

medium E. During the period of incubation, shake the tubes at least once daily.

(3) *Pyrogens*. Proceed as directed in § 436.32(d) of this chapter, using a solution containing 4,000 units of penicillin G per milliliter.

(4) [Reserved]

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

[42 FR 59868, Nov. 22, 1977, as amended at 43 FR 9799, Mar. 10, 1978; 50 FR 19918, 19919, May 13, 1985]

§ 440.255c Sterile penicillin G benzathine-penicillin G procaine suspension.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Sterile penicillin G benzathine-penicillin G procaine suspension is an aqueous mixture of penicillin G benzathine and penicillin G procaine with or without suitable and harmless buffer substances, suspending agents, and preservatives. Each container or each milliliter contains penicillin G benzathine and penicillin G procaine each equivalent to not less than 150,000 units of penicillin G. Its penicillin G benzathine content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of units of penicillin G that it is represented to contain. Its penicillin G procaine content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of units of penicillin G that it is represented to contain. It is sterile. It is nonpyrogenic. Its pH is not less than 5.0 and not more than 7.5. The penicillin G benzathine used conforms to the standards prescribed by § 440.55a (a)(1). The penicillin G procaine used conforms to the standards prescribed by § 440.74a(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 penicillin G benzathine used in making the batch for potency, mois-

ture, pH, penicillin G content, and crystallinity.

(b) The penicillin G procaine used in making the batch for potency, moisture, pH, penicillin G content, and crystallinity.

(c) The batch for penicillin G benzathine content, penicillin G procaine content, sterility, pyrogens, and pH.

(ii) Samples required:

(a) The penicillin G benzathine used in making the batch: 10 packages, each containing approximately 500 milligrams.

(b) The penicillin G procaine used in making the batch: 10 packages, each containing approximately 500 milligrams.

(c) 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) Total potency.* Assay for total potency by either 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, preparing the sample for assay as follows: Using a suitable hypodermic needle and syringe, place one dose of the drug in a 100-milliliter volumetric flask and add sufficient methyl alcohol to dissolve the benzathine penicillin G. Dilute to volume with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), and shake well. Immediately further dilute an aliquot 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, preparing the sample as follows: Using a suitable hypodermic needle and syringe, withdraw 2 one-dose portions of sample. Place one portion into an appropriate-sized volumetric flask and add 20 milliliters of 0.5N NaOH for each 300,000 units of benzathine penicillin G, mix well, being sure that all penicillin is in solution, and allow to stand for 15 minutes. Add 1 milliliter of 1.2N HCl for each 2 milliliters of 0.5N NaOH,

mix, and dilute with distilled water to obtain a concentration of 2,000 units per milliliter. Dilute the other portion, which is to be used as the blank solution, with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a concentration of approximately 2,000 units per milliliter.

(ii) *Penicillin G procaine content*—(a) *Reagents*—(1) *Sodium nitrite solution*. Dissolve 0.1 gram of sodium nitrite in 100 milliliters of distilled water. Prepare a fresh solution every week and store under refrigeration.

(2) *Ammonium sulfamate solution*. Dissolve 0.5 gram of ammonium sulfamate in 100 milliliters of distilled water and store under refrigeration.

(3) *N-(1-naphthyl)-ethylenediamine solution*. Dissolve 0.1 gram of *N*-(1-naphthyl)-ethylenediamine dihydrochloride in 100 milliliters of distilled water. Prepare fresh solutions every week and store under refrigeration.

(4) *Standard procaine solution*. Prepare a standard solution containing 27.55 milligrams of procaine hydrochloride U.S.P. in a liter of distilled water (each milliliter of the standard solution is equivalent to 60 units of penicillin G procaine).

(b) *Preparation of sample solution*. Using a suitable hypodermic needle and syringe, withdraw a one-dose portion of the sample and place it into an appropriate-sized volumetric flask. Add 20 milliliters of 0.5*N* NaOH for each 300,000 units of penicillin G benzathine, mix well, being sure that all penicillin is in solution, and allow to stand for 15 minutes. Add 1 milliliter of 1.2*N* HCl for each 2 milliliters of 0.5*N* NaOH, mix, and dilute with distilled water to obtain a concentration of 60 units of penicillin G procaine per milliliter. Transfer a 3.0-milliliter aliquot of this solution to a 50-milliliter volumetric flask and add 2 milliliters of water to give a volume of 5 milliliters.

(c) *Procedure*. Transfer respectively, 1.0, 2.0, 3.0, 4.0, and 5.0 milliliters of the standard procaine solution to each of five 50-milliliter volumetric flasks and transfer 5.0 milliliters of distilled water to a sixth 50-milliliter volumetric flask. Add 4.0, 3.0, 2.0, and 1.0 milliliters of water to the first four flasks, respectively, to give each a vol-

ume of 5 milliliters. To each flask of the standard and sample solutions, add 0.5 milliliter of 4*N* HCl, 1.0 milliliter of sodium nitrite solution, 1.0 milliliter of ammonium sulfamate solution, and 1.0 milliliter of *N*-(1-naphthyl)-ethylenediamine solution. Mix and wait two minutes after each addition. Dilute each flask to volume with distilled water. Using a suitable photoelectric colorimeter, determine the absorbancy of each solution at 550 nanometers. The instrument is balanced so that the zero concentration reads 0 absorbancy. Plot the standard curve on coordinate graph paper. Obtain the procaine penicillin content of the solution for assay directly from the point on the standard curve corresponding to its absorbancy.

(iii) *Penicillin G benzathine content*. The sum of the penicillin G procaine content determined as directed in paragraph (b)(1)(ii) of this section subtracted from the total potency determined as directed in paragraph (b)(1)(i) of this section represents the penicillin G benzathine content.

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use medium C in lieu of medium A, medium F in lieu of medium E, and during the period of incubation, shake the tubes at least once daily.

(3) *Pyrogens*. Proceed as directed in § 436.32(d) of this chapter, using a solution containing 4,000 units of penicillin G per milliliter.

(4) [Reserved]

(5) *pH*. Proceed as directed in § 436.202 of this chapter, using the undiluted aqueous suspension.

[42 FR 59868, Nov. 22, 1977, as amended at 43 FR 9799, Mar. 10, 1978; 50 FR 19918, 19919, May 13, 1985]

§ 440.255d Sterile penicillin G benzathine for suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Sterile penicillin G benzathine for suspension is a dry mixture of penicillin G benzathine and one or more suitable suspending or dispersing agents, and with or without one or more suitable preservatives and buffer substances. Its potency is satisfactory if it is not less than 90 percent and not