

(iv) *Preparation of sample solutions—(a) Product not packaged for dispensing (micrograms of azlocillin per milligram).* Dissolve and dilute an accurately weighed portion of the sample with sufficient distilled water to obtain a stock solution of 1.0 milligram of azlocillin per milliliter (estimated).

(b) *Product packaged for dispensing.* Determine both micrograms of azlocillin per milligram of the sample and milligrams of azlocillin per container. Use separate containers for preparation of each sample solution as described in paragraphs (b)(1)(iv)(b)(1) and (2) of this section.

(1) *Micrograms of azlocillin per milligram.* Dissolve and dilute an accurately weighed portion of the sample with sufficient distilled water to obtain a stock solution of 1.0 milligram of azlocillin per milliliter (estimated).

(2) *Milligrams of azlocillin per container.* Reconstitute as directed in the labeling using distilled water in lieu of the reconstituting fluid. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to a concentration of 1.0 milligram of azlocillin per milliliter (estimated).

(v) *Calculations—(a)* Calculate the micrograms of azlocillin per milligram of sample as follows:

$$\frac{\text{Micrograms of azlocillin per milligram of sample}}{\text{milligram of sample}} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u =Absorbance of sample solution;
 P_s =Potency of working standard solution in micrograms per milliliter;
 A_s =Absorbance of working standard solution;
 C_u =Milligrams of sample per milliliter of sample solution; and
 m =Percent moisture in sample.

(b) Calculate the azlocillin content of the single-dose vial as follows:

$$\text{Milligrams of azlocillin per vial} = \frac{A_u \times P_s \times d}{A_s \times 1,000}$$

where:

A_u =Absorbance of sample solution;
 P_s =Potency of working standard solution in micrograms per milliliter;
 A_s =Absorbance of working standard solution; and
 d =Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 100 milligrams of azlocillin per milliliter.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter, using the titration procedure and calculations described in paragraph (e)(2) of that section and preparing the sample as follows: Weigh the vial. Rapidly transfer a portion of the powder into the titration vessel, add the Karl Fischer reagent and restopper the vial immediately. Reweigh the vial to obtain the sample weight. A nitrogen purged glove bag or glove box should be used for preparing the sample.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams of azlocillin per milliliter.

(6) *Specific rotation.* Proceed as directed in § 436.210 of this chapter, using an aqueous solution containing 10 milligrams of azlocillin per milliliter and a 1.0-decimeter polarimeter tube. Calculate the specific rotation on an anhydrous basis.

(7) *Identity.* Proceed as directed in § 436.336 of this chapter.

[47 FR 53348, Nov. 26, 1982, as amended at 50 FR 1504, Jan. 11, 1985; 55 FR 11582, Mar. 29, 1990]

§ 440.2a Sterile amdinocillin.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Sterile amdinocillin is 4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[[[hexahydro-1*H*-azepin-1-yl)-methylene]amino]-3,3-dimethyl-7-oxo-, [2*S*-2*α*,5*α*,6*β*]-. It is so purified and dried that:

(i) If the amdinocillin is not packaged for dispensing, its amdinocillin potency is not less than 950 micrograms and not more than 1,050 micrograms of amdinocillin per milligram on an anhydrous basis. If the amdinocillin is packaged for dispensing, its amdinocillin potency is not less than 950 micrograms and not more than 1,050 micrograms of amdinocillin per milligram on an anhydrous basis and also, each container contains not less than 90 percent and not more than 120 percent of the number of milligrams of amdinocillin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) Its moisture content is not more than 0.5 percent.

(v) Its pH in an aqueous solution containing 100 milligrams of amdinocillin per milliliter is not less than 4.0 and not more than 6.2.

(vi) It is crystalline.

(vii) It gives a positive identity test for amdinocillin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for amdinocillin potency, and if packaged for dispensing, amdinocillin potency and container content, sterility, pyrogens, moisture, pH, crystallinity, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(a) If it is packaged for repacking or for use in the manufacture of another drug:

(1) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(2) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) If it is packaged for dispensing:

(1) For all tests except sterility: A minimum of 15 immediate containers.

