

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 0.1M 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 0.1M hydrochloric acid.

(3) *Procedure*. Using a suitable spectrophotometer and 0.1M hydrochloric acid as the blank, determine the absorbance of each standard and sample solution at the absorbance peak at approximately 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d =Dilution factor of sample withdrawn from beaker.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*. Use the dissolution acceptance table and interpretation in the United States Pharmacopeia XXI.

[50 FR 41679, Oct. 15, 1985]

§ 436.545 Acid resistance test for erythromycin particles in tablets.

(a) *Equipment*. Use Apparatus 2 as described in the United States Pharmacopeia XXI dissolution test.

(b) *Acid resistance medium*. Use 0.1N hydrochloric acid, 500 milliliters.

(c) *Procedure*. Warm the immersion fluid to a temperature of 37±0.5 °C. Place one tablet into a vessel containing 500 milliliters of acid resistance

medium. Rotate the paddle at the speed of 50 revolutions per minute for an accurately timed period of 1 hour. Withdraw a 50-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 No. 1 filter paper or equivalent, discarding the first 5.0 milliliters. Assay for dissolved erythromycin as directed in paragraph (d) of this section using the filtrate as the sample solution. Repeat the test on five additional tablets.

(d) *Arsenomolybdate colorimetric assay for dissolved erythromycin*—(1) *Apparatus*. Automatic analyzer consisting of (i) a liquid sampler, (ii) a proportioning pump, (iii) suitable spectrophotometers equipped with matched flow cells and analysis capability at 660 nanometers, (iv) a means of recording spectrophotometric readings, and (v) a manifold consisting of the components illustrated in the diagram in paragraph (d)(4) of this section.

(2) *Reagents*—(i) *Arsenomolybdate solutions*—(a) *Stock solution*. Dissolve 100 grams of ammonium molybdate in approximately 1,700 milliliters of water contained in a 2-liter volumetric flask. Insert an inert plastic coated stirring bar into the flask, and begin mixing. While mixing, slowly add 84 milliliters of sulfuric acid (temperature of solution should not exceed 50 °C). Dissolve 12 grams of sodium arsenate in 100 milliliters of water, and add to the solution in the flask. Remove the stirring bar, dilute with water to volume, and mix. Store in an amber bottle for 24 hours before using. (This solution should not be allowed to come into contact with rubber.)

(b) *Working solution*. Dilute 1 part of stock solution with 2 parts of water, and mix. This solution is freshly prepared on the day of use.

(ii) *Acetate buffer, pH 4.8*. Dissolve 133 grams of ACS grade sodium acetate crystals in about 3.5 liters of water. Adjust the pH to 4.8±0.1 with glacial acetic acid. Dilute with water to 4,000 milliliters, and mix.

(iii) *9N Sulfuric acid*. Place a 2-liter volumetric flask containing an inert plastic coated magnetic stirring bar and about 1,500 milliliters of water in

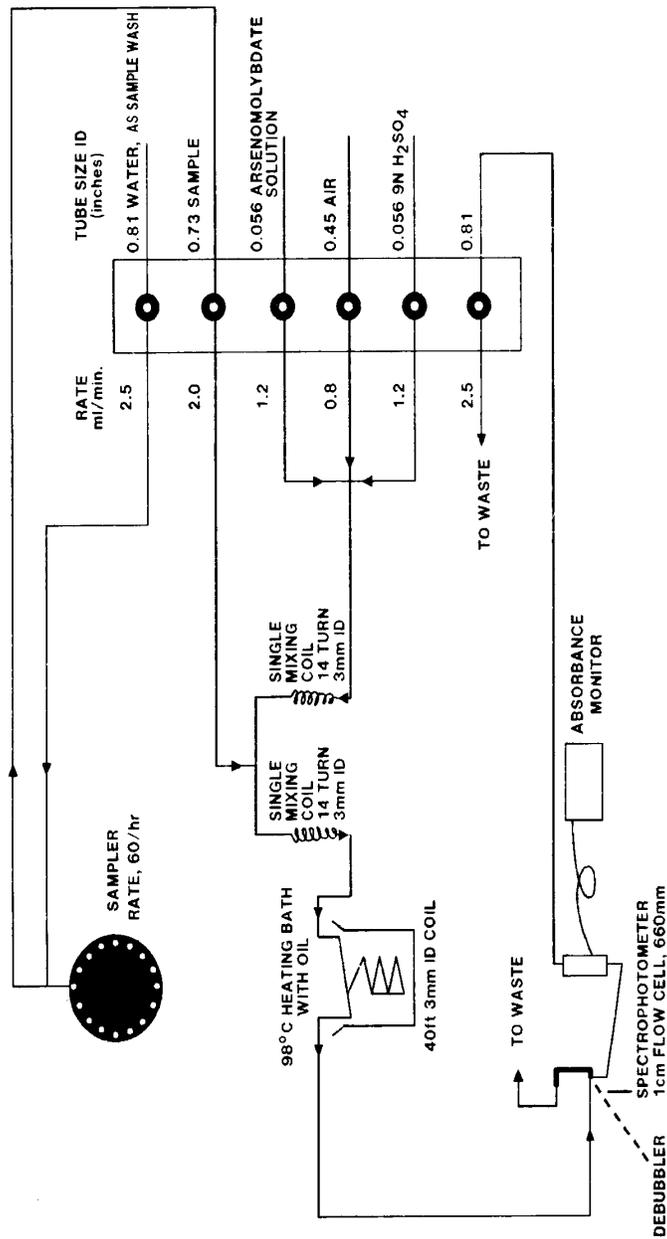
an ice bath, and begin mixing. While mixing, cautiously add 300 milliliters of sulfuric acid. Allow the solution to cool. Remove the stirring bar, dilute with water to volume, and mix.

