

Antibiotic	Diluent (solution number as listed in § 436.101(a))	Final concentration in milligrams per milliliter of sample
Amoxicillin trihydrate .....	Distilled water ...	1.0
Ampicillin .....	.....do .....	1.25
Ampicillin sodium .....	1 .....	1.25
Ampicillin trihydrate .....	Distilled water ...	1.25
Bacampicillin hydrochloride ...	.....do .....	1.2
Cefazolin sodium .....	1 .....	1.0
Cephaloridine .....	Distilled water ...	1.0
Cephalothin sodium .....	.....do .....	2.0
Cephapirin sodium .....	.....do .....	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

<sup>1</sup>The 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:

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

A<sub>1</sub>=Absorbance of sample solution.  
A<sub>2</sub>=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

the test on 20 additional tubes of ointment. The total number of metal particles exceeding 50 microns in any single dimension from the 30 tubes tested shall not exceed 150, with not more than three tubes containing more than eight such particles.

[39 FR 18944, May 30, 1974; 40 FR 11869, Mar. 14, 1975]

#### § 436.207 Residue on ignition.

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

(a) *Method 1.* Place approximately 1 gram of the sample, accurately weighed, in a tared porcelain crucible and carefully ignite at a low temperature until thoroughly charred. The crucible may be loosely covered with a porcelain lid during the charring. Add 2 milliliters of nitric acid and 5 drops of sulfuric acid to the contents of the crucible and cautiously heat until white fumes are evolved, then ignite, preferably in a muffle furnace, at 500° C. to 600° C. until the carbon is all burned off. Cool the crucible in a desiccator and weigh. From the weight of residue obtained, calculate the sulfated ash content.

(b) *Method 2.* Proceed as directed in paragraph (a) of this section, except use 2 milliliters of sulfuric acid and do not use the nitric acid.

#### § 436.208 Heavy metals determination.

(a) *Reagents*—(1) *Ammonia solution.* Prepare an aqueous solution containing not less than 9 grams and not more than 10 grams of ammonia (NH<sub>3</sub>) per 100 milliliters.

(2) *6 percent acetic acid.* Dilute 60 milliliters of glacial acetic acid with sufficient water to give a solution of 1,000 milliliters.

(3) *Hydrogen sulfide solution.* Prepare a saturated solution of hydrogen sulfide by passing hydrogen sulfide into cold water for a sufficient time. It is suitable if it produces an immediate copious precipitate when added to an equal volume of 1*N* ferric chloride. Prepare a fresh hydrogen sulfide solution each time a heavy metals test is to be performed.

(4) *Lead nitrate stock solution.* Dissolve 159.8 milligrams of lead nitrate with 100 milliliters of 0.15*N* nitric acid, and dilute with water to a volume of 1,000

milliliters. Prepare and store this solution in glass containers free from soluble lead salts.

(5) *Standard lead solution.* Dilute a 10-milliliter aliquot of the lead nitrate stock solution to 100 milliliters with water. This solution must be freshly prepared each time a heavy metals test is performed. One milliliter of this standard lead solution represents a lead level of 10 parts per million in a 1.0-gram sample or 20 parts per million in a 0.5-gram sample.

(b) *Preparation of the sample.* Use the sulfated ash obtained as described in § 436.207(a). If the heavy metal limit is greater than 30 parts per million, the sulfated ash may be obtained from a 0.5-gram sample. Add 2 milliliters of hydrochloric acid to the sulfated ash and slowly evaporate to dryness on a steam bath. Moisten the residue with 1 drop of hydrochloric acid, add 10 milliliters of hot water, and digest by heating on the steam bath for 2 minutes. After cooling to room temperature, add ammonia solution dropwise until a pH of 7.2 is reached, then add 2 milliliters of 6 percent acetic acid. Filter the solution, if necessary, and wash the crucible and the filter with about 10 milliliters of water. Combine the washings with the filtrate and dilute to exactly 25 milliliters with water.

(c) *Procedure.* Prepare a series of five standard lead solutions, in increments of 10 parts per million, in which the solution of lowest concentration contains 20 parts of lead per million less than the maximum limit of heavy metals permitted for the sample. Transfer the necessary quantities of standard lead solution described in paragraph (a)(5) of this section directly into metal-free 50-milliliter Nessler tubes of uniform diameter, add 2 milliliters of 6 percent acetic acid to each, and adjust each to a final volume of 25 milliliters with water. Transfer the 25-milliliter solution of the sample described in paragraph (b) of this section to another Nessler tube. Add 10 milliliters of hydrogen sulfide solution to each standard and sample solution, mix well, and allow to stand for 10 minutes. View downward over a white surface; the color of the solution of the sample should be no darker than the standard