

aseptically transfer approximately 300 milligrams of sample into a sterile 500-milliliter Erlenmeyer flask containing approximately 400 milliliters of diluting fluid D. Add at least 200,000 Levy units¹ of penicillinase. Repeat the process using 10 additional containers. Swirl both of the stoppered flasks to completely solubilize the suspension prior to filtration and proceed as directed in paragraph (e)(1)(ii) of that section. If the formulation cannot be filtered, proceed as directed in § 436.20(e)(2) of this chapter, except use medium B in lieu of medium A.

(3) *Pyrogens*. Proceed as directed in § 436.32(f) of this chapter, using a solution containing 20 milligrams of ampicillin per milliliter.

(4) [Reserved]

(5) *Loss on drying*. Proceed as directed in § 436.200(a) of this chapter.

(6) *pH*. Proceed as directed in § 436.202 of this chapter, using the solution obtained when the product is reconstituted as directed in the labeling.

[39 FR 18976, May 30, 1974, as amended at 49 FR 3459, Jan. 27, 1984; 50 FR 19918, 19919, May 13, 1985]

§ 440.209 Ampicillin sodium injectable dosage forms.

§ 440.209a Sterile ampicillin sodium.

The requirements for certification and the tests and methods of assay for sterile ampicillin sodium packaged for dispensing are described in § 440.9a.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59867, Nov. 22, 1977. Redesignated at 52 FR 42288, Nov. 4, 1987]

§ 440.209b Sterile ampicillin sodium and sulbactam sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Ampicillin sodium and sulbactam sodium is a dry mixture of ampicillin sodium and sulbactam sodium in which the ratio of ampicillin to sulbactam is 2:1. Its ampicillin potency is not less than 563 micrograms of ampicillin per milligram on an an-

¹One Levy unit of penicillinase inactivates 59.3 units of penicillin G in 1 hour at 25° C. and at a pH of 7.0 in a phosphate buffered solution of a pure alkali salt of penicillin G when the substrate is in sufficient concentration to maintain a zero order reaction.

hydrous basis. It contains not less than 280 micrograms of sulbactam per milligram on an anhydrous basis. Its ampicillin sodium content is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of ampicillin that it is represented to contain. Its sulbactam sodium content is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of sulbactam that it is represented to contain. It is sterile. It is nonpyrogenic. Its moisture content is not more than 2.0 percent. The pH of an aqueous solution containing 10 milligrams of ampicillin and 5 milligrams of sulbactam per milliliter is not less than 8.0 and not more than 10.0. It passes the identity test for ampicillin and sulbactam. The ampicillin sodium content conforms to the standards prescribed by § 440.9a(a)(1) of this chapter. The sulbactam content conforms to the standards prescribed by § 455.82a(a)(1) of this chapter.

(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 the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The ampicillin sodium used in making the batch for potency, sterility, pyrogens, moisture, pH, crystallinity, and identity.

(B) The sulbactam sodium used in making the batch for potency, sterility, pyrogens, moisture, crystallinity, and identity.

(C) The batch for ampicillin potency, sulbactam potency, sterility, pyrogens, moisture, pH, and identity.

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

(A) The ampicillin sodium used in making the batch: 12 packages, each containing approximately 300 milligrams.

(B) The sulbactam sodium used in making the batch: 12 packages, each containing approximately 300 milligrams.

(C) The batch:

(1) For all tests except sterility: A minimum of 10 immediate containers.

(2) For sterility testing: A minimum of 20 immediate containers collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Ampicillin and sulbactam content*. Proceed as directed in § 436.216 of this chapter, operating isothermally at 25 °C, using an ultraviolet detection system operating at a wavelength of 230 nanometers, a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl hydrocarbon bonded silica, a flow rate of 2.0 milliliters per minute, and a known injection volume of 10 microliters. Reagents, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(A) *1.0M Phosphoric acid*. Prepare by diluting 67.5 milliliters of reagent grade phosphoric acid (85 percent) in distilled water to 1 liter.

(B) *0.005M Tetrabutylammonium hydroxide*. Dilute 6.6 milliliters of tetrabutylammonium hydroxide (40 percent) to 1,800 milliliters with distilled water. Adjust the pH to 5.0 with 1.0M phosphoric acid and dilute with distilled water to 2 liters.

(C) *Mobile phase*. Mix 350 milliliters of acetonitrile with 1,650 milliliters of 0.005M tetrabutylammonium hydroxide. Filter and degas the mobile phase just prior to its introduction into the chromatograph pumping system. (Slight adjustments in pH and/or acetonitrile content may be made to achieve the system suitability parameters defined in paragraph (b)(1)(iii) of this section.)

