

(ii) *Solvent*. Transfer about 100 milligrams of dioxane, accurately weighed to a 100-milliliter volumetric flask, dilute with water to volume, and mix.

(iii) *Test preparation*. Dissolve about 200 milligrams of doxorubicin hydrochloride sample in 3.0 milliliters of solvent.

(iv) *Chromatographic system* (see *United States Pharmacopeia (U.S.P.) Chromatography (621)*). The gas chromatograph is equipped with a flame-ionization detector and a 4-millimeter X 2-meter column packed with 8-percent liquid phase G16 (see U.S.P. Chromatographic Reagents—Phases) on 100- to 120-mesh support S1AB (potassium hydroxide-washed) (see U.S.P. Chromatographic Reagents—Supports). The column is maintained at about 60 °C, and helium is used as the carrier gas. Adjust the column temperature and carrier gas flow rate so that dioxane elutes in about 6 minutes. Chromatograph the standard preparation, and record the peak responses as directed under procedure; the resolution (*R*) between adjacent peaks is not less than 2.0; the relative standard deviations of the ratios of the peak responses of the acetone and dioxane peaks and of the alcohol and dioxane peaks for replicate injections is not more than 4.0 percent; and the tailing factor for the alcohol peak is not more than 1.5.

(v) *Procedure*. (Note: Use peak areas where peak responses are indicated.) Separately inject equal volumes (about 1 microliter) of the standard preparation and the test preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.2 for acetone, 0.5 for alcohol, and 1.0 for dioxane. Calculate the percentage, by weight, of acetone and alcohol, respectively, in the sample as follows:

$$X = \text{Percent acetone or alcohol} = \frac{100(C_A C_D)(D_U W_U)(R_U/R_S)}{100(C_A C_D)(D_U W_U)(R_U/R_S)}$$

where:

C_A = Concentration of acetone or alcohol in the standard preparation in milligrams per milliliter;

C_D = Concentration of dioxane in the standard preparation in milligrams per milliliter;

D_U = Total quantity of dioxane in the test preparation, in milligrams;

W_U = Quantity of doxorubicin hydrochloride taken to prepare the test preparation, in milligrams;

R_U = Response ratio of the analyte peak (acetone or alcohol) to the dioxane peak obtained from the test preparation; and

R_S = Response ratio of the analyte peak (acetone/alcohol) to the dioxane peak obtained from the standard preparation.

The total of acetone and alcohol is not greater than 2.5 percent. Use the result obtained to calculate the doxorubicin hydrochloride content of the sample on the solvent-free basis.

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

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

(5) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 5 milligrams per milliliter.

(6) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

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

(8) *Chromatographic purity*. Proceed as directed in paragraph (b)(1) of this section, except prepare the sample solution by dissolving the sample to be tested in mobile phase to obtain a solution containing approximately 0.5 milligram of doxorubicin hydrochloride per milliliter. Calculate the percentage of impurities as follows:

$$\text{Percent total impurities} = \frac{(100 S)}{(S + r)}$$

where:

S = The sum of the responses of the minor component peaks; and

r = The response of the major doxorubicin hydrochloride peak.

The total related impurities detected is not more than 2.0 percent.

[41 FR 14184, Apr. 2, 1976; 41 FR 15844, Apr. 15, 1976, as amended at 42 FR 43063, Aug. 26, 1977; 43 FR 44836, Sept. 29, 1978; 47 FR 9396, Mar. 5, 1982; 47 FR 23710, June 1, 1982; 50 FR 19676, May 10, 1985; 53 FR 37292, Sept. 26, 1988; 59 FR 9639, Mar. 1, 1994]

§ 450.30 Idarubicin hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality,*

and purity. Idarubicin hydrochloride is the monohydrochloride salt of 5,12-Naphthacenedione,9-acetyl-7-[(3-amino-2,3,6-trideoxy- α -*L*-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,9,11-trihydroxy-(7*S*-*cis*). It is an orange-red powder. It is so purified and dried that:

(i) Its idarubicin hydrochloride content is not less than 960 micrograms and not more than 1,030 micrograms of idarubicin hydrochloride per milligram on the anhydrous basis.

(ii) Its moisture content is not more than 5.0 percent.

(iii) The pH of an aqueous solution containing 5 milligrams per milliliter is not less than 5.0 and not more than 6.5.

(iv) It is crystalline.

(v) The level of any individual impurity detected by high-performance liquid chromatography (HPLC) assay is not more than 1.0 percent.

(vi) The total of all detected impurities is not more than 3.0 percent.

(vii) It passes the identity test for idarubicin.

(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 the batch for idarubicin hydrochloride content, solvent residues, moisture, pH, crystallinity, related individual thin-layer chromatography and HPLC impurities, total impurities, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 14 packages, each containing approximately 40 milligrams.

