

the working standard in the same manner. Determine the percent relative ab-

sorptivity of the sample using the following calculation:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{Weight of standard in milligrams} \times \text{Potency of standard in micrograms per milligram} \times 10}{\text{Absorbance of standard} \times \text{Weight of sample in milligrams} \times (100 - m)}$$

where: *m*=percent moisture in the sample.

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

(7) *Identity*. Accurately weigh 40 milligrams of the sample and place into a 200-milliliter volumetric flask. Add 100 milliliters of 0.1*N* HCl and place on a shaker until the sample is dissolved. Dilute to volume with 0.1*N* HCl and mix well. Transfer a 5-milliliter aliquot of the solution to each of two 50-milliliter volumetric flasks. To one flask add 10 milliliters of 6*N* HCl and to the

other add 10 milliliters of 3*N* HCl. Place the acid-treated flasks into a boiling water bath for 20 minutes. Remove the flasks and place in a cold water bath. When cool, dilute to volume with water and mix well. Treat a portion of the standard in the same manner. Using a suitable spectrophotometer, place the 6*N* HCl-treated sample into the reference cell and read against the 3*N* HCl-treated sample at a wavelength of 368 nanometers. Reverse the order of the cells in the cell holder and read at a wavelength of 430 nanometers.

$$\frac{(A_{368} + A_{430} \text{ sample}) (\text{milligrams of standard per milliliter}) (100)}{(A_{368} + A_{430} \text{ standard}) (\text{milligrams of sample per milliliter}) (100 - m)} = 0.9 \text{ to } 1.1$$

where: *m*=percent moisture in the sample.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11155, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978; 50 FR 19920, May 13, 1985]

§ 446.20 Doxycycline hyclate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Doxycycline hyclate is [4S - 4α,4α,5α,5α,6α,12α]-4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacene-carboxamide hydrochloride hemihydrate. It is so purified and dried that:

- (i) Its potency is not less than 800 nor more than 920 micrograms of doxycycline per milligram on an “as is” basis.
- (ii) [Reserved]
- (iii) Its moisture content is not less than 1.4 nor more than 2.75 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 nor more than 3.0.

(v) It contains not less than 82 nor more than 90 percent doxycycline on an “as is” basis.

(vi) It gives a positive identity test for doxycycline hyclate.

(vii) It is crystalline.

(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, doxycycline content, identity, and crystallinity.
- (ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of doxycycline per milliliter (estimated). Further dilute with sterile distilled water to the reference concentration of 0.100 microgram of doxycycline per milliliter (estimated).

(2) [Reserved]

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

(4) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing the equivalent of 10 milligrams of doxycycline per milliliter.

(5) *Doxycycline content*—(i) *Equipment*—(a) *Sheet (chromatographic)*. Whatman No. 4 filter paper for chromatography, 15 × 57 centimeters.

(b) *Chamber (chromatographic)*. Square glass chromatography jar, 30 × 30 × 60 centimeters, equipped with 25-centimeter troughs for descending chromatography.

(ii) *Preparation of solutions*—(a) *0.05*N* Methanolic hydrochloric acid*. Dilute 4.2 milliliters of concentrated hydrochloric acid to 1 liter with methanol.

(b) *pH 4.2 buffer*. Mix 5.86 volumes of 0.1*M* citric acid with 4.14 volumes of 0.2*M* disodium phosphate.

(c) *Chromatographic system*. Mix toluene, pyridine, and pH 4.2 buffer in volumetric proportions of 20:3:10, respectively. Allow the phases to separate. Place the upper phase in the troughs near the top of the chamber. Place the lower phase in the bottom of the chamber. Saturate the atmosphere of the tightly sealed chamber for 24 hours before use by placing white blotters on two opposite sides of the chamber so that their ends are immersed in the lower phase in the bottom of the chamber. Replace the solvent in troughs before the chromatograms are to be developed.

(iii) *Preparation of the doxycycline standard solution*. Accurately weigh about 50 milligrams of the doxycycline working standard into a 5-milliliter volumetric flask and bring to volume with 0.05*N* methanolic hydrochloric

acid. Store in the refrigerator and use within 7 days.

(iv) *Preparation of sample*. Accurately weigh about 50 milligrams of the sample into a 5-milliliter volumetric flask and bring to volume with 0.05*N* methanolic hydrochloric acid.

