Ampicillin Capsules

Type of Posting	Revision Bulletin
Posting Date	21-Dec-2023
Official Date	22-Dec-2023
Expert Committee	Small Molecules 1

In accordance with the Rules and Procedures of the Council of Experts, the Small Molecules 1 Expert Committee has revised the Ampicillin Capsules monograph. The purpose of this revision is to add *Dissolution Test 2* to accommodate FDA-approved drug products with different dissolution conditions and/or tolerances than the existing dissolution test. *Labeling* information has been incorporated to support the inclusion of *Dissolution Test 2*.

- *Dissolution Test 2* was validated using the Triart C18 brand of column with L1 packing. The typical retention time for ampicillin is about 6 min.

The Ampicillin Capsules Revision Bulletin supersedes the currently official monograph.

Should you have any questions, please contact Yanyin Yang, Senior Scientist II, (301-692-3623 or yanyin.yang@usp.org).

Revision Bulletin
Official: December 22, 2023

Ampicillin Capsules

DEFINITION

Ampicillin Capsules contain an amount of ampicillin (anhydrous or as the trihydrate) equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of ampicillin ($C_{16}H_{19}N_3O_4S$).

IDENTIFICATION

- **A. Thin-Layer Chromatography**

Diluent: Acetone and 0.1 N hydrochloric acid (4:1)

Standard solution: 5 mg/mL of USP Ampicillin RS in *Diluent*

Sample solution: 5 mg/mL of ampicillin in *Diluent* from the contents of Capsules

Chromatographic system

(See *Chromatography* ⟨621⟩*, Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 2 µL

Developing solvent system: Acetone, toluene, glacial acetic acid, and water (650:100:25:100)

Spray reagent: 3 mg/mL of ninhydrin in alcohol

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Standard solution* and the *Sample solution* to the plate, and develop the chromatogram using the *Developing solvent system*. When the solvent front has moved about three-fourths of the length of the plate, remove the plate from the chamber, mark the solvent front, and allow to air-dry. Locate the spots on the plate by spraying lightly with *Spray reagent*, and dry at 90° for 15 min.

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

- **Procedure**

Standard solution: Prepare as directed for *Standard Preparation* in *Iodometric Assay—Antibiotics* ⟨425⟩, using USP Ampicillin RS.

Sample solution: Nominally 1.25 mg/mL of ampicillin prepared as follows. Place NLT 5 Capsules in a high-speed glass blender jar containing a suitable volume of water, and blend for 4 ± 1 min. Dilute a suitable aliquot with water.

Analysis: Proceed as directed for *Procedure* in *Iodometric Assay—Antibiotics* ⟨425⟩.

Calculate the percentage of the labeled amount of ampicillin ($C_{16}H_{19}N_3O_4S$) in the portion of Capsules taken:

$$\text{Result} = (B - I) \times (F_1/2) \times (1/C_U) \times F_2 \times 100$$

B = volume of 0.01 N sodium thiosulfate consumed in the *Blank Determination* (mL)

I = volume of 0.01 N sodium thiosulfate consumed in the *Inactivation and Titration* of the *Sample solution* (mL)

F_1 = factor as calculated in *Iodometric Assay—Antibiotics* ⟨425⟩

C_U = nominal concentration of ampicillin in the *Sample solution* (mg/mL)

F_2 = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

Change to read:

- **Dissolution** ▲(RB 22-Dec-2023) ⟨711⟩

▲Test 1: See *Dissolution* ⟨711⟩*, Procedure for a Pooled Sample*. ▲ (RB 22-Dec-2023)

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 45 min

Standard solution: *L*/900 mg/mL of USP Ampicillin RS in water, where *L* is the labeled amount of ampicillin in mg/Capsule

Sample solution: Use a filtered portion of the solution under test.

