

(ii) *Preparations of solutions*—(a) *Solvent*. Mix reagent grade chloroform with reagent grade absolute methanol in volumetric proportions of 1:1.

(b) *Spray A*. Mix 50 milliliters of freshly prepared 1.0 percent ferric chloride in water (weight per volume), just before spraying, with 50 milliliters of freshly prepared 1.0 percent potassium ferricyanide in water (weight per volume).

(c) *Spray B*. Dissolve 2.28 grams of periodic acid in 100 milliliters of water. Dilute one volume of this periodic solution with 10 volumes of acetone.

(d) *Spray C*. Dissolve 184 milligrams of benzidine in a solution of 0.6 milliliter of acetic acid, 4.4 milliliters of water, and 95 milliliters of acetone.

(iii) *Preparation of spotting solutions*—(a) *Plicamycin standard solution*. Weigh 5 milligrams of plicamycin working standard and dissolve in 10 milliliters of methanol. Use the solution the same day it is prepared.

(b) *Plicamycin for injection sample solution*. Dilute with methanol to a concentration of 0.5 milligram of plicamycin per milliliter. Centrifuge and use the supernatant for spotting.

(c) *Mannitol reference solution*. Suspend 100 milligrams of mannitol in 5 milliliters of methanol. Centrifuge and use the supernatant for spotting.

(iv) *Procedure*. Fill the chamber to a depth of 0.6 centimeter with freshly prepared solvent. Spot duplicate plates as follows: On a line 2.5 centimeters from the base of the silica gel plate, and at intervals of 2.0 centimeters, spot 100 microliters (in four 25-microliter aliquots) of the standard solution, the sample solution, and the mannitol reference solution. Allow each aliquot to dry before applying subsequent volumes. After all spots are thoroughly dry, place the silica gel plates in the chromatographic chamber and develop by the ascending technique for approximately 60 minutes. Allow several minutes for the plates to air dry. On one plate, locate and record the position of fluorescent spots by examining under long wave ultraviolet light. Apply spray A and record the position of blue spots on the yellow-green background. On the other plate, locate the mannitol by first applying spray B, followed by spray C. The spots appearing white are

mannitol. Measure the distance the solvent front traveled from the starting line and the distance the fluorescent spots are from the starting line. Calculate the R_f value by dividing the latter by the former. The plicamycin standard should have an R_f value of 0.7. If the standard has an R_f value greater than 0.8, the mobility of the standard may be decreased by increasing the ratio of the chloroform to methanol in the solvent to 3:2 or 3:1. Plicamycin appears as a single major component with the same R_f value as the plicamycin standard. It may show trace components at R_f values of about 0.5 and 0.4, and at the origin, which shall not be more intense than those shown by the plicamycin standard.

[39 FR 19145, May 30, 1974, as amended at 40 FR 1512, Jan. 8, 1975; 46 FR 60568, Dec. 11, 1981; 47 FR 9396, Mar. 5, 1982; 48 FR 11427, Mar. 18, 1983; 49 FR 5097, Feb. 10, 1984; 49 FR 24019, June 11, 1984; 50 FR 19676, May 10, 1985]

§ 450.245 Mitomycin for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Mitomycin for injection is a dry mixture of mitomycin and mannitol. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of mitomycin that it is represented to contain. It is sterile. It is nonpyrogenic. It contains no depressor substances. Its moisture content is not more than 5 percent. Its pH, when reconstituted as directed in the labeling, is not less than 6.0 and not more than 8.0. It passes the identity test for mitomycin. The mitomycin used conforms to the standards prescribed by § 450.45(a)(1).

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The mitomycin used in making the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(b) The batch for potency, sterility, pyrogens, depressor substances, moisture, pH, and identity.

(ii) Samples required:

(a) The mitomycin used in making the batch: Five packages, each containing approximately 100 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 25 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents from each container if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the solution thus obtained with sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Further dilute the stock solution with solution 1 to the reference concentration of 1 microgram of mitomycin per milliliter (estimated).

(2) *Sterility*. Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens*. Proceed as directed in §436.32(a) of this chapter, using a solution containing 0.5 milligram of mitomycin per milliliter.

(4) [Reserved]

(5) *Depressor substances*. Proceed as directed in §436.35 of this chapter.

(6) *Moisture*. Proceed as directed in §436.201 of this chapter.

(7) *pH*. Proceed as directed in §436.202 of this chapter using the drug reconstituted as directed in the labeling.

(8) *Identity*. Proceed as directed in §436.310 of this chapter.

[39 FR 19145, May 30, 1974, as amended at 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

PART 452—MACROLIDE ANTIBIOTIC DRUGS

Subpart A—Bulk Drugs

Sec.	
452.10	Erythromycin.
452.15	Erythromycin estolate.
452.25	Erythromycin ethylsuccinate.
452.25a	Sterile erythromycin ethylsuccinate.
452.30a	Sterile erythromycin gluceptate.
452.32a	Sterile erythromycin lactobionate.
452.35	Erythromycin stearate.
452.50	Clarithromycin.
452.60	Azithromycin.
452.75	Troleandomycin.

Subpart B—Oral Dosage Forms

452.110	Erythromycin oral dosage forms.
452.110a	Erythromycin tablets.
452.110b	Erythromycin enteric-coated tablets.
452.110c	Erythromycin capsules.
452.110d	Erythromycin particles in tablets.
452.115	Erythromycin estolate oral dosage forms.
452.115a	Erythromycin estolate tablets.
452.115b	Erythromycin estolate capsules.
452.115c	Erythromycin estolate oral suspension.
452.115d	Erythromycin estolate for oral suspension.
452.115e	Erythromycin estolate for pediatric drops.
452.115f	Erythromycin estolate chewable tablets.
452.115g	Erythromycin estolate and sulfisoxazole acetyl oral suspension.
452.125	Erythromycin ethylsuccinate oral dosage forms.
452.125a	Erythromycin ethylsuccinate chewable tablets.
452.125b	Erythromycin ethylsuccinate oral suspension.
452.125c	Erythromycin ethylsuccinate for oral suspension.
452.125d	Erythromycin ethylsuccinate tablets.
452.125e	Erythromycin ethylsuccinate-sulfisoxazole acetyl for oral suspension.
452.135	Erythromycin stearate oral dosage forms.
452.135a	Erythromycin stearate tablets.
452.135b	Erythromycin stearate oral suspension.
452.135c	Erythromycin stearate for oral suspension.
452.150	Clarithromycin oral dosage forms.
452.150a	Clarithromycin tablets.
452.150b	Clarithromycin granules for oral suspension.