

(B) The batch for idarubicin hydrochloride content, sterility, bacterial endotoxins, moisture, pH, and identity.

(ii) Samples required if requested by the Director, Center for Drug Evaluation and Research:

(A) The idarubicin hydrochloride used in making the batch: 14 packages, each containing approximately 40 milligrams.

(B) The batch:

(1) For all tests except sterility: A minimum of 34 immediate containers.

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

(b) *Tests and methods of assay.* Idarubicin hydrochloride is toxic. It must be handled with care in the laboratory. Transfer all dry powders into a suitable hood while wearing rubber gloves. Avoid inhaling fine particles of powder. Solutions should not be pipetted by mouth. If the substance contacts the skin, wash with soap and water. Dispose of all waste material by dilution with large volumes of dilute sodium hypochlorite (bleach) solution.

(1) *Idarubicin hydrochloride content (HPLC).* Proceed as directed in § 450.30(b)(1), preparing the sample solution and calculating the idarubicin hydrochloride as follows:

(i) *Sample solution.* Prepare the sample solution by rinsing the contents of the vial into an appropriate-sized volumetric flask with sufficient diluent to obtain a concentration of 0.5 milligram of idarubicin hydrochloride per milliliter (estimated).

(ii) *Calculations.* Calculate the idarubicin hydrochloride content per vial as follows:

$$\begin{array}{l} \text{Micrograms of} \\ \text{plicamycin} \\ \text{per milligram} \end{array} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u =Area of the idarubicin hydrochloride peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s =Area of the idarubicin hydrochloride peak in the chromatogram of the idarubicin hydrochloride working standard;

P_s =Idarubicin hydrochloride activity in the idarubicin hydrochloride working standard solution in micrograms per milliliter; and

d =Dilution factor of the sample.

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

(3) *Bacterial endotoxins.* Proceed as directed in the U.S.P. Bacteria endotoxin test. The specimen under test contains not more than 8.93 U.S.P. endotoxin units per milligram of idarubicin hydrochloride.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter, using the sample preparation method described in § 436.201(d)(4).

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using the sample obtained after reconstituting the drug as directed in the labeling, except use distilled water instead of saline.

(6) *Identity.* The high-performance liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the idarubicin hydrochloride working standard.

[58 FR 26665, May 4, 1993]

§ 450.240 Plicamycin for injection.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Plicamycin for injection is a dry mixture of plicamycin and mannitol with or without a suitable buffer substance. Each immediate container contains 2.5 milligrams of plicamycin. Its plicamycin content is satisfactory if it contains not less than 90 percent and not more than 110 percent of the number of milligrams of plicamycin that it is represented to contain. It is sterile. It is nonpyrogenic. Its moisture content is not more than 2.0 percent. It contains no depressor substances. Its pH when reconstituted as directed in the labeling is not less than 5.0 and not more than 7.5. It passes the identity test for plicamycin. The plicamycin used conforms to the standards prescribed by § 450.40(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. In addition, each package shall bear on its label or labeling the following as indicated:

(i) On the outside wrapper or container the statement "Store below 10° C. (50° F.)".

(ii) On the outside wrapper or container and on the immediate container the statement "Mandatory: Before using read enclosed professional information carefully for dosage instructions and warnings".

(iii) On the outside wrapper or container the statement "Warning: For hospital use only. To be used under direct supervision of a physician".

(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 plicamycin used in making the batch for plicamycin content, loss on drying, absorptivity, pH, identity, and crystallinity.

(b) The batch for plicamycin content, sterility, pyrogens, moisture, Ph, depressor substances, and identity.

(ii) Samples required:

(a) The plicamycin used in making the batch: 3 packages, each containing not less than 50 milligrams; and 2 packages, each containing not less than 100 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 21 immediate containers.

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

(b) *Tests and methods of assay.* Plicamycin is more toxic than the average drug and must be handled with care in the laboratory. Avoid inhaling fine particles of powder. If the substance contacts the skin, wash with soap and water. Plicamycin is hygroscopic and care should be exercised during storage and weighing of samples. Dispose of all waste materials by dilution with larger volumes of trisodium phosphate solution. The samples should be stored at 10° C. or less in a sealed light-resistant container with a desiccant. Solutions should not be pipetted by mouth.

(1) *Plicamycin content.* Proceed as directed in §436.341 of this chapter, except prepare the sample solution and calculate the plicamycin content as follows:

(i) *Preparation of sample solution.* Place approximately 5 milligrams of the sample, accurately weighed, into a 50-milliliter, amber volumetric flash

and dilute to volume with mobile phase and mix.

(ii) *Calculations.* Calculate the plicamycin content of the vial as follows:

$$\text{Milligrams of plicamycin per vial} = \frac{A_u \times P_s \times d}{A_s \times 1,000}$$

where:

A_u =Area of the plicamycin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s =Area of the plicamycin peak in the chromatogram of the plicamycin working standard;

P_s =Plicamycin activity in the plicamycin working standard solution in micrograms per milliliter; and

d =Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use the entire contents of each of the immediate containers tested.

(3) *Pyrogens.* Reconstitute the sample as directed in the labeling and proceed as directed in §436.32(b) of this chapter, using a solution containing 50 micrograms of plicamycin per milliliter.

(4) *Moisture.* Proceed as directed in §436.201 of this chapter, using the total contents of three to five vials.

(5) *pH.* Proceed as directed in §436.202 of this chapter, using the drug reconstituted as directed in the labeling. Allow the solution to remain in contact with the electrodes until a steady reading is obtained or for 5 minutes.

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

(7) *Thin layer chromatography identity test for plicamycin—(i) Equipment—(a) Plates.* Use 20 by 20 centimeter or 15 by 20 centimeter thin layer chromatographic plates coated with Silica Gel Mixture, Chromatographic, U.S.P., to a thickness of 250 microns. Activate the plates by heating at 110° C. for 75 minutes. Place the plates in a desiccator until cooled to room temperature. Plates may be stored in a desiccator for 7 days.

(b) *Chamber (chromatographic).* A suitable chamber, equipped for thin layer chromatography.

(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.

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§ 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.