

unit of one of the standard buffer solutions. Standardize the pH meter with the two buffer solutions. Make any necessary adjustment of the meter if the observed pH value of either standard solution differs by more than 0.05 pH units of its known value.

(c) *Sample preparation.* If necessary, dilute the sample with carbon dioxide-free distilled water to the concentration specified in the individual section for each antibiotic.

(d) *Test procedure.* Determine the pH of the sample at 25±2° C. Rinse the electrode(s) between determinations first with distilled water and then with a portion of the next sample to be tested. Store electrode(s) with tips immersed in water.

[39 FR 18944, May 30, 1974, as amended at 42 FR 29857, June 10, 1977; 42 FR 31449, June 21, 1977]

§ 436.203 Crystallinity.

Use the method specified in the individual section for each antibiotic.

(a) *Method 1.* To prepare the sample for examination, mount a few particles in mineral oil on a clean glass slide. Examine the sample by means of a po-

larizing microscope. The particles reveal the phenomena of birefringence and extinction positions on revolving the microscope stage.

(b) *Method 2.* Proceed as directed in paragraph (a) of this section, except to prepare the sample for examination, mount a few particles in mineral oil, add 1 drop of ethyl alcohol, and allow to react for about 30 seconds.

§ 436.204 Iodometric assay.

(a) *Reagents.* (1) 0.01*N* Sodium thiosulfate (2.482 grams Na₂S₂O₃·5H₂O and 125 milligrams Na₂CO₃ per liter).

(2) 1.0*N* Sodium hydroxide.

(3) 1.2*N* Hydrochloric acid.

(4) 0.01*N* Iodine solution (prepared from 0.1*N* iodine U.S.P.).

(5) Starch iodide paste, T.S. (U.S.P.).

(b) *Preparation of sample and working standard solutions—*(1) *Working standard solutions.* From the following table, select the initial solvent, diluent, and final concentration as listed for each antibiotic working standard. Dissolve and dilute an accurately weighed portion to the specified final concentration and proceed as directed in paragraphs (c) and (d) of this section.

Antibiotic	Initial solvent	Diluent (solution number as listed in § 436.101(a))	Final concentration in units or milligrams of activity per milliliter of standard solution
Amoxicillin	None	Distilled water	1.0 mg.
Ampicillindodo	1.25 mg.
Cephalexindodo	2 mg.
Cephaloridinedodo	2 mg.
Cephalothindodo	2 mg.
Cephapirindodo	2 mg.
Cloxacillindodo	1.25 mg.
Cyclacillindodo	1.0 mg.
Dicloxacillindo	1	1.25 mg.
Methicillindo	1	1.25 mg.
Nafcillindo	1	1.25 mg.
Oxacillindo	1	1.25 mg.
Penicillin Gdo	1	2,000 units.
Penicillin V potassiumdo	1	2,000 units.

(2) *Bulk antibiotic solutions.* From the following table, select the initial solvent, diluent, and final concentration as listed for each antibiotic. Dissolve an accurately weighed aliquot (approximately 30 to 60 milligrams) of the sample, dilute to the appropriate final concentration, and proceed as directed in paragraphs (c) and (d) of this section.

Antibiotic	Initial solvent	Diluent (solution number as listed in § 436.101(a))	Final concentration in units or milligrams of activity per milliliter of sample
Amoxicillin trihydrate	None	Distilled water	1.0 mg.
Ampicillindodo	1.25 mg.
Ampicillin sodiumdo	1	1.25 mg.
Ampicillin trihydratedo	Distilled water	125 mg.

Antibiotic	Initial solvent	Diluent (solution number as listed in § 436.101(a))	Final concentration in units or milligrams of activity per milliliter of sample
Bacampicillin hydrochloride	Nonedo	1.25 mg. ²
Cephalexindodo	2 mg.
Cephaloridinedodo	2 mg.
Cephalothin sodiumdodo	2 mg.
Cephapirin sodiumdodo	2 mg.
Cloxacillin sodium monohydratedodo	1.25 mg.
Cloxacillin sodium monohydratedodo	1.25 mg.
Dicloxacillin sodium monohydratedo	1	1.25 mg.
Cyclacillindo	Distilled water	1.0 mg.
Methicillin sodium monohydratedo	1	1.25 mg.
Mezlocillindo	Distilled water	2.0 mg.
Nafcillin sodium monohydratedo	1	1.25 mg.
Oxacillin sodium monohydratedo	1	1.25 mg.
Penicillin G benzathine blank solutiondo	Distilled water	2,000 units.
Penicillin G benzathine inactivated solutiondo	1N NaOH	2,000 units. ¹
Penicillin G potassiumdo	1	2,000 units.
Penicillin G procaine	2 ml methyl alcohol	1	2,000 units.
Penicillin G sodium	None	1	2,000 units.
Penicillin V	2 ml methyl alcohol	1	2,000 units.
Penicillin V potassiumdo	1	2,000 units.

¹ Allow to stand in 1N NaOH for 15 minutes before assaying.
²The final concentration of bacampicillin hydrochloride is calculated in milligrams of ampicillin activity per milliliter of sample. The ampicillin working standard is used for the assay of bacampicillin hydrochloride.

(3) *Finished product solutions.* Prepare the sample for assay as directed in the individual section for each antibiotic product to be tested by diluting to the concentration prescribed in the table in paragraph (b)(2) of this section and proceed as described in paragraphs (c) and (d) of this section.

(c) *Inactivated sample and standard solutions.* (1) Transfer 2.0 milliliters each of the sample and the appropriate working standard solutions to glass-stoppered Erlenmeyer flasks.

(2) Add 2.0 milliliters of 1N sodium hydroxide, except if the sample has been diluted in 1N sodium hydroxide, and allow to stand at room temperature for 15 minutes.

