

milliliters of distillate into an Erlenmeyer flask in about 20 minutes, adding water through the funnel as necessary. It is important to keep the liquid volume in the acetyl flask around 2 milliliters to 3 milliliters in order to obtain a quantitative recovery of the acetic acid. Collect a second fraction of distillate, about 10 milliliters in volume. As the second fraction is distilling, process the first fraction. Heat the first reaction and boil gently about 20 seconds. Add a few drops of BaCl<sub>2</sub> solution to check if any sulfate was distilled over. If the sulfate is present, discard and repeat the whole determination. If the sulfate is absent immediately titrate the solution with the 0.015 *N* NaOH solution to a faint pink endpoint, using one drop of phenolphthalein solution as the indicator. Repeat the above procedure with the second fraction. If the second fraction requires less than 0.10 milliliter of the 0.015 *N* NaOH solution and all the acetic acid has been distilled over, the determination is completed. If greater than this, collect a third fraction of approximately 10 milliliters and titrate this as before. Total volumes of NaOH used and calculate results as follows:

$$\frac{(\text{Milliliters of NaOH} \times N \text{ NaOH} \times 0.043 \times 100)}{\text{Weight sample in grams}} = \text{Percent acetyl.}$$

(7) *Crystallinity*. Proceed as directed in § 440.80a(b)(5)(iii) of this chapter.

**§ 436.516 Tetracycline-neomycin complex powder topical; tetracycline hydrochloride-neomycin sulfate powder topical.**

(a) *Potency*—(1) *Tetracycline-neomycin complex powder*—(i) *Tetracycline content*. Proceed as directed in § 436.514(a)(2), except use water in lieu of 0.1 *N* HCl for dissolving the sample. Its tetracycline content is satisfactory if it contains not less than 85 percent of the equivalent number of milligrams of tetracycline hydrochloride that it is represented to contain.

(ii) *Neomycin content*. Using 0.1 *M* potassium phosphate buffer, pH 8.0, dilute an appropriate aliquot of the aqueous solution, prepared as directed in paragraph (a)(1) of this section, to a final concentration of 1 μg. per milliliter (estimated), and proceed as directed in § 436.515(c)(1), except that the neomycin standard stock solution described

§ 436.517(b)(1)(iii) is used to prepare the standard curve, by further diluting with pH 8.0 buffer to final concentrations of 0.64, 0.80, 1.0, 1.25, and 1.56 μg. per milliliter. The 1.0 μg. per milliliter solution is the reference concentration. In lieu of the method described in this subparagraph, the neomycin content may also be determined as follows. Using the aqueous solution described, prepare the sample and proceed as directed in § 436.517(b)(1), except use *Staphylococcus aureus* (American Type Culture Collection 12715)<sup>1</sup> as the test organism, which is grown and maintained on agar containing 100 μg. of tetracycline hydrochloride per milliliter of agar. Its neomycin content is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(2) *Tetracycline hydrochloride-neomycin sulfate powder*—(i) *Tetracycline hydrochloride content*. Prepare the sample as directed in § 436.514(a)(2). Use an appropriate aliquot and proceed as directed in § 446.81a(b)(1) of this chapter. Its tetracycline hydrochloride content is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(ii) *Neomycin content*. Use an appropriate aliquot of the solution prepared in paragraph (a)(2)(i) of this section and proceed as directed in paragraph (a)(1)(ii) of this section. Its neomycin content is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(b) *Sterility*. Thoroughly cleanse with a suitable disinfectant the value (do not flame) of each container to be tested. Into each of two empty, sterile Erlenmeyer flasks stoppered with a cotton plug, spray quantities sufficient to yield a residue of approximately the equivalent of 50 milligrams from 10 separate cans by removing the plug temporarily and using aseptic technique while spraying; allow propellant to evaporate, add 250 milliliters to 500 milliliters of diluting fluid B in lieu of diluting fluid A, and swirl the flasks to dissolve the contents. Then proceed as

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

directed in § 436.20 of this chapter using the method described in paragraph (e)(1) of that section.

(c) *Moisture*. Proceed as directed in § 536.513(c) of this chapter.