(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) *Potency (HPLC).* Proceed as directed in § 436.216 of this chapter, using

ambient temperature, an ultraviolet detection system operating at a wavelength of 254 nanometers, a 4.6-millimeter by 25-centimeter column packed with microparticulate (5 to 10 micrometers in diameter) packing material such as trimethylsilane chemically bonded to porous silica, a flow rate of not more than 2.0 milliliters per minute, and a known injection volume of between 10 and 20 microliters. The retention time for idarubicin hydrochloride is between 14 and 16 minutes. The retention time for the resolution compound

4-demethoxydaunorubicinone (generated in situ) is between 6 and 9 minutes. Mobile phase, diluent, working standard and sample solutions, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Mobile phase.* Prepare a suitably sized quantity of a mixture of water, acetonitrile, and methanol (540:290:170). Dissolve 1 gram of sodium lauryl sulfate and 2 milliliters of 85 percent phosphoric acid per liter of this solution. Adjust with 2 *N* sodium hydroxide to a pH of 3.6±0.1. Filter through a suitable filter capable of removing particulate matter to 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(ii) *Diluent.* Prepare as mobile phase, excluding the sodium lauryl sulfate.

(iii) *Preparation of working standard solution.* Dissolve an accurately weighed quantity of idarubicin hydrochloride working standard in diluent to obtain a solution having a known concentration of 0.5 milligram of idarubicin hydrochloride per milliliter.

(iv) *Sample solution.* Transfer approximately 50 milligrams of sample, accurately weighed, to a 100-milliliter volumetric flask, add diluent to volume, and mix. This yields a solution containing 0.5 milligram of idarubicin hydrochloride per milliliter (estimated).

(v) *Resolution test solution.* To 2 milliliters of a 1.0 milligram per milliliter aqueous solution of idarubicin hydrochloride, add 20 microliters of 1 *N* hydrochloric acid. Hold for 30 minutes at 95 C in an oil bath. This procedure generates the aglycone of idarubicin, 4-demethoxydaunorubicinone. Transfer 1.0 milliliter of this solution to a 10-milliliter volumetric flask, add diluent

to volume, and mix. Use this solution to determine the resolution requirement for the chromatographic system.

(vi) *System suitability requirements*—
(A) *Asymmetry factor*. The asymmetry factor (A_s), measure data point 5 percent of the peak height from the baseline, is satisfactory if it is not less than 0.85 and not more than 1.1.

(B) *Efficiency of the column*. The absolute efficiency (h_r) is satisfactory if it is not more than 10.0 for the idarubicin hydrochloride peak, equivalent to 4,500 theoretical plates for a 25-centimeter column of 6-micrometer particles.

(C) *Resolution factor*. The resolution factor (R_s) between the peak for idarubicin and 4-demethoxydaunorubicinone (generated *in situ*) is satisfactory if it is not less than 9.5.

(D) *Coefficient of variation (relative standard deviation)*. The coefficient of variation (S_R in percent of 5 replicate injections) is satisfactory if it is not more than 2.0 percent.

(E) *Capacity factor*. The capacity factor (k') for idarubicin hydrochloride is satisfactory if it is not less than 5 and not more than 15. If the system suitability parameters have been met, proceed as described in § 436.216(b) of this chapter.

(vii) *Calculations*. Calculate the micrograms of idarubicin hydrochloride per milligram of sample as follows:

$$\text{Micrograms of idarubicin hydrochloride per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

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;

C_u =Milligrams of idarubicin hydrochloride sample per milliliter of sample solution;

m =Percent moisture content of the sample.

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

(3) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solu-

tion containing 5 milligrams per milliliter.

(4) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

(5) *HPLC impurities*. Proceed as directed in paragraph (b)(1) of this section. Calculate the percentage of impurities as follows:

$$\text{Percent individual impurity} = \frac{A_i \times 100}{A_t}$$

$$\text{Percent total HPLC impurities} = \frac{A \times 100}{A_t}$$

where:

A_i =Area of the individual impurity peak;

A =The sum of areas of all peaks minus the area due to the idarubicin hydrochloride peak and solvent peak; and

A_t =The sum of areas of all peaks in the chromatogram excluding the solvent peak.

(6) *Identity*. Proceed as directed in § 436.211 of this chapter, using a 1.0 percent potassium bromide disc prepared as directed in § 436.211(b)(1).

[58 FR 26664, May 4, 1993]

§ 450.40 Plicamycin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Plicamycin is a yellow compound and is so purified and dried that:

(i) Its plicamycin content is not less than 900 micrograms of plicamycin per milligram calculated on an anhydrous basis.

(ii) Its loss on drying is not more than 8 percent.

(iii) Its pH in an aqueous solution containing 0.5 milligram per milliliter is not less than 4.5 nor more than 5.5.

(iv) Its absorptivity on the anhydrous basis at the absorption maximum of 278 millimicrons is 100±5 percent of that of the plicamycin standard similarly treated.

(v) It gives a positive result to the identity tests for plicamycin.

(vi) It is crystalline.

(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 the statement “Store below 10° C. (50° F.)”.