

more than 2.0 percent. 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 azithromycin per milligram of sample on an anhydrous basis as follows:

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

where:

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

A_S = Area of the azithromycin peak in the chromatogram of the azithromycin working standard;

P_S = Azithromycin activity in the azithromycin 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.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous methanol (1:1) solution containing 2 milligrams per milliliter, prepared by diluting a methanol solution containing 4 milligrams of azithromycin dihydrate 1:1 with distilled water.

(4) *Residue on ignition.* Proceed as directed in § 436.207(b) of this chapter, except use a temperature of 800 °C instead of a temperature range of 500 to 600 °C.

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

(6) *Specific rotation.* Dissolve an accurately weighed sample with sufficient absolute ethanol to give a concentration of approximately 20 milligrams per milliliter. Proceed as directed in § 436.210 of this chapter, except dilute and maintain the test solution at 20 °C instead of 25 °C. Use a 1.0-decimeter polarimeter tube and calculate the specific rotation on an anhydrous basis.

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

(8) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 0.5 percent potassium bromide disc prepared as described in paragraph (b)(1) of that section.

[58 FR 26657, May 4, 1993]

§ 452.75 Troleandomycin.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Troleandomycin is the triacetyl ester of oleandomycin base or a mixture of two or more such esters. It is a white powder. It is so purified that:

(i) Its potency is not less than 750 micrograms of troleandomycin per milligram.

(ii) [Reserved]

(iii) Its loss on drying is not more than 1.0 percent.

(iv) Its pH in an aqueous alcohol solution containing 100 milligrams of troleandomycin per milliliter is not less than 7.0 and not more than 8.5.

(v) Its residue on ignition is not more than 0.1 percent.

(vi) It gives a positive identity test for oleandomycin.

(vii) Its R_f value by paper chromatography is approximately 0.85. If more than one spot appears on the paper chromatogram, determine its acetyl value, which is not less than 15.3 percent and not more than 16.0 percent.

(viii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) 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, loss on drying, pH, residue on ignition, identity, R_f value, acetyl value (only if more than one spot is present in the determination of R_f value), and crystallinity.

(ii) Samples of the batch: 10 packages, nine containing approximately equal portions of not less than 500 milligrams, and one containing not less than 2.0 grams.

(b) *Tests and methods of assay—(1) Potency.* Use either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Chemical method—(a) Reagents and equipment.* (1) Methyl orange reagent: Shake 0.5M boric acid solution for 12 hours (to ensure saturation) with an excess of methyl orange indicator. An alternative method is to heat the mixture to about 50° C. and shake for about

an hour. Then allow to cool. Filter the saturated dye solution and wash three times with chloroform. Store the dye solution over chloroform.

(2) Acid-alcohol solution: Add 2 milliliters of concentrated sulfuric acid to 98 milliliters of absolute methyl alcohol.

(3) Glycerin: Reagent grade.

(4) Chloroform.

(5) Glacial acetic acid.

(6) Centrifuge tubes: 40 milliliters, glass-stoppered.

(b) *Procedure.* Using the troleandomycin working standard which has been dried for 3 hours at 60° C. and a pressure of 5 millimeters or less, prepare a standard solution in chloroform containing 50.0 milligrams of oleandomycin base in 200 milliliters. Transfer 10.0 milliliters of the solution to a 100-milliliter volumetric flask and dilute to volume with chloroform. Transfer 2.0, 4.0, 6.0, and 8.0 milliliters of this solution to glass-stoppered centrifuge tubes (40-milliliter size) and dilute to a total volume of 20.0 milliliters each with chloroform. To the 20 milliliters of the solution present in each 40-milliliter size centrifuge tube, add 0.2 milliliter of glacial acetic acid, 0.2 milliliter of glycerin, and 0.4 milliliter of methyl orange reagent. Shake for 5 minutes and centrifuge for 3 minutes. Immediately transfer to another tube a 10.0-milliliter aliquot from the chloroform (lower) layer. Care must be exercised to see that no portion of the dye-glycerin phase is included with the chloroform aliquot. Add 1.0 milliliter of acid-alcohol solution to this chloroform aliquot, mix well, and read the absorbancy at 535 nanometers, using a 1-centimeter cell and a suitable photometer and using chloroform, similarly treated, as a blank. Prepare a standard curve, plotting the absorbance values of the standard solution against the concentration expressed in micrograms of oleandomycin base per aliquot. Accurately weigh the sample to be tested to give 50 milligrams (estimated) of oleandomycin base. Dissolve in chloroform and make to 200 milliliters with chloroform. Transfer 10.0 milliliters to a 100-milliliter volumetric flask and make to volume with chloroform. Transfer 5.0 milliliters to a glass-stoppered centrifuge tube and

proceed as above. Determine the potency of the sample from the standard curve.