(3) *Preparation of working standard solutions*—(i) *Working standard stock solution*. Accurately weigh approximately 400 milligrams of USP Erythromycin Reference Standard, previously dried at 60 °C for 3 hours under vacuum (pressure of 5 millimeters of mercury or less), and transfer to a 100-milliliter volumetric flask. Dissolve and dilute with acetate buffer, pH 4.8 to volume, and mix.

(ii) *Working standard solutions*. Pipet 5, 10, 15, and 20 milliliters of the standard stock solution into separate 500-milliliter volumetric flasks, add acetate buffer, pH 4.8 to volume, and mix. The approximate concentrations of these solutions (before adjusting for the standard potency) are 40, 80, 120,

and 160 micrograms of erythromycin per milliliter, respectively.

(4) *Procedure*. Use the working standard solutions prepared as described in paragraph (d)(3) of this section. The arrangement of the apparatus and flow of the samples and reagents are shown in the manifold diagram set forth following this paragraph. The sampler rate is usually 60 per hour, but may be varied. Establish a steady state by pumping reagents until the record trace becomes constant. Place cups containing the four concentrations of working standard solutions in the sampler followed by no more than 12 cups of sample solutions. Then place four more cups containing the four concentrations of working standard solutions in the sampler. Repeat the sequence above for additional samples by bracketing standards around no more than 12 sample solutions at a time.



(5) *System suitability test.* Perform a linear regression analysis of absorbance versus concentration in micrograms per milliliter of the stand-

ards. The system is suitable for calculation if the beginning baseline and the ending baseline after assaying a series of standard and sample solutions

does not vary by more than 2 percent transmittance, and the correlation coefficient for each standard curve is greater than 0.995.

(6) *Calculations.* (i) Calculate the concentration of each standard curve solution in micrograms of erythromycin per milliliter as follows:

$$\text{Concentration of each standard curve solution (micrograms of erythromycin per milliliter)} = \left(\frac{\text{Milligrams of working standard} \times \text{Potency of working standard (micrograms per milligram)}}{100} \right) \times \left(\frac{\text{Milliliters of standard stock solution}}{500} \right)$$

(ii) Calculate the percent of labeled amount of erythromycin released in 60 minutes as follows:

$$\text{Percent of labeled amount of erythromycin released in 60 minutes} = \left(\frac{500}{1,000} \right) \times \left(\frac{100}{\text{erythromycin content of tablet}} \right) \times \text{Micrograms of erythromycin per milliliter}$$

[51 FR 37721, Oct. 24, 1986]

PART 440—PENICILLIN ANTIBIOTIC DRUGS

Subpart A—Bulk Drugs

- Sec.
- 440.1a Sterile azlocillin sodium.
- 440.2a Sterile amdinocillin.
- 440.3 Amoxicillin trihydrate.
- 440.5 Ampicillin.
- 440.7 Ampicillin trihydrate.
- 440.7a Sterile ampicillin trihydrate.
- 440.8 Bacampicillin hydrochloride.
- 440.9a Sterile ampicillin sodium.
- 440.10 Benzylpenicilloyl-polylysine concentrate.
- 440.11 Carbenicillin indanyl sodium.
- 440.13a Sterile carbenicillin disodium.
- 440.15 Cloxacillin sodium monohydrate.
- 440.17 Cyclacillin.
- 440.19 Dicloxacillin sodium monohydrate.
- 440.19a Sterile dicloxacillin sodium monohydrate.
- 440.25 Hetacillin.
- 440.29 Hetacillin potassium.
- 440.29a Sterile hetacillin potassium.
- 440.36a Sterile methicillin sodium monohydrate.
- 440.37a Sterile mezlocillin sodium monohydrate.
- 440.41 Nafcillin sodium monohydrate.
- 440.41a Sterile nafcillin sodium monohydrate.
- 440.49 Oxacillin sodium monohydrate.
- 440.49a Sterile oxacillin sodium monohydrate.
- 440.55a Sterile penicillin G benzathine.

- 440.71 Penicillin V.
- 440.73 Penicillin V potassium.
- 440.74a Sterile penicillin G procaine.
- 440.80 Penicillin G potassium.
- 440.80a Sterile penicillin G potassium.
- 440.81a Sterile penicillin G sodium.
- 440.83a Sterile piperacillin sodium.
- 440.90a Sterile ticarcillin disodium.
- 440.91 Ticarcillin monosodium monohydrate.

Subpart B—Oral Dosage Forms

- 440.103 Amoxicillin oral dosage forms.
- 440.103a Amoxicillin trihydrate capsules.
- 440.103b Amoxicillin trihydrate for oral suspension.
- 440.103c Amoxicillin trihydrate chewable tablets.
- 440.103d Amoxicillin trihydrate and clavulanate potassium tablets.
- 440.103e Amoxicillin trihydrate and clavulanate potassium for oral suspension.
- 440.103f Amoxicillin trihydrate-clavulanate potassium chewable tablets.
- 440.105 Ampicillin oral dosage forms.
- 440.105a Ampicillin tablets.
- 440.105b Ampicillin chewable tablets.
- 440.105c Ampicillin capsules.
- 440.105d Ampicillin for oral suspension.
- 440.107 Ampicillin trihydrate oral dosage forms.
- 440.107a Ampicillin trihydrate chewable tablets.
- 440.107b Ampicillin trihydrate capsules.
- 440.107c Ampicillin trihydrate for oral suspension.
- 440.107d Ampicillin trihydrate-probenecid for oral suspension.