

$$\text{Calculated concentration, X, sample solution} = \text{antilog} \frac{0.405 - 0.914}{-0.553} = 8.32 \text{ micrograms per milliliter}$$

(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 solution containing 5 milligrams per milliliter.

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

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

[45 FR 75195, Nov. 14, 1980]

#### § 450.24 Doxorubicin hydrochloride.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity*. Doxorubicin hydrochloride is the monohydrochloride salt of (8S, 10S)-10-[(3-amino-2,3,6-trideoxy- $\alpha$ -L-lyxo-hexopyranosyl)oxy]-8-glycoloyl-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione. It is a red-orange, almost completely odorless, hygroscopic powder. It is so purified and dried that:

(i) Its doxorubicin hydrochloride content is not less than 970 micrograms and not more than 1,020 micrograms of doxorubicin hydrochloride per milligram on the anhydrous and solvent free basis.

(ii) Its total solvent residue (as acetone and alcohol) is not more than 2.5 percent.

(iii) It contains no depressor substances.

(iv) Its moisture content is not more than 4.0 percent.

(v) The pH of an aqueous solution containing 5 milligrams per milliliter is not less than 4.0 and not more than 5.5.

(vi) It is crystalline.

(vii) It passes the identity test for doxorubicin.

(viii) The total of any impurities detected by high-pressure liquid chromatography assay is not more than 3.0 percent.

(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 request shall contain:

(i) Results of tests and assays on the batch for doxorubicin hydrochloride content, solvent residue, depressor substances, moisture, pH, crystallinity, identity, and total impurities.

(ii) Samples required: 14 packages, each containing approximately 40 milligrams.

(b) *Tests and methods of assay*. Doxorubicin hydrochloride is toxic. It must be handled with care in the laboratory. Transfer all dry powders in 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) *Doxorubicin hydrochloride content (high-performance liquid chromatography)*. 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 X 25-centimeter column packed with micro-particulate (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. Mobile phase, working standard and sample solutions, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Mobile phase*. Prepare a suitable mixture of water, acetonitrile, methanol, and phosphoric acid (540:290:170:2). Dissolve 1 gram of sodium lauryl sulfate in 1,000 milliliters 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) *Preparation of working standard, sample, and resolution test solutions*—(A) *Working standard solution.* Dissolve an accurately weighed quantity of doxorubicin hydrochloride working standard in mobile phase to obtain a solution having a known concentration of 0.1 milligram of doxorubicin hydrochloride per milliliter.

(B) *Sample solution.* Transfer approximately 20 milligrams of sample, accurately weighed, to a 200-milliliter volumetric flask, add mobile phase to volume, and mix. This yields a solution containing 0.1 milligram of doxorubicin hydrochloride per milliliter (estimated).

(C) *Resolution test solution.* Use either of the following preparation methods:

(1) To 2 milliliters of a 1.0 milligram per milliliter solution of doxorubicin hydrochloride, add 20 microliters of 1*N* hydrochloric acid. Hold for 30 minutes at 95 °C in an oil bath.

(2) Dissolve about 10 milligrams of doxorubicin hydrochloride in 5 milliliters of water, add 5 milliliters of phosphoric acid, and allow to stand for about 30 minutes. Adjust with 2*N* sodium hydroxide (about 37 milliliters) to a pH of 2.6±0.1, add 15 milliliters of acetonitrile and 10 milliliters of methanol, mix, and filter. (Note: Portions of this solution may be frozen until needed, then thawed and mixed before use.)

(3) The procedures in paragraphs (b)(1)(ii)(C)(1) and (b)(1)(ii)(C)(2) of this section generate doxorubicinone, the aglycone of doxorubicin. Use this solution to determine the resolution requirement for the chromatographic system.

(iii) *System suitability requirements*—

(A) *Asymmetry factor.* The asymmetry factor ( $A_s$ ) for the doxorubicin peak measured at a point 5 percent of the peak height is not less than 0.7 and not more than 1.2.

(B) *Efficiency of the column.* The absolute column efficiency ( $h_r$ ) is satisfactory if it is not greater than 10.0, equivalent to 2,500 theoretical plates for a 25-centimeter column of 10-micrometer particles.

(C) *Resolution.* The resolution ( $R$ ) between the peaks of doxorubicin and doxorubicinone (generated in situ) is satisfactory if it is not less than 5.5.

(D) *Capacity factor.* The capacity factor ( $k$ ) for doxorubicin is satisfactory if it is in the range between 1.0 and 5.0.

(E) *Coefficient of variation.* The coefficient of variation (*relative standard of deviation* in percent) of 5 replicate injections is satisfactory if it is not more than 1.0 percent. If the system suitability parameters have been met, then proceed as described in §436.216(b) of this chapter.

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

$$\begin{array}{l} \text{Micrograms of} \\ \text{doxorubicin} \\ \text{hydrochloride} \\ \text{per milligram} \end{array} = \frac{A_U \times P_S \times 100}{A_S \times C_U \times (100 - m - X)}$$

where:

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

$A_S$  = Area of the doxorubicin hydrochloride peak in the chromatogram of the doxorubicin hydrochloride working standard;

$P_S$  = Doxorubicin hydrochloride activity in the doxorubicin hydrochloride working standard solution in micrograms per milliliter;

$C_U$  = Milligrams of the sample per milliliter of sample solution;

$m$  = Percent moisture content of the sample; and

$X$  = Percent solvent residue determined as directed in paragraph (b)(2) of this section.

(2) *Residue solvent (as acetone and alcohol)*—(i) *Standard preparation.* Transfer to a 100-milliliter volumetric flask

about 200 milligrams of acetone, 300 milligrams of dehydrated alcohol, and 1,000 milligrams of dioxane, each accurately weighed, and mix. Dilute with water to volume, and mix. Transfer 5.0 milliliters of the resulting solution to a 50-milliliter volumetric flask, dilute with water to volume, and mix. This solution contains about 0.2 milligram of acetone, 0.3 milligram of alcohol, and 1 milligram of dioxane per milliliter.

(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 or 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.

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#### § 450.30 Idarubicin hydrochloride.

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