

(ii) *Calculations.* Calculate the micrograms of dactinomycin per milligram of sample as follows:

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

where:

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

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

P_s = Dactinomycin activity in the dactinomycin working standard solution in micrograms per milliliter;

C_u = Milligrams of sample per milliliter of sample solution; and

m = Percent moisture content of the sample.

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *Absorptivity*—(i) *Procedure.* Accurately weigh approximately 15 milli-

grams of the sample "as is" and 15 milligrams of the working standard dried as directed in § 436.200(a) of this chapter. Transfer each weighing to separate 100-milliliter volumetric flasks. Dissolve the material and bring to volume with spectrophotometric-grade methyl alcohol. Mix well. Pipette 5.0 milliliters of each solution into separate 25-milliliter volumetric flasks, dilute to volume with spectrophotometric-grade methyl alcohol. Mix well. Using a suitable spectrophotometer and 1-centimeter absorption cells, determine the absorbance of the sample solution at the 240-nanometer and at the 445-nanometer absorption peaks (the exact position of the peaks should be determined for the particular instrument used). Determine the absorbance of the standard at the 445-nanometer absorption peak.

(ii) *Calculations.* Calculate the relative absorptivity and the ratio for the absorbances of the sample as follows:

$$\text{Relative absorptivity at 445 nanometers} = \frac{A_2 \times \text{milligrams of standard} \times \text{potency of the standard in micrograms per milligram}}{A_3 \times \text{milligrams of sample} \times (100 - M) \times 10}$$

$$\text{Ratio for the absorbances of the sample at 240 and 445 nanometers} = \frac{A_1}{A_2}$$

where:

A_1 = Absorbance at 240 nanometers for the sample;

A_2 = Absorbance at 445 nanometers for the sample;

A_3 = Absorbance at 445 nanometers for the standard;

M = Percent moisture in the sample.

(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) of this section compares qualitatively to that of the dactinomycin working standard.

[49 FR 6092, Feb. 17, 1984, as amended at 49 FR 24018, June 11, 1984; 50 FR 19675, May 10, 1985]

§ 450.22 Daunorubicin hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Daunorubicin hydrochloride is the monohydrochloride salt of (1*s*,3*s*)-3-acetyl-1,2,3,4,6,11-hexahydro-3,5,12-trihydroxy-10-methoxy-6,11-dioxo-1-naphthaceny-3-amino-2,3,6-trideoxy- α -L-*lyxo*-hexopyranoside. It is a red-orange, hygroscopic powder. It is so purified and dried that:

(i) Its potency is not less than 842 micrograms and not more than 1,030 micrograms of daunorubicin per milligram.

(ii) Its moisture content is not more than 3.0 percent.

(iii) Its pH in an aqueous solution containing 5 milligrams per milliliter is not less than 4.5 and not more than 6.5.

(iv) It is crystalline.

(v) It passes the identity test for daunorubicin.

(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 potency, moisture, pH, crystallinity, and identity.

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

(b) *Tests and methods of assay.* Daunorubicin hydrochloride is toxic. It must be handled with care in the laboratory. Transfer all dry powders in a suitable hood. Wear rubber gloves, protective gowns, head coverings, and protective eye goggles when handling dry powders. Avoid inhaling fine particles of powder. Solutions should not be pipetted by mouth. If the substance contacts the skin, promptly wash with soap and water. Dispose of all waste material by dilution with large volumes of sodium hypochlorite solution.

(1) *Potency.* Use either of the following methods; however, the results obtained from the high-pressure liquid chromatography shall be conclusive.

(i) *High-pressure liquid chromatography.* Proceed as directed in § 436.322 of this chapter, except in lieu of the mobile phase and pH described in paragraph (b)(2) of that section, use a mixture of water: acetonitrile (62:38) adjusted to pH 2.2±0.2 with phosphoric acid. Prepare the sample and standard solutions and calculate the daunorubicin content as follows:

(a) *Preparation of sample and working standard solutions.* Accurately weigh approximately 25 milligrams of the sample and of the daunorubicin working standard and dissolve each in 25 milliliters of the internal standard solution prepared as directed in § 436.322(b)(3) of this chapter.

(b) *Calculations.* Calculate the daunorubicin content as follows:

$$\text{Micrograms of daunorubicin per milligram} = \frac{R_u \times W_s \times P \times 100}{R_s \times W_u \times (100 - M)}$$

where:

R_u =Area of the daunorubicin sample peak/
Area of the internal standard peak;

R_s =Area of the daunorubicin standard peak/
Area of the internal standard peak;

W_s =Weight of the daunorubicin working standard in milligrams;

W_u =Weight of the sample in milligrams;

M =Moisture content of the sample in percent;

P =Potency of the daunorubicin working standard in micrograms per milligram.

