

chloride in 500 milliliters of distilled water.

(2) Saline phosphate buffer, pH 7.6: Dissolve 9 grams of sodium chloride and 1.38 grams monobasic sodium phosphate in 900 milliliters of distilled water, adjust to pH 7.6 and dilute to 1 liter with distilled water.

(b) *Preparation of sample solution.* Transfer 1 milliliter of the benzylpenicilloyl-polylysine concentrate into a 500-milliliter volumetric flask and dilute to volume with saline phosphate buffer, pH 7.6.

(c) *Procedure.* Transfer 3 milliliters of the sample solution into a spectrophotometric cell. Using a suitable spectrophotometer and the saline phosphate buffer, pH 7.6, as a blank, determine the initial absorbance at 282 nanometers. Thereafter, react the diluted benzylpenicilloyl-polylysine solution with 0.02-milliliter portions of the mercuric chloride solution. Determine the absorbance at 282 nanometers at 1 and 3 minutes after each addition of mercuric chloride solution. The increased absorbance at 282 nanometers is used in calculating the benzylpenicilloyl content. Calculate the benzylpenicilloyl content by means of the following formula:

$$\text{Molar benzylpenicilloyl content} = \frac{(A_1 - A_2) \times 500}{22,325}$$

where:

A₁=The highest absorbance at 282 nanometers

A₂=The initial absorbance at 282 nanometers

22,325=The molar absorptivity of the penamaldate formed by the reaction of the benzylpenicilloyl moiety with the mercuric chloride at a pH of 7.6.

Percent benzylpenicilloyl substitution=(Molar benzylpenicilloyl content × 100)/Molar lysine content

(2) *Penicillenate and penamaldate content.* Dilute 1 milliliter of the benzylpenicilloyl-polylysine concentrate to 50 milliliters with saline phosphate buffer, pH 7.6. Using a suitable spectrophotometer and the saline phosphate buffer, pH 7.6, as a blank, determine the absorbance at 322 and 282 nanometers. Calculate the penicillenate content by the following formula:

$$\text{Molar penicillenate content} = \frac{\text{Absorbance at 322 nanometers} \times 50}{26,600}$$

where:

26,600=Molar absorptivity of the penicillenate moiety at 322 nanometers at a pH of 7.6

Calculate the penamaldate content by the following formula:

$$\text{Molar penamaldate content} = \frac{\text{Absorbance at 282 nanometers} \times 50}{22,325}$$

where:

22,325=Molar absorptivity of the penamaldate moiety at 282 nanometers at a pH of 7.6.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

[39 FR 35346, Oct. 1, 1974; 39 FR 38370, Oct. 31, 1974; 39 FR 39871, Nov. 12, 1974; 39 FR 40946, Nov. 22, 1974]

§ 440.11 Carbenicillin indanyl sodium.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Carbenicillin indanyl sodium is the monosodium salt of *N*-(2-carboxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] hept-6-yl)-2-phenylmalonamic acid, 1-(5-indanyl) ester. It is so purified and dried that:

(i) Its potency is not less than 659 micrograms and not more than 769 micrograms of carbenicillin per milligram on an anhydrous basis at the time of certification, and not less than 630 micrograms of carbenicillin per milligram on an anhydrous basis at any time during the expiration period.

(ii) [Reserved]

(iii) Its moisture content is not more than 2.0 percent.

(iv) Its pH in an aqueous solution containing 100 milligrams per milliliter is not less than 5.0 nor more than 8.0.

(v) It gives a positive result to the identity test for carbenicillin indanyl sodium.

(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 potency, moisture, pH, and identity.

(ii) Samples required: Five packages, each containing approximately 1.0 gram and one package containing approximately 2.5 grams.

(b) *Tests and methods of assay—(1) Potency.* Proceed as directed in §436.300 of this chapter.

(2) [Reserved]

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

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

(5) *Identity.* Proceed as directed in §436.211 of this chapter, using the 0.5-percent potassium bromide disc prepared as described in paragraph (b)(1) of that section.

[39 FR 18976, May 30, 1974, as amended at 50 FR 19918, May 13, 1985]

§440.13a Sterile carbenicillin disodium.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Carbenicillin disodium is the disodium salt of α -carboxybenzylpenicillin. It is so purified and dried that:

(i) It contains not less than 770 micrograms of carbenicillin per milligram on an anhydrous basis. If it is packaged for dispensing, its carbenicillin content is not less than 90 percent and not more than 120 percent of the number of milligrams of carbenicillin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its moisture content is not more than 6 percent.

(vi) Its pH in an aqueous solution containing 10 milligrams of carbenicillin per milliliter (or if packaged for dispensing, after reconstitution as directed in the labeling) is not less than 6.0 and not more than 8.0.

(vii) It gives a positive identity test for carbenicillin disodium.

(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 the batch for potency, sterility, pyrogens, moisture, pH, and identity.

(ii) Samples required:

(a) If the batch is packaged for re-packing or for use in the manufacture of another drug:

(1) For all tests except sterility: 10 packages, each containing approximately 300 milligrams; and 5 packages, each containing approximately 1 gram.

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

(b) If the batch is packaged for dispensing:

(1) For all tests except sterility: A minimum of 15 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.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration; 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. If it is a single dose container, use a separate needle and syringe for each container. Dilute with sufficient solution 1 to give a stock solution of convenient concentration. Further dilute the stock solution with solution 1 to the reference concentration of 20.0 micrograms of carbenicillin per milliliter (estimated).