(v) *Preparation of the chromatogram*. Dip the chromatographic sheets into pH 4.2 buffer and lightly blot each sheet between clean nonfluorescing, white blotters. Use separate sheets for the doxycycline standard solution, for each doxycycline sample solution, and for blanks without standard or sample application. Care must be taken so that the moist sheets do not become too dry; a period of 5 to 10 minutes between impregnating the paper and placing it in the chromatographic chamber is usually satisfactory. Evenly apply a 0.100-milliliter aliquot of a doxycycline solution to the origin line of a sheet as a 14-centimeter-long streak. Place the sheets in the chamber and develop them in a descending manner for 2 hours. The doxycycline band should move approximately 12.5 centimeters from the origin line. Remove the sheets from the chamber and air-dry for about 10 minutes.

(vi) *Processing the chromatogram*. Examine each sheet under 366-nanometer ultraviolet light. Outline the fluorescent bands with a pencil. The main marked area should be approximately 10 × 15 centimeters in size. Outline areas on the blank sheet approximately equal in size and in the same locations as those outlined on the standard sheet. Exposure of the sheets to ammonia or other alkaline vapors must be avoided. Cut the marked areas from the sheets and then cut them into approximately 2-centimeter squares. For each sheet, place the squares from each of the following areas into separate 125-milliliter Erlenmeyer flasks: The main doxycycline band of the sample, the main doxycycline band of the standard, all the other bands of the standard, the area of the blank sheet corresponding to the main band of the standard, the other area of the blank sheet corresponding to the other bands of the standard. The time between removing the sheets from the chamber and placing the squares into the Erlenmeyer

flasks should be minimal, since excessive drying of the paper can lead to erratic elutions.

(vii) *Elution.* To each flask add 50 milliliters of 0.05*N* methanolic hydrochloric acid and agitate on a reciprocating shaker for 1 hour. Decant the contents of each flask into another flask by pouring through a small funnel fitted with a glass wool plug.

(viii) *Doxycycline standard solution for direct measurement of absorbance.* Pipette a 0.100-milliliter aliquot of the doxycycline standard solution into

each of three 125-milliliter Erlenmeyer flasks. Add 50 milliliters of 0.05*N* methanolic hydrochloric acid to each of these flasks.

(ix) *Absorbance measurement.* Using a suitable spectrophotometer and 0.05*N* methanolic hydrochloric acid as the reference solvent, determine the absorbance of each eluate and of each doxycycline standard solution at the absorption maximum at about 349 nanometers.

(x) *Calculation of percent doxycycline in samples.* Calculate as follows:

$$\text{Percent doxycycline} = \frac{(A_u - A_b)(W_s)}{(A_s - A_b)(W_u)} \times \text{Doxycycline content of the working standard}$$

where:

A_u =Absorbance of the eluate from the main doxycycline band of the sample sheet.

A_s =Absorbance of the eluate from the main doxycycline band on the standard sheet.

A_b =Absorbance of the eluate from the area of the blank sheet corresponding to the area of the doxycycline band of the standard sheet.

W_u =Weight in milligrams of sample.

W_s =Weight in milligrams of doxycycline working standard.

(xi) *Recovery of the doxycycline standard from the chromatogram.* As follows:

$$\text{Percent recovery} = \frac{A_s - A_b}{A_p} \times \frac{100}{F}$$

where:

A_p =Absorbance of the doxycycline standard solution described in paragraph (b)(5)(viii) of this section.

F =The fractional purity of doxycycline standard solution described in paragraph (b)(5)(xii) of this section.

If the recovery of the doxycycline standard from the chromatogram is less than 95 percent, repeat the chromatogram.

(xii) *Determination of the fractional purity of the doxycycline working standard.* Determine F by means of the following equation:

$$F = 1 - \frac{A_c - A_{cb}}{A_c - A_{cb} + A_s - A_b}$$

where:

A_c =Absorbance of the eluate from sections of the standard chromatogram containing non-doxycycline 349 nanometers-absorbing contaminants.

A_{cb} =Absorbance of the eluates from the sections of the blank sheets corresponding to those sections of the non-doxycycline-absorbing contaminants of the standard sheets.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using the 0.25 potassium bromide mixture described in paragraph (b)(1) of that section.

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

[39 FR 19076, May 30, 1974, as amended at 43 FR 11155, Mar. 17, 1978; 50 FR 19920, May 13, 1985]

§ 446.20a Sterile doxycycline hyclate.

(a) *Requirements for certification—(1) Standards of identity, strength, equality, and purity.* Sterile doxycycline hyclate is [4S - (4 α ,4 α ,5 α ,5 α ,6 α ,12 α)] - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro - 3,5,10,12,12a - pentahydroxy - 6 - methyl - 1,11 - dioxo - 2 - naphthacenicarboxamide hydrochloride hemihydrate. It is so purified and dried that:

(i) Its potency is not less than 800 nor more than 920 micrograms of doxycycline per milligram on an "as is" basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]