

(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.

Antibiotic	Diluent (solution number as listed in § 436.101(a))	Final concentration in milligrams per milliliter of sample
Amoxicillin trihydrate	Distilled water ...	1.0
Ampicillindo	1.25
Ampicillin sodium	1	1.25
Ampicillin trihydrate	Distilled water ...	1.25
Bacampicillin hydrochloridedo	1.2
Cefazolin sodium	1	1.0
Cephaloridine	Distilled water ...	1.0
Cephalothin sodiumdo	2.0
Cephapirin sodiumdo	1.0
Cloxacillin sodium monohydrate.	1	1.25
Cyclacillin	Distilled water ...	1.25
Dicloxacillin sodium monohydrate.do	1.25
Methicillin sodium monohydrate.	1	1.25
Nafcillin sodium monohydrate	1	1.25
Oxacillin sodium monohydrate	1	1.25
Penicillin G potassium	1	1.25
Penicillin G procaine	17	2.0
Penicillin G sodium	1	1.25
Penicillin V	17	1.25
Penicillin V potassium	1	1.25

¹The final concentration of bacampicillin hydrochloride is calculated in milligrams of ampicillin per milliliter of sample. The ampicillin working standard is used for the assay of bacampicillin hydrochloride.

(d) *Procedure.* Using a volume of from 1 to 2 milliliters of standard or sample solution, add an equal volume of water and mix. Add the following reagents in the specified volumetric proportions with respect to the sample or standard solutions: Add 1.25 volumes of neutral hydroxylamine reagent and allow to react for 5 minutes. Add 1.25 volumes of ferric ammonium sulfate reagent, mix, and after 3 minutes determine the absorbance of the resulting solution at the wavelength of 480 millimicrons, using a suitable spectrophotometer and a reagent blank prepared by treating a volume of water in the same manner as the standard or sample solution. The time elapsed after the addition of the ferric ammonium sulfate reagent and the reading of the absorbance must be precisely the same (within 10 seconds) for each solution. Calculate the potency of the sample in units or micrograms per milligram as follows:

$$\text{Units or micrograms per milligram of sample} = \frac{A_1 \times \text{Potency (in units or micrograms per milliliter of standard solution)}}{A_2 \times \text{Milligrams of sample per milliliter of sample solution}}$$

A₁=Absorbance of sample solution.
A₂=Absorbance of standard solution.

[39 FR 18944, May 30, 1974, as amended at 39 FR 34032, Sept. 23, 1974; 39 FR 44012, Dec. 20, 1974; 42 FR 59856, Nov. 22, 1977; 44 FR 10378, Feb. 20, 1979; 45 FR 16474, Mar. 14, 1980; 46 FR 2981, Jan. 13, 1981; 46 FR 25602, May 8, 1981; 46 FR 61072, Dec. 15, 1981; 49 FR 34350, Aug. 30, 1984]

§ 436.206 Test for metal particles in ophthalmic ointments.

(a) *Procedure.* Extrude the contents of each of 10 tubes as completely as practicable into separate, clear, glass Petri dishes (60 millimeters in diameter), cover the dishes, and heat to 80° C. to 85° C. for at least 2 hours or until the ointment has melted completely and evenly in the dishes. A higher temperature of 100° C.±2° C. may be used if necessary to allow adequate settling of metal particles. Allow the ointment to

cool to room temperature without agitation. Invert each Petri dish on the stage of a suitable microscope adjusted to furnish 30 times magnification and equipped with an eye-piece micrometer disc which has been calibrated at the magnification being used. In addition to the usual source of light, direct an illuminator from above the ointment at a 45° angle. Examine the entire bottom of the Petri dish for metal particles. By varying the intensity of the illuminator from above, such metal particles are recognized by their characteristic reflection of light. Count the total number of metal particles exceeding 50 microns in any single dimension.

(b) *Evaluation.* The batch is acceptable if (1) a total of not more than 50 such particles is found in 10 tubes; and (2) not more than one tube is found to contain more than eight such particles. If the batch fails the above test, repeat