(ii) *Microbiological turbidimetric assay.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 80 percent isopropyl alcohol solution (solution 15) to obtain a stock solution containing 1,000 micrograms per milliliter. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 25 micrograms of troleandomycin 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 a saturated solution prepared by adding 100 milligrams of troleandomycin per milliliter of water-ethyl alcohol (1:1) diluent.

(5) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter, except use a silica crucible.

(6) *Identity.* Dissolve about 10 milligrams in 5 milliliters of hydrochloric acid and heat the solution in a boiling water bath; a greenish yellow color is produced.

(7) *Rf value*—(i) *Apparatus and reagents.* (a) Chromatographic chamber (cylinder, glass-stoppered museum jar, 11.5 inches x 3.5 inches).

(b) Chromatographic paper (8 inches x 8 inches, Whatman No. 1).

(c) 0.1N hydrochloric acid.

(d) Resolving solvent: Butyl acetate, benzene, nitromethane, pyridine (5:5:5:1 by volume).

(e) Spray developing reagent: Place 1.0 milliliter of 10 percent platinum chloride solution and 25.0 milliliters of 4 percent potassium iodide solution in a 250-milliliter volumetric flask. Fill to mark with distilled water and mix well.

(ii) *Procedure.* Dissolve the sample in chloroform to give a solution containing 10 to 20 milligrams of oleandomycin base equivalent per milliliter. Prepare a sheet of chromatographic paper by drawing a line of origin parallel to and 1 inch from the edge of the paper. Wet the paper thoroughly with the 0.1N hydrochloric acid and blot it firmly between

sheets of absorbent paper. Starting 2 inches in from the edge and at 1-inch intervals, apply 3 to 5 microliters of the sample solutions to the starting line. Allow a few minutes for the paper to dry partially. While it is still damp, form a cylinder by bringing the outer edges together, allowing about 1-inch overlap, and secure with a paper clip. Stand the paper in the chromatographic chamber, which has been filled to a depth of one-half of an inch with the resolving solvent. After the solvent front rises to a height of 4 to 5 inches above the origin, remove the paper from the tank and hang it up to air dry. Spray the dried paper with the developing reagent. Hang the paper in a 100° C. oven for 3 minutes. A purple spot becomes visible for troleandomycin at an R_f value of about 0.85. The approximate R_f values for diacetyloleandomycin, monoacetyloleandomycin, and oleandomycin are, respectively, 0.72, 0.27, and 0.13.

(8) *Acetyl determination*—(i) *Apparatus and reagents.* (a) One 3-necked Pyrex flask of approximately 45 milliliters capacity, pear-shaped with T-joints, agar inlet tube, glass-stoppered funnel, glass condenser, and bubble counter.

(b) 50-milliliter Pyrex Erlenmeyer flask.

(c) 10-milliliter buret, calibrated to 0.02 milliliter.

(d) Anhydrous methyl alcohol, reagent grade.

(e) 2*N* sodium hydroxide solution.

(f) Sulfuric acid solution prepared by adding 100 milliliters of concentrated H_2SO_4 to 200 milliliters of water.

(g) 1*N* barium chloride solution.

(h) Phenolphthalein solution (1 percent in ethyl alcohol).

(i) Water-pumped nitrogen.

(j) NaOH solution 0.015*N*.