Solution A: 1 in 1000 solution of polyoxyethylene (23) lauryl ether in water

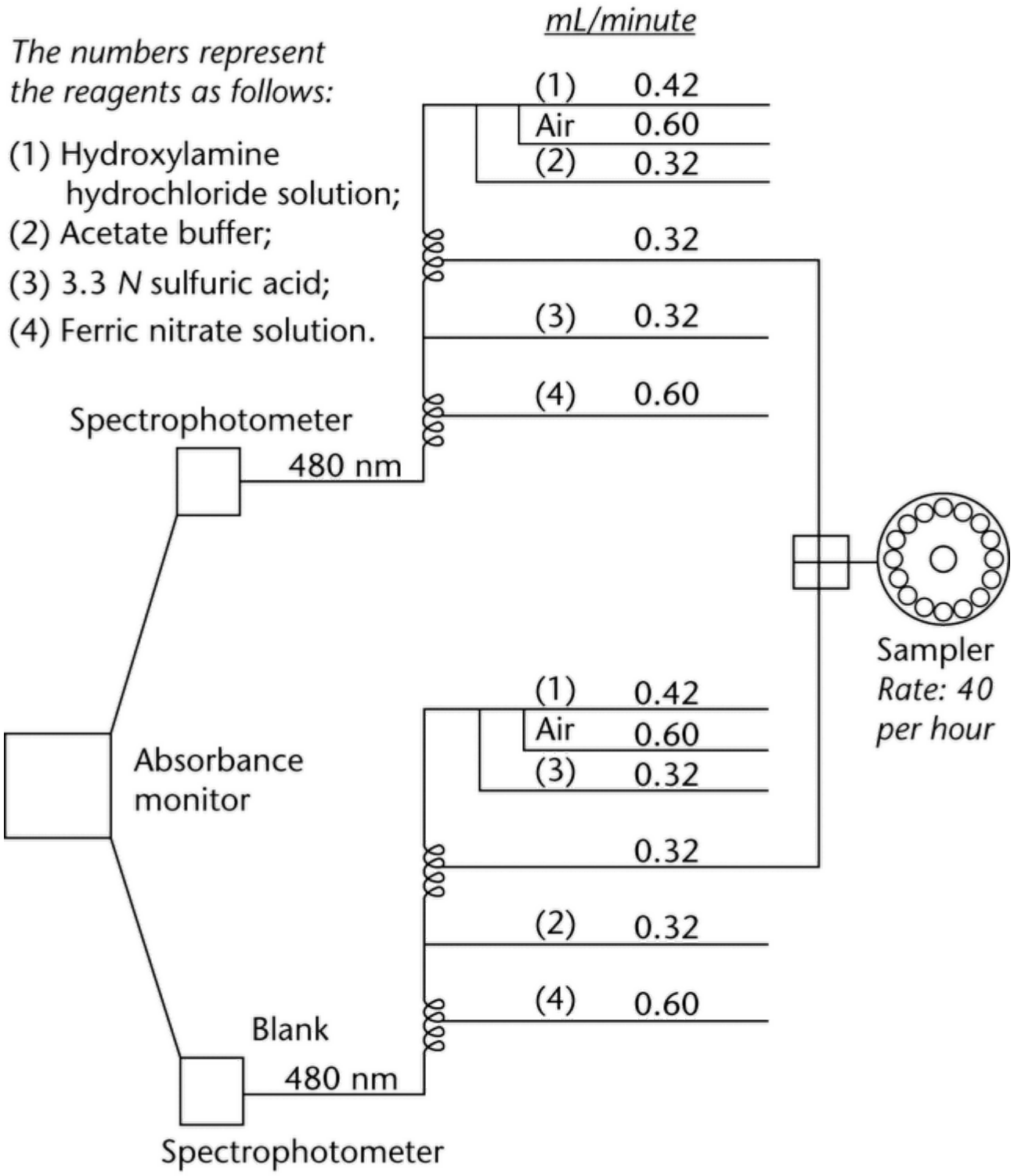
Solution B: Dissolve 20 g of hydroxylamine hydrochloride in 5 mL of *Solution A*, and add water to make 1000 mL.

Buffer: 26 mg/mL of sodium hydroxide and 3.1 mg/mL of sodium acetate in water

Ferric nitrate solution: Suspend 233 g of ferric nitrate in about 600 mL of water, add 2.8 mL of sulfuric acid, stir until the ferric nitrate is dissolved, add 1 mL of polyoxyethylene (23) lauryl ether, dilute with water to 1000 mL, and mix.