(ii) *Preparation of working standard and sample solutions*—(A) *Working standard solution*. Accurately weigh a portion of the ampicillin working standard containing the equivalence of approximately 75 milligrams of ampicillin activity and transfer into a 25-milliliter volumetric flask. Accurately weigh a portion of the sulbactam working standard containing 35 milligrams of sulbactam and transfer into the 25-milliliter volumetric flask containing the ampicillin. Dissolve and dilute to volume with mobile phase. Further dilute 5 milliliters to 25 milliliters with mobile phase.

(B) *Sample solution*. Dissolve an accurately weighed sample in sufficient mobile phase to give a stock solution containing 1 milligram of sample per milliliter (estimated); and, also, if it is packaged for dispensing, reconstitute 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 with mobile phase to yield a solution containing about 0.30 milligram sulbactam and about 0.60 milligram ampicillin per milliliter.

(iii) *System suitability requirements*—(A) *Tailing factor*. The tailing factor (*T*) is satisfactory if it is not more than 1.5 at 10 percent of peak height in lieu of 5 percent of peak height.

(B) *Efficiency of the column*. The efficiency of the column (*n*) is satisfactory if it is greater than 3,500 theoretical plates for sulbactam for a 30-centimeter column.

(C) *Resolution*. Dissolve 17.5 milligrams of sulbactam in 50 milliliters of 0.01N sodium hydroxide and let stand for 30 minutes. Adjust the pH of the solution to 5.0 with concentrated phosphoric acid. Transfer a 5-milliliter aliquot of the resulting solution to a 25-milliliter volumetric flask, add 4.25 milliliters of acetonitrile, and dilute to volume with 0.005M tetrabutylammonium hydroxide as described in paragraph (b)(1)(i)(B) of this section. Transfer 2 milliliters of this solution to a 50-milliliter flask, add 30 milligrams of ampicillin potency, dissolve and dilute to volume with mobile phase. Use this solution to determine the resolution factor. The resolution (*R*) between the peaks for ampicillin and sulbactam alkaline degradation product is satisfactory if it is not less than 1.2.

(D) *Coefficient of variation (relative standard deviation)*. The coefficient of variation (*S_r* in percent) of 5 replicate injections is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in § 436.216(b) of this chapter.

Alternate chromatographic conditions are acceptable provided reproducibility and resolution are comparable to the system. However, the sample preparation described in paragraph (b)(1)(ii)(b) of this section should not be changed.

(iv) *Calculations.* (A) Calculate the micrograms of ampicillin or subactam per milligram of sample as follows:

$$\begin{array}{l} \text{Micrograms of} \\ \text{ampicillin or} \\ \text{subactam per} \\ \text{milligram} \end{array} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

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

A_s =Area of the ampicillin or subactam peak in the chromatogram of the ampicillin or subactam working standard;

P_s =Ampicillin or subactam activity in the ampicillin-subactam 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 ampicillin or subactam content of the container as follows:

$$\begin{array}{l} \text{Milligrams of} \\ \text{ampicillin or subactam} \\ \text{per container} \end{array} = \frac{A_u \times P_s \times d}{A_s \times 1,000}$$

where:

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

A_s =Area of the ampicillin or subactam peak in the chromatogram of the ampicillin or subactam working standard;

P_s =Ampicillin or subactam activity in the ampicillin-subactam 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(b) of this chapter, using a solution containing 20 milligrams of subactam and 40 milligrams of ampicillin 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 10 milligrams of ampicillin and 5 milligrams of subactam per milliliter.

(6) *Identity.* The high-performance liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the ampicillin-subactam working standard.

[52 FR 42288, Nov. 4, 1987, as amended at 54 FR 47205, Feb. 20, 1989; 55 FR 11582, Mar. 29, 1990]

§ 440.210 Benzylpenicilloyl-polylysine injection.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Benzylpenicilloyl-polylysine injection is an aqueous solution of benzylpenicilloyl-polylysine. It contains one or more suitable and harmless buffers. Its benzylpenicilloyl content is satisfactory if it is not less than $5.4 \times 10^{-5} M$ and not more than $7.0 \times 10^{-5} M$, except that for the issuance of a certificate for a batch, the benzylpenicilloyl content must be not less than $6.4 \times 10^{-5} M$. It is sterile. It is nonpyrogenic. Its pH is not less than 6.5 and not more than 8.5. The benzylpenicilloyl-polylysine concentrate used conforms to the standards prescribed by § 440.10(a)(1).

(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:

(a) The benzylpenicilloyl-polylysine concentrate used in making the batch for percent benzylpenicilloyl substitution, benzylpenicilloyl content, penamaldate content, penicillenate content, and pH.

(b) The batch for benzylpenicilloyl content, sterility, pyrogens, and pH.

(ii) Samples required:

(a) The benzylpenicilloyl-polylysine concentrate used in making the batch: 2 vials, each containing not less than 5 milliliters.

(b) The batch:
(1) For all tests except sterility: A minimum of 60 immediate containers.