

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

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

where:

A=buffered crystalline penicillin content of the product.

B=total number of units of penicillin per milliliter as determined in paragraph (b)(2) of this section.

C=number of units of procaine penicillin per milliliter as determined in paragraph (b)(3) of this section.

F=appropriate dilution factor depending on the dilution made in the preparation of the solution for assay.

The content of buffered crystalline penicillin in the batch is satisfactory when determined by the method described in this paragraph if it is not less than 85 percent of that which it is represented to contain.

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

(d) *Total potency of a one-dose container*. Wash out the material remaining in the 10-milliliter volumetric flask referred to in paragraph (b)(1) of this section with 1-percent phosphate buffer, pH 6.0. Dilute to give a concentration of approximately 2,000 units per milliliter, and assay by the iodometric method described in §440.80a (b)(5)(iv)(a) of this chapter. Obtain the total potency by adding the number of units found in this solution (units per milliliter × volume) to the number of units found (units per milliliter × volume) in the solution assayed in accordance with paragraph (b)(2) of this section.

§ 436.504 Penicillin-bacitracin ointment.

(a) *Potency*—(1) *Penicillin content*. Proceed as directed in §540.380a(b)(1) of this chapter, except the last sentence

of that paragraph. Its content of penicillin is satisfactory if it contains not less than 85 percent of the number of units it is represented to contain.

(2) *Bacitracin content*. Proceed as directed in §448.510a(b)(1) of this chapter, except that sufficient penicillinase is added to the sample under test to completely inactivate the penicillin present. Its content of bacitracin is satisfactory if it contains not less than 85 percent of the number of units it is represented to contain.

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

[39 FR 18944, May 30, 1974, as amended at 40 FR 13497, Mar. 27, 1975]

§ 436.505 Penicillin-streptomycin-bacitracin ointment; penicillin-dihydrostreptomycin-bacitracin ointment; penicillin-streptomycin-bacitracin methylene disalicylate ointment; penicillin-dihydrostreptomycin-bacitracin methylene disalicylate ointment.

(a) *Potency*—(1) *Content of penicillin, streptomycin, and dihydrostreptomycin*. Proceed as directed in §536.501(a) of this chapter.

(2) *Bacitracin content*. Proceed as directed in §448.510a(b)(1) of this chapter, except that:

(i) Sufficient penicillinase is added to the sample under test to completely inactivate the penicillin present.

(ii) Use as the test organism the streptomycin dihydrostreptomycin resistant strain of either *Micrococcus flavus* (ATCC 10240A)¹ or *Sarcina subflava* (ATCC 7468/d),¹ grown and maintained in media containing 500 micrograms of streptomycin or dihydrostreptomycin per milliliter of media, or calculate from the quantity of streptomycin or dihydrostreptomycin found, using the method prescribed by paragraph (a)(1) of this section, the quantity that would be present when the sample is diluted to contain one unit of bacitracin (labeled potency) per milliliter. Prepare the bacitracin standard curve by adding the calculated quantity of streptomycin or dihydrostreptomycin to each concentration of bacitracin used for

¹Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

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.

[39 FR 18944, May 30, 1974, as amended at 40 FR 13497, Mar. 27, 1975]

§ 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