

(3) *Pyrogens*. Proceed as directed in § 436.32(b) of this chapter, using a solution containing 30 milligrams of erythromycin per milliliter.

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

(5) *pH*. Proceed as directed in § 436.202 of this chapter, using a concentration of 50 milligrams of erythromycin per milliliter.

(6) *Residue on ignition*. Proceed as directed in § 436.207(a) of this chapter.

(7) *Heavy metals*. Proceed as directed in § 436.208 of this chapter.

(8) *Identity*. Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(3) of that section.

[51 FR 35215, Oct. 2, 1986, as amended at 55 FR 11584, Mar. 29, 1990]

§ 452.35 Erythromycin stearate.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity*. Erythromycin stearate is the odorless, white or slightly yellow powder of the stearic acid salt of erythromycin. It is practically insoluble in water but is soluble in alcohol, methyl alcohol, chloroform, and ether. It is so purified and dried that:

(i) It contains not less than 550 micrograms of erythromycin per milligram, calculated on an anhydrous basis.

(ii) [Reserved]

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

(iv) Its pH is not less than 6.0 and not more than 11.0.

(v) Its residue on ignition is not more than 1.0 percent.

(vi) It gives positive identity tests for erythromycin stearate.

(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 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 residue on ignition, identity, and crystallinity.

(ii) Samples required: A minimum of 10 containers, each consisting of 500 milligrams.

(b) *Tests and methods of assay—(1) Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient methyl alcohol to give a concentration of 1 milligram of erythromycin base per milliliter (estimated). Further dilute with 0.1M potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of erythromycin base 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 a 1 percent slurry of erythromycin stearate in water.

(5) *Residue on ignition*. Proceed as directed in § 436.207(a) of this chapter.

(6) *Identity*. Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(2) of that section.

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

[39 FR 19149, May 30, 1974, as amended at 50 FR 19920, 19921, May 13, 1985]

§ 452.50 Clarithromycin.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity*. Clarithromycin is 6-O-methylerythromycin A. It is so purified and dried that:

(i) Its potency is not less than 960 micrograms of clarithromycin activity per milligram, on an anhydrous basis.

(ii) Its moisture content is not more than 2.0 percent.

(iii) The pH of a 0.2 percent (weight per volume) slurry in aqueous methanol (95:5) is not less than 7.5 and not more than 10.0.

(iv) Its residue on ignition is not more than 0.3 percent.

(v) Its heavy metals content is not more than 20 parts per million.

(vi) Its specific rotation in chloroform containing 10 milligrams of clarithromycin per milliliter at 20 °C is between -89° and -95°, calculated on an anhydrous basis.

(vii) It gives a positive identity test.

(viii) 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 clarithromycin potency, moisture, pH, residue on ignition, heavy metals, specific rotation, identity, and crystallinity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.216 of this chapter, using a constant column temperature of 50 °C, a suitable ultraviolet detection system operating at 210 nanometers, an analytical column 3 to 30 centimeters long packed with a reversed phase packing material such as octadecyl hydrocarbon bonded silicas (3 to 10 micrometers in diameter), the inlet of this column is connected to a guard column 1 to 5 centimeters in length packed with the same material of 5- to 30-micrometer particle size, a constant flow rate of 0.7 to 1.0 milliliters per minute, and a known injection volume of between 10 and 20 microliters. The retention time for clarithromycin is between 5 and 6 minutes and the retention time for 6,11-Di-O-methylerythromycin A (resolution compound) is between 7 and 8 minutes. Mobile phase, system suitability solution, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Mobile phase.* Add 650 milliliters of methanol and 350 milliliters of 0.067 *M* potassium phosphate (monobasic) to a suitable container, mix well, and adjust the pH to 4.0 with phosphoric acid. 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 system suitability solution.* Prepare a methanol solution containing approximately 625 micrograms per milliliter each of clarithromycin and 6,11-Di-O-methylerythromycin A. Quantitatively transfer an aliquot of this solution to a suitable volumetric flask and dilute it to volume with mobile phase to obtain a solution containing approximately

125 micrograms each of clarithromycin and 6,11-Di-O-methylerythromycin A.

(iii) *Preparation of working standard solution.* Dissolve (by shaking or sonication) an accurately weighed portion of the clarithromycin working standard in sufficient methanol to obtain a known solution containing about 625 micrograms of clarithromycin activity per milliliter. Quantitatively transfer an aliquot of this solution to a suitable volumetric flask and dilute to volume with mobile phase and mix to obtain a known solution containing approximately 125 micrograms of clarithromycin activity per milliliter. Filter through a suitable filter capable of removing particulate matter to 0.5 micron in diameter.