Apparatus: Automatic analyzer consisting of (1) a liquid sampler, (2) a proportioning pump, (3) suitable spectrophotometers equipped with matched flow cells and analysis capability at 480 nm, (4) a means of recording spectrophotometric readings, and/or computer for data retrieval and

calculation, and (5) a manifold consisting of the components illustrated in *Figure 1*.



Click image to enlarge

Figure 1.

Analysis: With the sample line pumping water, the other lines pumping their respective reagents, and the spectrophotometer set at 480 nm, standardize the system until a steady absorbance baseline has been established. Transfer portions of the *Standard solution* and the *Sample solution* to sampler cups, and place in the sampler. Start the sampler, and conduct determinations of the *Standard*

solution and the *Sample solution* typically at the rate of 40/h using a ratio of about 2:1 for sample and wash time.

Calculate the percentage of the labeled amount of ampicillin ($C_{16}H_{19}N_3O_4S$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times P \times F \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Ampicillin RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

P = potency of ampicillin in USP Ampicillin RS (µg/mg)

F = conversion factor, 0.001 mg/µg

L = label claim (mg/Capsule)

Tolerances: NLT 75% (Q) of the labeled amount of ampicillin ($C_{16}H_{19}N_3O_4S$) is dissolved.

▲**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.1 N hydrochloric acid; 500 mL

Apparatus 1: 100 rpm

Time: 20 min

Buffer: Dissolve 1.36 g of potassium phosphate, monobasic in 1000 mL of water. Add 0.6 mL of glacial acetic acid. Adjust with 1 N sodium hydroxide solution or 10% (v/v) phosphoric acid to a pH of 3.5.

Mobile phase: Acetonitrile and *Buffer* (10:90)

Diluent: 87 g/L of potassium phosphate, dibasic in water

Standard stock solution: 1 mg/mL of USP Ampicillin RS in *Medium*. Sonicate to dissolve. Ensure the temperature of the water bath in the sonicator does not exceed 20°. Prepare the *Standard solution* as quickly as possible from the *Standard stock solution*.

Standard solution

For Capsules labeled to contain 250 mg: 0.417 mg/mL of USP Ampicillin RS in *Diluent* from the *Standard stock solution* prepared as follows. Immediately dilute 10 mL of the *Standard stock solution* with *Medium* to 20 mL. Immediately transfer 10 mL of the resulting solution into a stoppered glass tube containing 2 mL of *Diluent* and mix. Store this solution in the refrigerator.

For Capsules labeled to contain 500 mg: 0.833 mg/mL of USP Ampicillin RS in *Diluent* from the *Standard stock solution* prepared as follows. Immediately transfer 10 mL of the *Standard stock solution* into a stoppered glass tube containing 2 mL of *Diluent* and mix. Store this solution in the refrigerator.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, discarding the first 3 mL of the filtrate. Immediately, transfer 5 mL of the filtered solution into a stoppered glass tube containing 1 mL of the *Diluent* and mix. Store this solution in the refrigerator.

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Temperatures

Autosampler: 6°

Column: 50°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

Run time: NLT 1.9 times the retention time of ampicillin

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ampicillin ($C_{16}H_{19}N_3O_4S$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times P \times F \times D \times (1/L) \times 100$$

r_U = peak response of ampicillin from the *Sample solution*

r_S = peak response of ampicillin from the *Standard solution*

C_S = concentration of USP Ampicillin RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 500 mL

P = potency of ampicillin in USP Ampicillin RS (µg/mg)

F = conversion factor, 0.001 mg/µg

D = dilution factor for the *Sample solution*

L = label claim (mg/Capsule)

Tolerances: NLT 80% (Q) of the labeled amount of ampicillin ($C_{16}H_{19}N_3O_4S$) is dissolved. ▲ (RB 22-Dec-2023)

- **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

SPECIFIC TESTS

- **Water Determination** ⟨921⟩**,** ***Method I*****:** NMT 4.0% where the Capsules contain anhydrous ampicillin, or between 10.0% and 15.0% where the Capsules contain ampicillin trihydrate

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.

Change to read:

- **Labeling:** Label the Capsules to indicate whether the ampicillin therein is in the anhydrous form or is the trihydrate. ▲When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.▲ (RB 22-Dec-2023)

- **USP Reference Standards** ⟨11⟩

  USP Ampicillin RS

Not Applicable

Current DocID: