

(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 penicillin G sodium, buffered, used in making the batch for potency, loss on drying, pH, penicillin G content, crystallinity, and heat stability.

(b) The batch for potency, sterility, pyrogens, loss on drying, and pH.

(ii) Samples required:

(a) The penicillin G sodium, buffered, used in making the batch: 10 packages, each containing approximately 60 milligrams.

(b) The batch:

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

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

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* 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 solution 1 to give a stock solution of convenient concentration.

(ii) *Assay procedures.* Assay for potency by any of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(a) *Microbiological agar diffusion assay.* Proceed as directed in §436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 unit of penicillin G per milliliter (estimated).

(b) *Iodometric assay.* Proceed as directed in §436.204 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(c) *Hydroxylamine colorimetric assay.* Proceed as directed in §436.205 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the meth-

od described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in §436.32(b) of this chapter, using a solution containing 20,000 units of penicillin G per milliliter.

(4) [Reserved]

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

(6) *pH.* Proceed as directed §436.202 of this chapter, using an aqueous solution containing 60 milligrams per milliliter.

[42 FR 59872, Nov. 22, 1977; 43 FR 2393, Jan. 17, 1978, as amended at 45 FR 22922, Apr. 4, 1980; 50 FR 19918, 19919, May 13, 1985]

§ 440.283 Sterile piperacillin sodium.

The requirements for certification and the tests and methods of assay for sterile piperacillin sodium packaged for dispensing are described in §440.83a [47 FR 15770, Apr. 13, 1982]

§ 440.290 Ticarcillin disodium injectable dosage forms.

§ 440.290a Sterile ticarcillin disodium.

The requirements for certification and the tests and methods of assay for sterile ticarcillin disodium packaged for dispensing are described in §440.90a.

[43 FR 9800, Mar. 10, 1978. Redesignated at 50 FR 33518, Aug. 20, 1985]

§ 440.290b Sterile ticarcillin disodium and clavulanate potassium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Ticarcillin disodium and clavulanate potassium is a dry mixture of ticarcillin disodium and clavulanate potassium, in which the ratio of ticarcillin to clavulanic acid is 15:1 or 30:1. Its ticarcillin potency is not less than 755 micrograms of ticarcillin per milligram on an anhydrous basis if the ratio is 30:1 and 733 micrograms of ticarcillin per milligram on an anhydrous basis if the ratio is 15:1. Its ticarcillin disodium content is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of ticarcillin that it is represented to contain. Its clavulanate potassium content is satisfactory if it contains not less than 85 percent and not more than 120 percent of the number of milligrams of

clavulanic acid that it is represented to contain. It is sterile. It is nonpyrogenic. Its moisture content is not more than 4.2 percent. Its pH of an aqueous solution containing 100 milligrams per milliliter is not less than 5.5 and not more than 7.5. The ticarcillin disodium conforms to the standards prescribed by § 440.90a(a)(1) except that it contains not less than 840 micrograms of ticarcillin per milligram on an anhydrous basis. The clavulanate potassium conforms to the standards prescribed by § 455.15a(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 ticarcillin disodium used in making the batch for potency, sterility, pyrogens, moisture, pH, and identity.

(b) The clavulanate potassium used in making the batch for potency, sterility, pyrogens, moisture, pH, identity, and clavam-2-carboxylate content.

(c) The batch for ticarcillin potency, ticarcillin content, clavulanic acid content, sterility, pyrogens, moisture, and pH.

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

(a) The ticarcillin disodium used in making the batch: 12 packages, each containing approximately 300 milligrams.

(b) The clavulanate potassium 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) Ticarcillin and clavulanic acid contents.* Determine micrograms of ticarcillin per milligram of sample and milligrams of both ticarcillin and

clavulanic acid per container. Proceed as directed in § 436.355 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength between 220 and 230 nanometers, and a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl silane bonded silicas. Reagents, working standard and sample solutions, system suitability requirements, and calculations for ticarcillin or clavulanic acid content are as follows:

(i) *Reagents—(a) 0.1M Monobasic sodium phosphate buffer solution, pH 4.3.* Transfer 13.8 grams of monobasic sodium phosphate monohydrate to a 1-liter volumetric flask and dissolve in 900 milliliters of distilled water. Adjust the pH to 4.3±0.1 with 18N phosphoric acid or 10N sodium hydroxide. Dilute to volume with distilled water. Mix well.

(b) *Mobile phase.* Mix acetonitrile: 0.1M monobasic sodium phosphate buffer solution, pH 4.3 (5:95 v/v) and mix for no less than two minutes. Degas by passing through a 0.5-micrometer filter with vacuum. The mobile phase may be sparged with the helium through a 2-micrometer metal filter for the duration of the analysis. Adjust the ratio of acetonitrile to aqueous buffer as necessary to obtain satisfactory separation of the peaks.

(c) *Diluent.* 0.05M monobasic sodium phosphate buffer solution, pH 6.4 Transfer 6.9 grams of monobasic sodium phosphate to a 1-liter volumetric flask and dissolve in 900 milliliters of water. Adjust the pH to 6.4 with sodium hydroxide (10N). Dilute to volume with distilled water. Mix well. Use this diluent to prepare the working standard and sample solutions described in paragraph (b)(1)(ii) of this section.

(ii) *Working standard and sample solutions—(a) Preparation of working standard solution.* Accurately weigh a quantity of the ticarcillin working standard containing the equivalent of approximately 90 milligrams of ticarcillin activity and transfer into a 100-milliliter volumetric flask. Prepare a solution of the clavulanic acid working standard containing the equivalent of 30 milligrams or 60 milligrams of clavulanic acid activity in a 100-milliliter volumetric flask. Dissolve and dilute to

volume with diluent. Transfer 10 milliliters of this solution into the flask containing the ticarcillin standard. Dilute the combined standard solution to volume with diluent. Mix. Use within 8 hours after preparation.

(b) *Preparation of sample solutions—(1) Ticarcillin potency (micrograms of ticarcillin per milligram).* Accurately weigh the total contents of a container and dissolve with sufficient diluent to obtain a stock solution containing approximately 30 milligrams of ticarcillin per milliliter. Further dilute this solution with diluent to obtain a final concentration of 0.9 milligrams of ticarcillin per milliliter (estimated).

(2) *Ticarcillin and clavulanic acid content (milligrams of ticarcillin and clavulanic acid per container).* Reconstitute the container with an appropriate volume of distilled water. Using a suitable hypodermic syringe, remove all of the withdrawable contents. Dilute with diluent to obtain a stock solution containing approximately 30 milligrams of ticarcillin per milliliter and 1 or 2 milligrams of clavulanic acid per milliliter. Further dilute this solution with the diluent to obtain a final concentration of 0.9 milligram of ticarcillin per milliliter. The final solution will contain either 0.03 or 0.06 milligram of clavulanic acid per milliliter (estimated) depending on the initial ticarcillin to clavulanic acid ratio.

(iii) *System suitability requirements—(a) Tailing factor.* The tailing factor (T) is satisfactory if it is not more than 2.0.

(b) *Efficiency of the column.* The efficiency of the column (n) is satisfactory if it is greater than 1,000 theoretical plates in a 25-centimeter column.

(c) *Resolution factor.* The resolution factor (R) between the clavulanic acid and ticarcillin peaks is satisfactory if it is not less than 5.0.

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

If the system suitability requirements have been met, then proceed as described in §436.355(b) of this chapter.

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

$$\frac{\text{Micrograms of ticarcillin per milligram}}{= \frac{A_u \times P_s}{A_s \times C_u}}$$

where:

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

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

P_s =Ticarcillin activity in the ticarcillin working standard solution in micrograms of anhydrous ticarcillin free acid per milliliter; and

C_u =Milligrams of sample per milliliter of sample solution.

(b) Calculate the ticarcillin or clavulanic acid anhydrous free acid content of the container as follows:

$$\frac{\text{Milligrams of anhydrous ticarcillin or clavulanic acid free acid per container}}{= \frac{A_u \times P_s \times d}{A_s \times 1,000}}$$

where:

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

A_s =Area of the ticarcillin or clavulanic acid peak in the chromatogram of the ticarcillin or clavulanic acid working standard;

P_s =Anhydrous ticarcillin or clavulanic free acid activity in the ticarcillin-clavulanic acid 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 100 milligrams of ticarcillin 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 a solution containing 100 milligrams per milliliter.

[50 FR 34418, Aug. 20, 1985; 50 FR 42156, Oct. 18, 1985; 50 FR 43384, Oct. 25, 1985; 50 FR 45403, Oct. 31, 1985, as amended at 55 FR 11582, Mar. 29, 1990]