(ii) *Procedure.* Weigh accurately (to 0.01 milligram) approximately 30 milligrams of the sample into the three-necked acetyl flask. Add 2.0 milliliters of methyl alcohol to dissolve the sample; then add slowly, with gentle swirling, 1.0 milliliter of NaOH solution. Connect the gas inlet tube with bubble counter attached and adjust nitrogen flow to about two bubbles a second. Put glass-stoppered funnel in centerneck of acetyl flask and put

about 5 milliliters of H_2O in the funnel. Add a boiling chip to the solution and attach condenser in the refluxing position with water cooling. Adjust burner flame under acetyl flask to reflux solution gently. Reflux for 30 minutes. Cool assembly slightly; then rinse down condenser (still in reflux position) with a few milliliters of H_2O . Reassemble condenser to the distillation position and add water through the funnel to make a total of approximately 5 milliliters of H_2O added to acetyl flask. Adjust burner flame so that about 5 milliliters of H_2O and methyl alcohol is distilled over in approximately 10 minutes. Discard this distillate. Cool acetyl flask slightly. Acidify solution in flask by adding 1 milliliter of the sulfuric acid solution through the funnel. Adjust burner flame and distill over approximately 20 milliliters of distillate into an Erlenmeyer flask in about 20 minutes, adding water through the funnel as necessary. It is important to keep the liquid volume in the acetyl flask around 2 to 3 milliliters in order to obtain a quantitative recovery of the acetic acid. Collect a second fraction of distillate, about 10 milliliters in volume. As the second fraction is distilling, process the first fraction. Heat the first fraction and boil gently about 20 seconds. Add a few drops of $BaCl_2$ solution to check if any sulfate was distilled over. If the sulfate is present, discard and repeat the whole determination. If the sulfate is absent, immediately titrate the solution with the 0.015*N* NaOH solution to a faint-pink endpoint, using one drop of phenolphthalein solution as the indicator. Repeat the above procedure with the second fraction. If the second fraction requires less than 0.10 milliliter of the 0.015*N* NaOH solution and all the acetic acid has been distilled over, the determination is completed. If greater than this, collect a third fraction of approximately 10 milliliters and titrate this as before. Total volumes of NaOH used and calculate results as follows:

$$\frac{(\text{Milliliters of NaOH} \times N \text{ NaOH} \times 0.043 \times 100)}{(\text{Weight sample in grams})} = \text{Percent acetyl.}$$

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

[39 FR 19149, May 30, 1974, as amended at 48 FR 3960, Jan. 28, 1983; 50 FR 19920, 19921, May 13, 1985]

Subpart B—Oral Dosage Forms

§ 452.110 Erythromycin oral dosage forms.

§ 452.110a Erythromycin tablets.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity*. Erythromycin tablets are erythromycin with suitable and harmless buffer substances, diluents, binders, lubricants, colorings, flavorings, and suitable preservatives. The potency of each tablet is 250 milligrams or 500 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. Tablets shall disintegrate within 1 hour. The loss on drying is not more than 5.0 percent. The erythromycin used in making the batch conforms to the standards prescribed by § 452.10(a)(1), except heavy metals.

(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:

(a) The erythromycin used in making the batch for potency, pH, moisture, residue on ignition, crystallinity, and identity.

(b) The batch for potency, disintegration time, and loss on drying.

(ii) Samples required:

(a) The erythromycin used in making the batch: 10 packages, each containing 500 milligrams.

(b) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay—(1) Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend a representative number of tablets in a high-speed glass blender for 2 to 3 minutes with 200 milliliters of methyl alcohol. Add 300 milliliters of 0.1M potassium phos-

phate buffer, pH 8.0 (solution 3), and blend again for 2 to 3 minutes. Further dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

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

(3) *Disintegration time*. Proceed as directed in § 436.212 of this chapter, using the procedure described in paragraph (e)(2) of that section.

[39 FR 19149, May 30, 1974, as amended at 42 FR 59068, Nov. 15, 1977; 50 FR 19921, May 13, 1985]

§ 452.110b Erythromycin enteric-coated tablets.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity*. Erythromycin enteric-coated tablets are enteric-coated tablets composed of erythromycin, suitable and harmless buffer substances, diluents, binders, lubricants, colorings, and flavorings. Each tablet contains 100, 250, 333, or 500 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. The tablets shall disintegrate within 2 hours. The moisture content is not more than 6 percent. The erythromycin base used in making the batch conforms to the standards of § 452.10(a)(1) (i), (iii), (iv), (v), (vii), and (viii).

(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:

(a) The erythromycin used in making the batch for potency, moisture, pH, residue on ignition, crystallinity, and identity.

(b) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(a) The erythromycin used in making the batch: 10 packages, each containing 500 milligrams.

(b) The batch: A minimum of 36 tablets.