(ii) *Microbiological turbidimetric assay for daunorubicin—(a) Preparation of working standard stock solutions and standard response line concentrations.*

Dissolve an accurately weighed portion of the working standard with sufficient 0.054M sodium phosphate buffer, pH 6.9 (solution 18), as described in § 436.101(a)(18) of this chapter, to obtain a stock solution containing 1 milligram of daunorubicin activity per milliliter. The working standard stock solution may be stored under refrigeration for 1 week. Further dilute an aliquot of the stock solution with solution 18 to obtain standard response line concentrations of 4, 8, and 16 micrograms of daunorubicin activity per milliliter. The 8-micrograms-per-milliliter concentration is the reference concentration of the assay.

(b) *Preparation of sample solution.* Dissolve an accurately weighed portion of the sample with sufficient 0.054M sodium phosphate buffer, pH 6.9 (solution 18), as described in § 436.101(a)(18) of this chapter, to obtain a stock solution containing 1 milligram of daunorubicin activity per milliliter (estimated). Further dilute an aliquot of the stock solution with solution 18 to the reference concentration of 8 micrograms of daunorubicin activity per milliliter (estimated).

(c) *Procedure for assay.* Place 1.0 milliliter of each concentration of the standard response line and of the sample solution in each set of replicate tubes (as described in § 436.100(b)(1) of this chapter). Eighteen tubes are used for the three-point standard response line and six for each sample. To each tube, add 9 milliliters of medium 3 (as listed in § 436.102(b)(3) of this chapter), inoculated with 2 milliliters of a suspension of test organism I per liter of medium 3. The suspension of test organism I is prepared as described in § 436.103 of this chapter, except incubate the slants and Roux bottle for 16 to 18 hours at 37° C. Place the inoculated tubes immediately in a water bath at 37° C for approximately 3 hours.

The absorbance value for the growth control should be approximately 0.70-0.75 and the absorbance values for the 16 and 4 micrograms per milliliter standard doses should be approximately 0.25-0.35 and 0.55-0.65, respectively. An adjustment of the inoculum may be necessary in order to obtain absorbance values to these approximate levels in a 3-hour time period. Remove the tubes from the water bath and add 0.5 milliliter of a 12-percent formaldehyde solution to each tube. Determine the absorbance value of each tube in a suitable spectrophotometer, at a wavelength of 530 nanometers. Set the instrument at zero absorbance with an uninoculated blank composed of the same amounts of medium 3, solution 18, and formaldehyde used in the assay.

(d) *Estimation of potency.* Estimate the potency of the sample as follows: Using the three x values and the three corresponding y values, calculate Σx , Σx^2 , $(\Sigma x)^2$, Σy and Σxy . Calculate b, the slope (regression coefficient), and a, the Y-intercept of the standard response line by the following equations:

$$b = \frac{n \sum xy - (\sum x)(\sum y)}{n \sum x^2 - (\sum x)^2}$$

$$a = \frac{\sum y - b \sum x}{n}$$

| | | | | |
|--|---------|---------|---------|--------------------------|
| Standard doses (micrograms per milliliter) | 16.0 | 8.0 | 4.0 | n = 3 |
| Log doses (x) | 1.20412 | 0.90309 | 0.60206 | $\Sigma x = 2.70927$ |
| x^2 | 1.4499 | 0.81557 | 0.36248 | $(\Sigma x)^2 = 7.34014$ |
| Absorbance readings | 0.247 | 0.483 | 0.583 | $\Sigma x^2 = 2.62795$ |
| | 0.236 | 0.414 | 0.584 | |
| | 0.241 | 0.446 | 0.574 | |
| | 0.236 | 0.423 | 0.555 | |
| | 0.233 | 0.416 | 0.578 | |
| | 0.243 | 0.413 | 0.559 | |
| Mean responses (y) | 0.239 | 0.433 | 0.572 | $\Sigma y = 1.244$ |
| xy | 0.28778 | 0.39104 | 0.34438 | $\Sigma xy = 1.0232$ |

$$b = \frac{3(1.0232) - (2.70927)(1.244)}{3(2.62795) - (2.70927)^2} = -0.553$$

$$a = \frac{1.244 - (-0.553)(2.70927)}{3} = 0.914$$

where:

- n=Number of standard doses;
- x=Logarithm of the concentration in micrograms per milliliter of each dose of the standard curve;
- y=Mean response of the six absorbance values for each dose of the standard.

Calculate the concentration of the sample solution X corresponding to the observed mean response of the sample solution Y by the following equation:

$$x = \text{antilog} \frac{Y - a}{b}$$

where:

- X=The concentration of the sample solution in micrograms per milliliter;
- Y=The mean response of the six absorbance values for reference concentration sample solutions.

Calculate the potency of the daunorubicin sample as follows:

$$\text{Potency of daunorubicin sample} = \frac{X \times F}{W}$$

in micrograms per milligram

where:

- F=125, the appropriate dilution factor of the daunorubicin sample;
- W=Weight of sample in milligrams.

The following example illustrates the mathematical calculations of the potency of a sample solution:

Mean response, Y, of sample solution=0.405.

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

(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- α -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