(2) For sterility testing: 25 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay—(1) Amdinocillin potency and container content.* Proceed as directed in § 436.353 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 220 nanometers, a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl hydrocarbon bonded silicas, e.g., a Whatman ODS-3 column (25-centimeter column having an inside diameter of 4.6 millimeters and 5 micrometer particle size or equivalent), a flow rate of 1.0 milliliter per minute, and an injection volume of 20 microliters. Reagents, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Reagents—(a) Buffer solution 0.01M pH 5.0.* Transfer 1.36 grams of monobasic potassium phosphate in sufficient water to make 1,000 milliliters of solution. Adjust the pH to 5.0 ± 0.1 with 18N phosphoric acid or 10N sodium hydroxide.

(b) *Mobile phase.* Mix acetonitrile (high-pressure liquid chromatography grade): 0.01M pH 5.0 phosphate buffer (15:85).

(ii) *Working standard and sample solutions—(a) Preparation of working standard solution.* Prepare the working standard solution fresh before injection by dissolving an accurately weighed portion of the amdinocillin working standard with sufficient distilled water to obtain a stock solution containing approximately 100 micrograms of amdinocillin per milliliter.

(b) *Preparation of sample solutions—(1) Product not packaged for dispensing (micrograms of amdinocillin per milligram).* Dissolve an accurately weighed portion of the sample with sufficient distilled water to obtain a solution containing 100 micrograms of amdinocillin per milliliter (estimated).

(2) *Product packaged for dispensing.* Determine both micrograms of amdinocillin per milligram of the sample and milligrams of amdinocillin per container. Use separate containers for preparation of each sample solution as described in paragraphs (b)(1)(ii)(b)(2)(i) and (ii) of this section.

(i) *Micrograms of amdinocillin per milligram.* Dissolve an accurately weighed

portion of the sample with sufficient distilled water to obtain a solution containing 100 micrograms of amdinocillin per milliliter (estimated).

(ii) *Milligrams of amdinocillin per container.* Reconstitute the sample as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the solution thus obtained with sufficient distilled water to obtain a solution containing 100 micrograms of amdinocillin per milliliter (estimated).

(iii) *System suitability requirements—(a) Tailing factor.* The tailing factor (*T*) is satisfactory if it is not more than 2.5 at 5 percent of peak height:

(b) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 1,500 theoretical plates.

(c) *Resolution factor.* The resolution factor (*R*) between the peak for amdinocillin and its nearest eluting impurity is satisfactory if it is not less than 2.5.

(d) *Coefficient of variation.* The coefficient of variation (*S_R* in percent) of five replicate injections is satisfactory if it is not more than 2.0 percent.

If the system suitability parameters have been met, then proceed as described in §436.353(b) of this chapter.

(iv) *Calculations.* (a) Calculate the micrograms of amdinocillin per milligram of sample as follows:

$$\frac{\text{Micrograms of amdinocillin per milligram}}{\text{milligram}} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u=Area of the amdinocillin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the amdinocillin peak in the chromatogram of the amdinocillin working standard;

P_s=Amdinocillin activity in the amdinocillin working standard solution in micrograms per milliliter;

C_u=Milligrams of sample per milliliter of sample solution; and

m=Percent moisture content of the sample.

(b) Calculate the amdinocillin content of the container as follows:

$$\frac{\text{Milligrams of amdinocillin per container}}{\text{per container}} = \frac{A_u \times P_s \times d}{A_s \times 1,000}$$

where:

A_u=Area of the amdinocillin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the amdinocillin peak in the chromatogram of the amdinocillin working standard;

P_s=Amdinocillin activity in the amdinocillin working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in §436.32(a) of this chapter, using a solution containing 40 milligrams of amdinocillin per milliliter.

(4) *Moisture.* Proceed as directed in §436.201 of this chapter.

(5) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 100 milligrams of amdinocillin per milliliter.

(6) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

(7) *Identity.* Proceed as directed in §436.211 of this chapter, using a potassium bromide disc containing 1 milligram of amdinocillin in 300 milligrams of potassium bromide, prepared as described in paragraph (b)(1) of that section.

[50 FR 7765, Feb. 26, 1985; 50 FR 10220, Mar. 14, 1985; 50 FR 18243, Apr. 30, 1985; 55 FR 11582, Mar. 29, 1990]

§ 440.3 Amoxicillin trihydrate.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Amoxicillin trihydrate is the trihydrate form of D(-) *α*-amino-*p*-hydroxybenzyl penicillin. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms and not more than 1,050 micrograms of amoxicillin per milligram on an anhydrous basis.

(ii) [Reserved]