the Standard preparation, respectively.

Acceptance criteria: 845–988 μg/mg on the anhydrous basis.
IMPURITIES
Organic Impurities
• Procedure 1: Limit of Methylene Chloride
  Internal standard solution: 2.1 mg/mL of dioxane in dimethyl sulfoxide
  Standard solution: 0.33 mg/mL of methylene chloride in dioxane
  Sample solution: 166.7 mg/mL of Ampicillin Sodium in dioxane

Chromatographic system
  (See Chromatography (621), System Suitability.)
  Mode: GC
  Detector: Flame ionization
  Column: 1.8-m x 4-mm glass column packed with a 10% phase G39 on unsilanized support S1A
  Temperature
    Column: 65°
    Injector: 100°
  Detector block: 260°
  Carrier gas: Nitrogen
  Flow rate: 60 mL/min
  Injection size: 1 µL

System suitability
  Sample: Standard solution
  [NOTE—The relative retention times for methylene chloride and dioxane are 0.5 and 1.0, respectively.]

Suitability requirements
  Resolution: NLT 4 between methylene chloride and dioxane
  Relative standard deviation: NMT 5%

Analysis
  Samples: Standard solution and Sample solution
  Calculate the percentage of methylene chloride in the portion of Ampicillin Sodium taken:
  \[ \text{Result} = \left( \frac{R_s}{R_U} \right) \times \left( \frac{C_S}{C_U} \right) \times 100 \]
  \( R_s \) = peak response ratio of methylene chloride to dioxane from the Sample solution
  \( R_U \) = peak response ratio of methylene chloride to dioxane from the Standard solution
  \( C_S \) = concentration of methylene chloride in the Standard solution (mg/mL)
  \( C_U \) = nominal concentration of Ampicillin Sodium in the Sample solution (mg/mL)

  Acceptance criteria: NMT 0.2%

• Procedure 2: Dimethylaniline (223): Meets the requirements

SPECIFIC TESTS
• Crystallinity (695): Meets the requirements. [NOTE—Ampicillin Sodium in the freeze-dried form is exempt from this requirement.]
  \( \text{pH} \) (791): 8.0–10.0
  Sample solution: 10.0 mg/mL

• Water Determination, Method I (921): NMT 2.0%

• Sterility Tests (71): Where the label states that Ampicillin Sodium is sterile, it meets the requirements.

• Bacterial Endotoxins Test (85): Where the label states that Ampicillin Sodium is sterile or the label states that Ampicillin Sodium must be subjected to further processing during the processing of injectable dosage forms, it contains NMT 0.15 USP Endotoxin Unit/mg of ampicillin.

ADDITIONAL REQUIREMENTS
• Packaging and Storage: Preserve in tight containers.
• Labeling: Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

USP Reference Standards (11)
  USP Ampicillin RS
  USP Ampicillin Sodium RS
  USP Endotoxin RS

Ampicillin and Sulbactam for Injection

Ampicillin and Sulbactam for Injection is a sterile, dry mixture of Ampicillin Sodium and Sulbactam Sodium. It contains the equivalent of not less than 90.0 mg per ml and not more than 115.0 mg per ml of Ampicillin (C_{16}H_{19}N_{3}O_{5}S) and Sulbactam (C_{8}H_{11}NO_{3}S), the labeled amounts representing proportions of ampicillin to sulbactam of 2:1. It contains not less than 563 µg of ampicillin and 280 µg of sulbactam per mg, calculated on the anhydrous basis.

Packaging and storage—Preserve in Containers for Sterile Solids as described under Injections (1).

USP Reference standards (11)—
  USP Ampicillin RS
  USP Endotoxin RS
  USP Sulbactam RS

Constituted solution—At the time of use, it meets the requirements for Constituted Solutions under Injections (1).

Identification—The retention times of the major peaks in the chromatogram of the Assay preparation correspond to those in the chromatogram of the Standard preparation, as obtained in the Assay.

Bacterial endotoxins (85)—It contains not more than 0.17 USP Endotoxin Unit in a portion equivalent to 1 mg of a mixture of ampicillin and sulbactam (0.67 and 0.33 mg, respectively).

Sterility (71)—It meets the requirements when tested as directed for Membrane Filtration under Test for Sterility of the Product to be Examined.

pH (791): between 8.0 and 10.0, in a solution containing 10 mg of ampicillin and 5 mg of sulbactam per mL.

Water, Method I (921): not more than 2.0%.

Particulate matter (788): meets the requirements for small-volume injections.

Other requirements—It meets the requirements for Uniformity of Dosage Units (905) and for Labeling under Injections (1).

Assay—
  0.005 M Tetrabutylammonium hydroxide—Add 0.6 mL of a 40% solution of tetrabutylammonium hydroxide with water to obtain 1800 mL of solution. Adjust with 1 M phosphoric acid to a pH of 5.0 ± 0.1, dilute with water to 2000 mL, and mix.

  Mobile phase—Prepare a filtered and degassed mixture of 0.005 M Tetrabutylammonium hydroxide and acetonitrile (1650:350). Make adjustments if necessary (see System Suitability under Chromatography (621)).

  Standard preparation—Quantitatively dissolve accurately weighed quantities of USP Ampicillin RS and USP Sulbactam RS in Mobile phase to obtain a solution having known concentrations of about 0.6 mg of ampicillin per mL and 0.3 mg of sulbactam per mL. [NOTE—Inject this solution promptly.]

  Resolution solution—Prepare a solution of USP Sulbactam RS in 0.01 N sodium hydroxide containing 0.3 mg per mL, and allow to stand for 30 minutes. Adjust with phosphoric acid to a pH of 5.0 ± 0.1. Transfer 5 mL of the solution to a 25-mL volumetric flask, add 4.25 mL of acetonitrile, dilute with 0.005 M Tetrabutylammonium hydroxide to volume, and mix. Transfer 1 mL of this solution to a second 25-mL volumetric flask, add