

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

(9) *Absorptivity*. Determine the absorbance of the sample and standard solutions in the following manner: Dissolve an accurately weighed portion of approximately 40 milligrams each of the sample and standard in approximately 150 milliliters of distilled water and mix thoroughly. Dilute each to exactly 250 milliliters with distilled water and mix thoroughly. Transfer a 10.0-milliliter aliquot of each of these solutions to separate 100-milliliter vol-

umetric flasks. Add approximately 75 milliliters of distilled water and 5.0 milliliters of 5*N* NaOH to each flask, and then dilute to volume with water and mix thoroughly. Exactly 6 minutes after the addition of the NaOH, determine the absorbance of each solution at 380 nanometers, using a suitable spectrophotometer and distilled water as the blank. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

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

where *m*=percent moisture in the sample.

(10) *Identity*. Place approximately 100 milligrams of the sample to be tested in a test tube, and 5 milliliters of 1*N* NaOH, and heat gently to boiling for about 15 seconds. (The musty, aminelike odor of pyrrolidine is detectable.) Allow to cool to room temperature. A deep burgundy-red color of the clear solution indicates the presence of rolitetracycline.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11158, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978; 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§ 446.76a Sterile rolitetracycline nitrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Sterile rolitetracycline nitrate is [4*S*-(4*α*,4*αα*,5*αα*,6*β*, 12*αα*)] - 4 - (dimethylamino) - 1,4,4*a*,5,5*a*,6,11,12*a* - octahydro - 3,6,10,12,12*a* - pentahydroxy - 6 - methyl - 1,11 - dioxo - *N* - (1 - pyrrolidinylmethyl) - 2 - naphthacenicarboxamide mononitrate sesquihydrate. It is so purified and dried that:

- (i) It contains not less than 765 micrograms of rolitetracycline per milligram on an "as is" basis.
- (ii) It is sterile.
- (iii) It is nonpyrogenic.

- (iv) [Reserved]
- (v) It contains no depressor substances.
- (vi) Its moisture content is not more than 5.0 percent.
- (vii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 3.5 and not more than 5.5.
- (viii) It is crystalline.
- (ix) When calculated on an anhydrous basis, its absorptivity at 380 nanometers relative to that of the rolitetracycline standard treated is 89.2±4.0 percent.
- (x) It gives a positive result to the identity tests for rolitetracycline nitrate.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(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, sterility, pyrogens, depressor substances, moisture, pH, crystallinity, absorptivity, and identity.
- (ii) Samples required:
 - (a) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(b) For sterility testing: 20 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 sterile distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of rolitetracycline per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this subchapter, 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 subchapter, using a solution containing 5.0 milligrams of rolitetracycline per milliliter.

(4) [Reserved]

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

(6) *Moisture*. Proceed as directed in § 436.201 of this subchapter.

(7) *pH*. Proceed as directed in § 436.202 of this subchapter, using an aqueous

solution containing 10 milligrams per milliliter.

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

(9) *Absorptivity*. Determine the absorbance of the sample and standard solutions in the following manner: Dissolve an accurately weighed portion of approximately 40 milligrams each of the sample and standard in approximately 150 milliliters of distilled water and mix thoroughly. Dilute each to exactly 250 milliliters with distilled water and mix thoroughly. Transfer a 10.0-milliliter aliquot of each of these solutions to representative 100-milliliter volumetric flasks. Add about 75 milliliters of distilled water and 5.0 milliliters of 5N NaOH to each and then dilute to volume with water and mix thoroughly. Exactly 6 minutes after the addition of the NaOH, determine the absorbance of each solution at 380 nanometers, using a suitable spectrophotometer and distilled water as the blank. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

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

where: m = percent moisture in the sample.

(10) *Identity*—(i) *Rolitetracycline*. Place approximately 100 milligrams of the sample to be used in a test tube, add 5 milliliters of 1N NaOH, and heat gently to boiling for about 15 seconds. (The musty, amine-like odor of pyrrolidine is detectable.) Allow to cool to room temperature. A deep burgundy-red color of the clear solution indicates the presence of rolitetracycline.

(ii) *Nitrate identity*. Transfer approximately 1 gram of sample to a 250-milliliter beaker, add 100 milliliters of water, and acidify with 1 milliliter of acetic acid. Heat to boiling and, with constant stirring, add 10 milliliters of a

10-percent solution of nitron (1,4-diphenyl-3,5-endo-anilino-4,5-dihydro-1,2,4-triazole) $C_{20}H_{16}N_4$ ⁷ in 1N acetic acid. Allow to cool. A heavy precipitate indicates the presence of nitrate.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11159, Mar. 17, 1978; 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§ 446.80 Tetracycline.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Tetracycline is [4S -

⁷Nitron is available from J. T. Baker Laboratory Chemicals, North Phillipsburg, N.J. 08865.