

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.

in a photoelectric colorimeter. The suspension may be used for 2 weeks if it is stored under refrigeration. *Staphylococcus epidermidis* (ATCC 12228),<sup>1</sup> which is maintained on agar as described in § 440.80a(b)(1)(ii)(a) of this chapter, may also be used as the test organism. From a stock slant, inoculate a Roux bottle containing this medium and incubate for 24 hours at 32° C.-35° C. Wash the resulting growth from the agar surface, using approximately 30 milliliters of sterile sodium chloride solution. Standardize the suspension by determining the dilution that will permit 80 percent light transmission through a filter of 6500 Angstrom units in a photoelectric colorimeter. The suspension may be stored for 2 weeks under refrigeration.

(vi) *Preparation of plates.* Using the agar described in subdivision (ii) of this subparagraph and approximately a 0.5 percent inoculum of the suspension described in paragraph (b)(1)(v) of this section, prepare the plates as directed in § 440.80a(b)(1)(v) of this chapter.

(vii) *Assay.* Dissolve volumetrically in 0.1 M potassium phosphate buffer, pH 7.8 to 8.0, the sample to be tested to make a convenient stock solution. Further dilute volumetrically this solution with 0.1 M potassium phosphate buffer, pH 7.8 to 8.0, to a final concentration of 10.0 micrograms (estimated) per milliliter, if the test organism is *Staphylococcus aureus* or 1.0 microgram per milliliter (estimated) if the test organism is *Staphylococcus epidermidis*.

(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 to approximately 200 micrograms per milliliter with physiological salt solution.

(3) *Moisture.* In an atmosphere of about 10 percent relative humidity, transfer about 100 milligrams of the finely powdered sample to a tared weighing bottle equipped with ground-glass top and stopper. Weigh the bottle and place it in a vacuum oven, tilting the stopper on its side so that there is no closure during the drying period. Dry at a temperature of 60° C. and a pressure of 5 millimeters of mercury or less for 3 hours. At the end of the drying period fill the vacuum oven with air dried by passing it through a drying

agent such as sulfuric acid or silica gel. Replace the stopper and place the weighing bottle in a desiccator over a desiccating agent such as phosphorous pentoxide or silica gel, allow to cool to room temperature, and reweigh. Calculate the percent loss.

(4) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using a solution containing 33 milligrams per milliliter.

**§ 436.542 Acid resistance/dissolution test for enteric-coated erythromycin pellets.**

(a) *Equipment.* Use Apparatus 1 as described in the United States Pharmacopeia XX dissolution test.

(b) *Immersion fluids.* All immersion fluids may be degassed by heating immediately prior to use.

(1) *Acid resistance medium.* Use 0.06N hydrochloric acid, pH 1.2.

(2) *Dissolution medium.* Dissolve 6.8 grams of monobasic potassium phosphate in 250 milliliters of water. Add 109 milliliters of 0.2N sodium hydroxide and 400 milliliters of water and adjust the resulting solution with 0.2N sodium hydroxide to a pH of 6.8±0.1. Dilute to 1 liter.

(c) *Procedure.* Warm the immersion fluids to a temperature of 37° ±5.0° C. Place the contents of one capsule into the basket. Lower the basket into 900 milliliters of acid resistance medium contained in the beaker. Ensure that all air is displaced from the immersed basket and that the pellets remain in the basket. Rotate the basket at the speed of 50 revolutions per minute for an accurately timed period of 1 hour. Remove the basket from the fluid and immediately lower the basket into 900 milliliters of dissolution medium contained in the beaker. Again ensure that all air is displaced from the immersed basket and that the pellets remain in the basket. Rotate the basket at 50 revolutions per minute for an accurately timed dissolution period of 45 minutes. Withdraw a 25-milliliter sample of the dissolution medium from a point midway between the stirring shaft and the wall of the vessel and approximately midway in depth. Filter the sample through a Whatman 541 filter paper or equivalent, discarding the first 2 milliliters. Assay for erythromycin using