(d) *Tetracycline-neomycin complex used in making the drug*—(1) *Potency*—(i) *Tetracycline content*. Dissolve the sample to be tested in sufficient water to give a convenient stock solution. Using an appropriate aliquot, proceed as directed in § 446.81a(b)(1).

(ii) *Neomycin content*. Using an aliquot of the stock solution prepared as directed in paragraph (d)(1)(i) of this paragraph, proceed as directed in paragraph (a)(2) of this section, except the last sentence of that subparagraph.

(2) *Toxicity*. Proceed as directed in § 440.80a(b)(4) of this chapter using 0.5 milliliter of a solution prepared by diluting the sample with physiological sodium chloride solution to contain 200  $\mu$  g. of neomycin per milliliter (estimated).

(3) *pH*. Using a 1-percent aqueous solution, proceed as directed in § 440.80a(b)(5)(ii) of this chapter.

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**§ 436.517 Bacitracin-neomycin tablets; zinc bacitracin-neomycin tablets; bacitracin methylene disalicylate-neomycin tablets.**

(a) *Tablets*—(1) *Potency*—(i) *Bacitracin, zinc bacitracin, or bacitracin methylene disalicylate content*. Proceed as directed in § 448.110a(b)(1). Its content of bacitracin, zinc bacitracin, or bacitracin methylene disalicylate is satisfactory if it contains not less than 85 percent of the number of units per tablet that it is represented to contain.

(ii) *Neomycin content*. Place 5 tablets in a blending jar and add thereto 200 milliliters of a 500-milliliter quantity of 0.10-percent phosphate buffer pH 8.0. After blending for 1 minute with a high-speed blender, add the remainder of the buffer. Blend again for 1 minute and make the proper estimated dilutions in the buffer and proceed as directed in paragraph (b)(1) of this section. Its content of neomycin is satisfactory if it contains not less than 85 percent of the number of milligrams of activity that it is represented to contain.

(2) *Moisture*. Proceed as directed in § 440.80a(b)(5)(i) of this chapter.

(3) *Disintegration time*. Proceed as directed in § 440.180a(b)(3).

(b) *Neomycin used in making the tablets*—(1) *Potency*—(i) *Cylinders (cups)*. Use cylinders described under § 440.80a(b)(1)(i) of this chapter.

(ii) *Culture medium*. Use the medium described in § 440.80a(b)(1)(ii)(a) of this chapter for both the base and seed layers, except its pH after sterilization is 7.8 to 8.0.

(iii) *Working standard*. Dry the working standard (obtained from the U.S.P. Reference Standards Committee, 46 Park Avenue, New York 16, N.Y.) for 3 hours at 60° C. and a pressure of 5 millimeters or less and weigh out a sufficient quantity to make a convenient stock solution by diluting with a 0.1 M potassium phosphate buffer, pH 7.8 to 8.0. The stock solution, when stored at a temperature of approximately 15° C., or less, may be used for a period not exceeding 1 month.

(iv) *Standard curve*. Using the stock solution, prepare a daily standard curve as directed in § 444.70a(b)(1)(iv) of this chapter, using solutions of the neomycin working standard in 0.1M potassium phosphate buffer, pH 8.0, in concentrations of 6.4, 8.0, 10.0, 12.5, and 15.6 micrograms per milliliter if the test organism is *Staphylococcus aureus* (ATCC 6538P),<sup>1</sup> or in concentrations of 0.64, 0.80, 1.0, 1.25, and 1.56 micrograms per milliliter if the test organism is *Staphylococcus epidermidis* (ATCC 12228).<sup>1</sup> The 10.0 micrograms per milliliter and the 1.0 microgram per milliliter concentrations are used as the reference points.

(v) *Preparation of test organism*. The test organism is *Staphylococcus aureus* (ATCC 6538P),<sup>1</sup> which is maintained on agar described in § 440.80a(b)(1)(ii)(a) of this chapter. From a stock slant inoculate a Roux bottle containing this same agar and incubate for 24 hours at 32° C.–35° C. Wash the resulting growth from the agar surface with about 50 milliliters of sterile sodium chloride solution. Standardize this suspension by determining the dilution that will permit 80 percent light transmission through a filter at 6500 Angstrom units

<sup>1</sup>See footnote 1 to § 436.516.