

in a photoelectric colorimeter. The suspension may be used for 2 weeks if it is stored under refrigeration. *Staphylococcus epidermidis* (ATCC 12228),¹ 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

the filtrate as the test solution as directed in §436.105. Repeat the test on five additional capsules.

(d) *Evaluation.* Use the interpretation described in the United States Pharmacopeia XX dissolution test.

[46 FR 16678, Mar. 13, 1981, as amended at 50 FR 47213, Nov. 15, 1985; 52 FR 35912, Sept. 24, 1987; 54 FR 41824, Oct. 12, 1989]

§436.543 Acid resistance test for pellet-filled doxycycline hyclate capsules.

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

(b) *Acid resistance medium.* Use 0.06*N* hydrochloric acid, pH 1.2. May be degassed by heating immediately prior to use.

(c) *Procedure.* Warm the acid resistance medium to a temperature of 37±2.0 ° C. Place the contents of one pellet-filled capsule into the basket. Lower the basket into a beaker containing 900 milliliters of acid resistance medium. Ensure that all air is displaced from the immersed basket and that the contents of the pellet-filled capsule remain in the basket.

Rotate the basket at the speed of 50 revolutions per minute for an accurately timed period of 20 minutes. Withdraw a 5-milliliter sample of the acid resistance medium from a point midway between the stirring shaft and the wall of the vessel and approximately midway in depth (this is the sample solution). Assay the sample solution for doxycycline as described in paragraph (d) of this section. Repeat the test on five additional pellet-filled capsules.

(d) *Doxycycline content*—(1) *Preparation of working standard solution.* Dissolve an accurately weighed portion of doxycycline hyclate working standard with 0.1*M* hydrochloric acid to obtain a concentration of 0.01 milligram per milliliter.

(2) *Preparation of sample solution.* Dilute the 5-milliliter sample portion to 25 milliliters with 0.1*M* hydrochloric acid.

(3) *Procedure.* Using a suitable spectrophotometer and 0.1*M* hydrochloric acid as the blank, determine the absorbance of each standard and sample solution at the absorbance peak at ap-

proximately 345 nanometers. Determine the exact position of the absorption peak for the particular instrument used.

(4) *Calculations.* Determine the total amount of doxycycline dissolved as follows:

$$\text{Milligrams of doxycycline dissolved} = \frac{A_u \times c \times d \times 900^*}{A_s}$$

where:

A_u =Absorbance of sample;

A_s =Absorbance of standard;

c =Concentration of working standard in milligrams; and

d =Dilution factor of sample withdrawn from beaker.

*If more than 15 milliliters of dissolution medium is removed, correct for the volume removed.

(e) *Evaluation.* The pellet-filled capsule passes the test if no more than 50 percent of the drug is dissolved at 20 minutes. If one pellet-filled capsule fails to meet this requirement, repeat the test on six additional pellet-filled capsules. No more than 2 pellet-filled capsules in 12 may exceed 50 percent of the drug dissolved at 20 minutes.

[50 FR 41679, Oct. 15, 1985; 50 FR 45603, Nov. 1, 1985]

§436.544 Dissolution test for pellet-filled doxycycline hyclate capsules.

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

(b) *Dissolution medium.* Prepare the dissolution medium as follows: Dissolve 10.21 grams of potassium biphthalate and 1.4 grams of sodium hydroxide in approximately 950 milliliters of distilled water and adjust the pH to 5.5 using 1*M* sodium hydroxide solution. Dilute with distilled water to 1,000 milliliters.

(c) *Procedure.* Proceed as directed in the United States Pharmacopeia XXI dissolution test. Ensure that all air is displaced from the immersed basket and that the contents of the pellet-filled capsule remain in the basket. Rotate the basket at the speed of 50 revolutions per minute for an accurately timed period of 30 minutes. Withdraw a 5-milliliter sample of the dissolution medium from a point midway between the stirring shaft and the wall of the