

the curve. Use this standard curve to calculate the bacitracin content of the sample.

(3) *Bacitracin methylene disalicylate content.* Proceed as directed in paragraph (a)(2) of this section, except prepare the sample as follows: Place a representative portion of the sample (usually approximately 1 gram, accurately weighed) or the entire contents of a single-dose container in blending jar, add 99 milliliters of a 2.0-percent aqueous solution of sodium bicarbonate and 1 milliliter of a 10-percent aqueous solution of polysorbate 80 and blend for 3 minutes in a high-speed blender. Allow the foam to subside, remove an aliquot of the solution, and dilute to 1 unit per milliliter with 1.0-percent phosphate buffer, pH 6.0.

(b) *Moisture.* Proceed as directed in § 436.201.

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§ 436.506 Benzathine penicillin G and buffered crystalline penicillin for aqueous injection.

(a) *Total potency (except in single-dose containers).* Proceed as directed in § 440.80a(b)(1) of this chapter, except if the bioassay method is used prepare the sample by diluting 1.0 milliliter of the drug suspension with sufficient dimethyl formamide, formamide, or methyl alcohol to dissolve the benzathine penicillin. Make to 100 milliliters with buffer. Shake well and dilute to 1.0 unit per milliliter. If the iodometric method is used, proceed as directed in § 440.55a(b) of this chapter, except in preparing the blank solution dilute 1.0 milliliter of the drug suspension to 250 milliliters with 1-percent phosphate buffer at pH 6.0. In preparing the solution for inactivation dissolve 1.0 milliliter of the drug suspension in approximately 20 milliliters of 0.5 *N* NaOH. Allow to stand for 15 minutes. Dilute to 250 milliliters with distilled water. Pipette a 2.0-milliliter aliquot into a 125-milliliter glass-stoppered Erlenmeyer flask and add 2.0 milliliters 1.2 *N* HCl and 10 milliliters 0.01 *N* iodine.

(b) *Buffered crystalline penicillin content.* Place 1.0 milliliter of the drug suspension in a 10-milliliter volumetric flask and add 20 percent sodium sulfate

to make 10 milliliters. Shake well and centrifuge to obtain a clear, or reasonably clear, solution. Dilute a 5.0-milliliter aliquot to 50 milliliters with buffer and proceed as directed in § 440.80a(b)(1) of this chapter to determine the number of units per milliliter of this solution, and from this value calculate the number of units per milliliter of the drug. The content of buffered crystalline penicillin is satisfactory if it is not less than 85 percent of that which it is represented to contain.

(c) *Benzathine penicillin G content.* The benzathine penicillin G content of the batch is the difference between the total potency as described in paragraph (a) or (d) of this section and the content of buffered crystalline penicillin determined by the method prescribed in paragraph (b) of this section. The content of benzathine penicillin G is satisfactory if it is not less than 85 percent of that which it is represented to contain.

(d) *Total potency of a single-dose container.* Add sufficient distilled water to the material remaining in the 10-milliliter volumetric flask referred to in paragraph (b) of this section to bring the volume back to 10 milliliters and determine the number of units per milliliter of this suspension. If the iodometric method is used, 2.0-milliliter aliquots are placed in 50-milliliter volumetric flasks (one blank and one to be inactivated). Obtain the total potency by adding the number of units found in the 10-milliliter volumetric flask to one-half the content of buffered crystalline penicillin found in paragraph (b) of this section.

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

(f) *Moisture.* Proceed as directed in § 440.74a(b)(5) of this chapter.

(g) *Pyrogens.* Proceed as directed in § 436.500.

(h) *Toxicity.* Proceed as directed in § 440.55a(b)(3) of this chapter.

(i) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using the suspension resulting when the

product is reconstituted as directed in the labeling.

§ 436.507 Benzathine - procaine - buffered crystalline penicillins for aqueous injection.

(a) *Potency*—(1) *Total potency*. Proceed as directed in § 440.80a(b)(1) of this chapter, except if the bioassay method is used prepare the sample by diluting one dose of the drug suspension with sufficient dimethyl formamide or formamide or methyl alcohol to dissolve the benzathine penicillin G. Make to 100 milliliters with 1-percent phosphate buffer, pH 6.0. Shake well, and dilute to 1.0 unit per milliliter with buffer. If the iodometric method of assay is used, add the indicated amount of distilled water to the contents of a vial of the sample, shake well, and proceed as follows (except for single-dose containers):

(i) Using a standardized hypodermic syringe, withdraw one dose and dilute with 1-percent phosphate buffer, pH 6.0, to give a concentration of approximately 2,000 units per milliliter. Use 2.0 milliliters of this suspension as the blank in the iodometric assay procedure described in § 440.80a(b)(5)(iv)(a) of this chapter.

(ii) Using a standardized hypodermic syringe, withdraw another dose, place in a flask, and add 20 milliliters of 0.5 *N* NaOH for each 300,000 units of benzathine penicillin, 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 the same volume as was used in paragraph (a)(1)(i) of this section. Place 2.0 milliliters in a 125-milliliter glass-stoppered Erlenmeyer flask, add 10 milliliters of 0.01 *N* iodine, allow to stand for 15 minutes, and titrate with 0.01 *N* sodium thiosulfate as directed in the iodometric assay procedure in § 440.80a(b)(5)(iv)(a) of this chapter. The total potency of the batch is satisfactory if it contains not less than 85 percent of that which it is represented to contain.

(2) *Procaine penicillin content (except for single-dose containers)*. Make suitable dilutions of the solution prepared in paragraph (a)(1)(ii) of this section to obtain approximately 60 units of pro-

caine penicillin per milliliter. Determine the procaine penicillin content by the colorimetric procedure described in § 436.503(b)(3). The content of procaine penicillin is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(3) *Buffered crystalline penicillin content*—(i) *Preparation of the solution for assay*. (a) Add the indicated amount of distilled water to the contents of a vial of the sample, and shake well. Withdraw one dose of the suspension with a hypodermic syringe and place in a 10-milliliter volumetric flask. Add 20-percent sodium sulfate solution almost to the mark, centrifuge sufficiently to see the meniscus, make to volume with 20-percent sodium sulfate solution, shake well, and centrifuge to obtain a clear or reasonably clear solution; or

(b) If the original product contains more than 600,000 units, place it in a 50-milliliter volumetric flask, add 20-percent sodium sulfate to the mark, shake well, place a 10-milliliter portion in a centrifuge tube, and centrifuge to obtain a reasonably clear solution.

(c) Dilute a 5.0-milliliter aliquot of the clear solution obtained in paragraph (a) (3)(i) (a) or (b) of this section with 1-percent phosphate buffer, pH 6.0, to give a solution for assay of approximately 2,000 units per milliliter.

(ii) *Iodometric assay for total penicillin in the solution for assay*. Determine the total quantity of penicillin in the solution for assay by the iodometric assay procedure described in § 440.80a(b)(5)(iv)(a) of this chapter.

(iii) *Colorimetric determination of procaine penicillin in the solution for assay*. Proceed as directed in § 436.503 (b)(3). The content of procaine penicillin in the batch is satisfactory if it is not less than 85 percent of that which it is represented to contain.

(iv) The buffered crystalline penicillin in one dose of the product is calculated as follows:

$$A=(B-C)F,$$

where:

A=the buffered crystalline penicillin content of the product.

B=the number of units of penicillin per milliliter as determined in paragraph (a)(3)(ii) of this section.