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

  Internal standard solution

  Sample solution: 166.7 mg/mL of Ampicillin Sodium in

  Internal standard solution

  Chromatographic system

  (See Chromatography (621), System Suitability.)

  Mode: GC

  Detector: Flame ionization

  Column: 1.8-m × 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:

  \[
  \text{Result} = \left( \frac{R_u}{R_s} \right) \times \left( \frac{C_s}{C_0} \right) \times 100
  \]

  \( R_u = \) peak response ratio of methylene chloride to dioxane from the **Sample solution**

  \( R_s = \) peak response ratio of methylene chloride to dioxane from the **Standard solution**

  \( C_s = \) concentration of methylene chloride in the **Standard solution** (mg/mL)

  \( C_0 = \) nominal concentration of Ampicillin Sodium in the **Sample solution** (mg/mL)

  Acceptance criteria: NMT 0.2%

• **PROCEDURE 2: DIMETHYLALINE (223):** Meets the requirement

SPECIFIC TESTS

• **CRYSTALLINITY (695):** Meets the requirements. [NOTE—Ampicillin Sodium in the freeze-dried form is exempt from this requirement.]

• **pH (791):** 8.0–10.0

• **WATER DECOMPOSITION (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% of the labeled amounts of ampicillin (C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O<sub>4</sub>S) and sulbactam (C<sub>8</sub>H<sub>11</sub>NO<sub>5</sub>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—Dilute 6.6 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
15 mg of USP Ampicillin RS, dilute with Mobile phase to volume, and mix. [NOTE—Inject this solution promptly.]

Assay preparation 1—Mix the contents of a container of Ampicillin and Sulbactam for Injection. Quantitatively dissolve an accurately weighed portion of the powder in Mobile phase to obtain a solution having a concentration of about 1 mg of the powder per mL. [NOTE—Inject this solution promptly.]

Assay preparation 2 (where it is represented as being in a single-dose container)—Constitute a container of Ampicillin and Sulbactam for Injection with a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw the total withdrawable contents from the container, using a suitable hypodermic needle and syringe, and dilute quantitatively, and stepwise if necessary, with Mobile phase to obtain a solution containing about 0.6 mg of ampicillin per mL and 0.3 mg of sulbactam per mL. [NOTE—Inject this solution promptly.]

Assay preparation 3 (where the label states the quantities of ampicillin and sulbactam in a given volume of constituted solution)—Constitute a container of Ampicillin and Sulbactam for Injection with a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively, and stepwise if necessary, with Mobile phase to obtain a solution containing about 0.6 mg of ampicillin per mL and 0.3 mg of sulbactam per mL. [NOTE—Inject this solution promptly.]

Chromatographic system (see Chromatography (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column containing packing L1. The flow rate is about 2 mL per minute. Chromatograph the Resolution solution, and record the responses as directed for Procedure: the relative retention times are about 0.7 for ampicillin and 1.0 for sulbactam alkaline degradation product; and the resolution, R, between ampicillin and sulbactam alkaline degradation product is not less than 4.0. Chromatograph the Standard preparation, and record the responses as directed for Procedure: the relative retention times are about 0.35 for ampicillin and 1.0 for sulbactam; the column efficiency determined from the sulbactam peak is not less than 3500 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the Standard preparation and the appropriate Assay preparation into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantities, in µg, of ampicillin (C₁₆H₁₉N₃O₄S) and of sulbactam (C₈H₁₁NO₅S) in the portion of Ampicillin and Sulbactam for Injection taken by the formula:

\[
(C_P / C_S)(r_D / r_S)
\]

in which C₃ is the concentration, in mg per mL, of the appropriate USP Reference Standard in the Standard preparation; P is the assigned content, in µg per mg, of the appropriate USP Reference Standard; C₄ is the concentration, in mg per mL, of Ampicillin and Sulbactam for Injection in the Assay preparation, based on the weight, in mg, of powder removed from the container and the extent of dilution; and r₀ and r₁ are the peak areas for the appropriate analyte obtained from Assay preparation and the Standard preparation, respectively. Calculate the quantity of ampicillin (C₁₆H₁₉N₃O₄S) and of sulbactam (C₈H₁₁NO₅S) withdrawn from the container, or in the volume of constituted solution taken by the same formula:

\[
(L / D)(C_P / C_S)(r_D / r_S)
\]

in which L is the labeled quantity, in mg, of ampicillin or sulbactam, as appropriate, in the container or in the volume of constituted solution taken; D is the concentration, in mg per mL, of ampicillin or sulbactam in Assay preparation 2 or Assay preparation 3, on the basis of the labeled quantity, in mg, of ampicillin or sulbactam, as appropriate, in the container and the extent of dilution; r₀ and r₁ are the peak areas for the appropriate analyte obtained from Assay preparation 2 or Assay preparation 3 and the Standard preparation, respectively; and the other terms are as defined above.

Amprolium

\[
C_{14}H_{19}ClN_4 \cdot HCl
\]

1-[4-(Amino-2-propyl-5-pyrimidinyl)methyl]-2-methylpyridinium chloride monohydrate

1-[4-(Amino-2-propyl-5-pyrimidinyl)methyl]-2-picolinium chloride monohydrate [121-25-5].

Amprolium contains not less than 97.0 per cent and not more than 101.0 per cent of amprolium (C₁₄H₁₉ClN₄ · HCl), calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards (11)—USP Ampromilum RS

Identification—

A: Infrared Absorption (197K); previously dried.

B: Ultraviolet Absorption (197U)—

Solution: 10 µg per mL.

Medium: 0.1 N hydrochloric acid.

Absorbances at 246 nm, calculated on the dried basis, do not differ by more than 3.0%.

Loss on drying (731)—Dry it at a pressure not exceeding 5 mm of mercury at 100° for 3 hours: it loses not more than 1.0% of its weight.

Assay—

Diluent—Prepare a mixture of 500 mL of water, 450 mL of methanol, and 50 mL of acetonitrile.

Mobile phase—Dissolve 6 g of sodium 1-heptanesulfonate in 500 mL of water, add 12 mL of glacial acetic acid, 2.0 mL of triethylamine, 450 mL of methanol, and 50 mL of acetonitrile, and mix. Pass through a suitable filter of 0.5 µm or finer porosity. Make adjustments if necessary (see System Suitability under Chromatography (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Amprolium RS in Diluent to obtain a solution having a known concentration of about 0.5 mg per mL.

Assay preparation—Transfer about 50 mg of Amprolium, accurately weighed, to a 100-mL volumetric flask, add Diluent to volume, and mix.

Resolution solution—Prepare a solution in Diluent containing about 0.5 mg of USP Amprolium RS and 0.2 mg of 2-picoline per mL.

Chromatographic system (see Chromatography (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column containing packing L13. The flow rate is about 0.6 mL per minute. Chromatograph the Resolution solution, and record the responses as directed for Procedure: the resolution, R, between amprolium and 2-picoline is not less than 7; the column efficiency determined from the amprolium peak is not less than 6500 theoretical plates; the tailing factor for the analyte peak is not more than 2.3; and the relative standard deviation of the amprolium responses for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of

Amprolium