(iv) *Sample solution.* Dissolve (by shaking or sonication) an accurately weighed portion of the sample in sufficient methanol to obtain a solution containing 625 micrograms of clarithromycin activity per milliliter (estimated). Quantitatively transfer an aliquot of this solution to a suitable volumetric flask and dilute to volume with mobile phase and mix to obtain a known solution containing approximately 125 micrograms of clarithromycin activity per milliliter (estimated). Filter through a suitable filter capable of removing particulate matter to 0.5 micron in diameter.

(v) *System suitability requirements*—(A) *Asymmetry factor.* The asymmetry factor (A_s) is satisfactory if it is not less than 0.9 and not more than 1.5 for the clarithromycin peak.

(B) *Efficiency of the column.* The absolute efficiency (h_r) is satisfactory if it is not more than 40.0 for the clarithromycin peak.

(C) *Resolution factor.* The resolution factor (R) between the peak for clarithromycin and the peak for 6,11-Di-O-methylerythromycin A is satisfactory if it is not less than 2.0.

(D) *Coefficient of variation (relative standard deviation).* The coefficient of variation (S_R in percent of 5 replicate injections) is satisfactory if it is not more than 2.0 percent.

(E) *Capacity factor.* Calculate the clarithromycin capacity factor (k) as follows:

$$k' = (t_r/t_0) - 1$$

where:

t_r = Retention time of the clarithromycin peak; and
 t_0 = Void volume time.

The capacity factor is satisfactory if it is not less than 1.3 and not more than 4.0. If the system suitability parameters have been met, then proceed as described in § 436.216(b) of this chapter.

(vi) *Calculations.* Calculate the micrograms of clarithromycin per milligram of sample on an anhydrous basis as follows:

$$\frac{\text{Micrograms of clarithromycin per milligram}}{A_S \times C_U \times (100 - m)} = \frac{A_U \times P_S \times 100}{A_S \times C_U \times (100 - m)}$$

where:

A_U = Area of the clarithromycin peak (at a retention time equal to that observed for the clarithromycin standard) in the chromatogram of the sample;
 A_S = Area of the clarithromycin peak in the chromatogram of the clarithromycin working standard;
 P_S = Clarithromycin activity in the clarithromycin 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) *Moisture.* Proceed as directed in § 436.201 of this chapter, using the sample preparation described in paragraph (d)(1) of that section and the titration procedure described in paragraph (e)(3) of that section, except that instead of adding 20 milliliters of solvent A before starting the titration, add a sufficient volume of solvent C to cover the electrodes in the dry titrating vessel.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, except standardize the pH meter with pH 7.0 and pH 10.0 buffers and prepare the sample as follows: Transfer 200 milligrams of the sample to a 150-milliliter beaker. Add 5 milliliters of methanol and then 95 milliliters of distilled water. Place the pH electrodes in the slurry and stir at the slowest speed possible to ensure mixing but no vortex. After 10 minutes, while still stirring, determine the pH.

(4) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(5) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

(6) *Specific rotation.* Dilute an accurately weighed sample with sufficient chloroform to give a concentration of approximately 10 milligrams of clarithromycin per milliliter. Proceed as directed in § 436.210 of this chapter, using a 1.0-decimeter polarimeter tube, maintaining the solution at 20 °C, and calculate the specific rotation on an anhydrous basis.

(7) *Identity.* Proceed as directed in § 436.211 of this chapter, preparing the sample as follows: Prepare a 5-percent solution of the sample in chloroform and use 0.1 millimeter matched absorption cells.

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

[58 FR 26653, May 4, 1993]

§ 452.60 Azithromycin.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Azithromycin is the dihydrate form of (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl- α -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)- β -*D*-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one. It is so purified and dried that:

(i) Its potency is not less than 945 micrograms and not more than 1,030 micrograms of azithromycin activity per milligram, on the anhydrous basis.

(ii) Its moisture content is not less than 4.0 percent and not more than 5.0 percent.

(iii) The pH of an aqueous methanol (1:1) solution containing 2 milligrams per milliliter is not less than 9 and not more than 11.

(iv) Its residue on ignition is not more than 0.3 percent.

(v) Its heavy metals content is not more than 25 parts per million.

(vi) The specific rotation in absolute ethanol containing 20 milligrams of azithromycin per milliliter at 20 °C is between -45° to -49°, calculated on an anhydrous basis.

(vii) It is crystalline.

(viii) It gives a positive identity test.

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