(3) Add 2.0 milliliters of 1.2N hydrochloric acid.

(4) Add 10.0 milliliters of 0.01N iodine solution, stopper, allow to stand at room temperature for 15 minutes, and proceed as directed in paragraph (e) of this section.

(d) *Blank determination.* Transfer 2.0 milliliters each of the sample and the appropriate working standard solutions to glass-stoppered Erlenmeyer flasks. Add 10.0 milliliters of 0.01N iodine solution and immediately proceed as directed in paragraph (e) of this section.

(e) *Titration procedure.* Titrate the excess iodine using 0.01N sodium thiosulfate. Toward the end of the ti-

tration, add 1 drop of the starch iodide paste. Continue the titration by the addition of 0.01- to 0.02-milliliter portions of 0.01N sodium thiosulfate, shaking vigorously after each addition. The endpoint is reached when the blue color of the starch-iodine complex is discharged. Calculate the antibiotic content as described in paragraph (f) of this section.

(f) *Calculations—(1) F factor determination.* Using the appropriate working standard for the particular antibiotic to be tested, assay the standard as directed in this section. Calculate the F factor (the units of micrograms of activity equivalent of each milliliter of 0.01N sodium thiosulfate consumed) by means of the following formula:

$$F = \frac{W_s \times P}{V_s}$$

where:

W_s=Actual weight in milligrams of standard in the 2.0 milliliters titrated;

P=Potency of the working standard in units or micrograms per milligram;

V_s=Milliliters of sodium thiosulfate used in the working standard blank determination minus the milliliters of sodium thiosulfate used in the titration of the inactivated working standard solution (the difference is the equivalent of the number of milliliters of 0.01N iodine absorbed by the inactivated standard).

(2) *Bulk antibiotic.* Calculate the potency of the sample in units or micrograms per milligram by means of the following formula:

$$\frac{V_u \times F}{W_u}$$

where:

V_u =Milliliters of sodium thiosulfate used in the sample blank determination minus the milliliters of sodium thiosulfate used in the titration of the inactivated sample solution (the difference is the equivalent of the number of milliliters of 0.01*N* iodine absorbed by the inactivated sample);

W_u =Actual weight in milligrams of sample in the 2.0 milliliters titrated.

(3) *Finished products.* Calculate the potency of the sample in units or milligrams by means of the appropriate one of the following formulas:

$$\text{Units of antibiotic per dose or item} = \frac{V_u \times F \times d}{2n}$$

$$\text{Milligrams of antibiotic per dose or item} = \frac{V_u \times F \times d}{n \times 2,000}$$

where:

d =Dilution factor for the sample;

n =Number of doses or items in the sample assayed.

[39 FR 18944, May 30, 1974, as amended at 39 FR 34032, Sept. 23, 1974; 42 FR 59856, Nov. 22, 1977; 44 FR 10378, Feb. 20, 1979; 46 FR 2980, Jan. 13, 1981; 46 FR 25602, May 8, 1981; 46 FR 46312, Sept. 18, 1981; 46 FR 58298, Dec. 1, 1981; 46 FR 61072, Dec. 15, 1981; 49 FR 6091, Feb. 17, 1984]

§ 436.205 Hydroxylamine colorimetric assay.

(a) *Reagents*—(1) *Hydroxylamine hydrochloride solution.* Dissolve 350 grams of hydroxylamine hydrochloride in sufficient distilled water to make 1 liter.

(2) *Buffer.* Dissolve 173 grams of sodium hydroxide and 20.6 grams of sodium acetate in sufficient distilled water to make 1 liter.

(3) *Neutral hydroxylamine.* Mix 1 volume each of hydroxylamine hydrochloride solution described in paragraph (a)(1) of this section and the buffer described in paragraph (a)(2) of this section. Check the pH and if necessary adjust to pH 7.0±0.1 by adding an additional amount of one of the compo-

nents. To 1 volume of this neutralized solution add 8 volumes of distilled water and 2 volumes of 95 percent ethanol. This solution should be used for 1 day only.

(4) *Ferric ammonium sulfate.* Dissolve 272 grams of ferric ammonium sulfate in a mixture of 26 milliliters of concentrated sulfuric acid and sufficient distilled water to make 1 liter. This reagent may be used for 1 week when stored in a brown bottle at room temperature.

(b) *Preparation of working standard solutions.* From the following table, select the diluent and final concentration as listed for each antibiotic working standard. Dissolve and dilute an accurately weighed portion to the specified final concentration and proceed as directed in paragraph (d) of this section.

Antibiotic	Diluent (solution number as listed in § 436.101(a))	Final concentration in milligrams per milliliter of standard solution
Amoxicillin	Distilled water ...	1.0
Ampicillindo	1.25
Cefazolin ¹do	1.0
Cephaloridine	Distilled water ...	1.0
Cephalothindo	2.0
Cephapirindo	1.0
Cloxacillin	1	1.25
Cyclacillin	Distilled water ...	1.25
Dicloxacillin	1	1.25
Methicillin	1	1.25
Nafcillin	1	1.25
Oxacillin	1	1.25
Penicillin G	1	1.25
Penicillin G procaine	17	2.0
Penicillin V Potassium	1	1.25

¹To prepare the working standard solution, proceed as directed in the individual section of the antibiotic drug regulation in this chapter for the antibiotic to be tested.

(c) *Preparation of sample solutions.* From the following table, select the diluent and final concentration as listed for each antibiotic. Dissolve an accurately weighed portion of the sample, dilute to the appropriate final concentration, and proceed as directed in paragraph (d) of this section; if the product is packaged for dispensing, dilute an aliquot of the stock solution (prepared as described in the individual monograph) to the appropriate concentration and then proceed as directed in paragraph (d) of this section.