

sterile distilled water to the reference concentration of 0.06 microgram of chlortetracycline hydrochloride per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens*. Proceed as directed in § 436.32(b) of this chapter, using a solution containing 5 milligrams of chlortetracycline hydrochloride per milliliter.

(4) [Reserved]

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

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

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

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

(9) *Identity*. To 1.0 milligram of sample, add 2.0 milliliters of concentrated sulfuric acid. In the presence of chlortetracycline, a deep blue color is produced that becomes dark green.

[43 FR 11154, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978, as amended at 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§ 446.15 Demeclocycline.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Demeclocycline is [4S - (4 α ,4a α ,5a α ,6 β ,12a α)] - 7 - chloro - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11, 12a - octahydro - 3,6,10,12,12a - pentahydroxy - 1, 11 - dioxo - 2 - naphthacene-carboxamide. It is so purified and dried that:

(i) Its potency is not less than 970 micrograms of demeclocycline hydrochloride equivalent per milligram on the anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not less than 4.3 percent and not more than 6.7 percent.

(iv) Its pH is an aqueous solution containing 10 milligrams per milliliter is not less than 4 and not more than 5.5.

(v) When calculated on the anhydrous basis, its absorptivity at 380 nanometers relative to that of the

demeclocycline hydrochloride working standard is 107.4 \pm 3.88.

(vi) It is crystalline.

(vii) It passes the identity test.

(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, absorptivity, crystallinity, and identity.

(ii) Samples required: 10 packages, each containing approximately 250 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 0.1N hydrochloric acid to obtain a concentration of 1,000 micrograms of demeclocycline hydrochloride per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.100 microgram of demeclocycline hydrochloride 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 10 milligrams per milliliter.

(5) *Absorptivity*. Determine the percent absorptivity of the sample relative to that of the standard in the following manner: Dissolve an accurately weighed portion of approximately 40 milligrams of the sample in 2 milliliters of 0.1N HCl, dilute to exactly 250 milliliters with distilled water, and mix thoroughly. Transfer a 10-milliliter aliquot of this solution to a 100-milliliter volumetric flask. Add about 75 milliliters of distilled water and 5 milliliters of 5N NaOH, dilute to volume with distilled water, and mix thoroughly. Exactly 6 minutes after the addition of the NaOH, determine the absorbance of the solution at a wavelength of 380 nanometers, using a suitable spectrophotometer and distilled water as the blank. Treat a portion of the demeclocycline hydrochloride

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

tivity 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*. Proceed as directed in § 446.16(b)(7). The value yielded by calculation ranges between 0.97 and 1.17.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11155, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978; 46 FR 16683, Mar. 13, 1981; 50 FR 19920, May 13, 1985]

§ 446.16 Demeclocycline hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Demeclocycline hydrochloride is [4S - (4 α 4 α ,5 α ,6 β ,12 α)] - 7 - chloro - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro-3,6,10,12,12a - pentahydroxy - 1,11 - dioxo - 2 - naphthacenicarboxamide monohydrochloride. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram on the anhydrous basis.

(ii) [Reserved]

(iii) Its loss on drying is not more than 2 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2 and not more than 3.

(v) When calculated on the anhydrous basis, its absorptivity at 380 nanometers relative to that of the demeclocycline hydrochloride standard is 100 \pm 4.2 percent.

(vi) It is crystalline.

(vii) It passes the identity test.

(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, loss on drying, pH, absorptivity, crystallinity, and identity.

(ii) Samples required: 10 packages, each containing approximately 250 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 0.1N hydrochloric acid to obtain a concentration of 1,000 micrograms of demeclocycline hydrochloride per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.100 microgram of demeclocycline hydrochloride per milliliter (estimated).

(2) [Reserved]

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

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

(5) *Absorptivity*. Determine the percent absorptivity of the sample relative to that of the standard in the following manner: Dissolve an accurately weighed portion of approximately 40 milligrams of the sample in 2 milliliters of 0.1N HCl, dilute to exactly 250 milliliters with distilled water, and mix thoroughly. Transfer a 10 milliliter aliquot of this solution to a 100-milliliter volumetric flask. Add about 75 milliliters of distilled water and 5 milliliters of 5N NaOH, dilute to volume with distilled water, and mix thoroughly. Exactly 6 minutes after the addition of the NaOH, determine the absorbance of the solution at a wavelength of 380 nanometers, using a suitable spectrophotometer and distilled water as the blank. Treat a portion of