

(6) *pH*. Proceed as directed in § 436.202 of this chapter, using a solution containing 25 milligrams of sodium novobiocin per milliliter.

(7) *Residue on ignition*. Proceed as directed in § 436.207(b) of this chapter, calculating on the basis of an anhydrous sample weight.

(8) *Specific rotation*. Accurately weigh approximately 1.25 grams of the sample in a 25-milliliter glass-stoppered volumetric flask. Prepare an acid-methyl alcohol solution by diluting 1.0 milliliter of concentrated hydrochloric acid to a volume of 100 milliliters with absolute methyl alcohol and mix well. Dissolve the sample in about 15-milliliters of the acid-methyl alcohol solution. Adjust to volume with the acid-methyl alcohol solution and mix well. Proceed as directed in § 436.210 of this chapter, using a 2.0-decimeter polarimeter tube. Calculate the specific rotation on an anhydrous basis.

(9) *Identity*. (i) Using 0.1M aqueous sodium borate as a diluent, prepare 10 milliliters of a solution containing the equivalent of 1 milligram (approximate) of novobiocin per milliliter.

(ii) Prepare a saturated aqueous solution of *N*,2,6-trichloroquinoneimine by shaking continuously for 30 minutes in a dark bottle 25 milligrams of *N*,2,6-trichloroquinoneimine in 100 milliliters of distilled water. Let stand 2 hours after shaking. Store in the dark bottle.

(iii) Add 2.0 milliliters of the saturated *N*,2,6-trichloroquinoneimine solution to 4 milliliters of the novobiocin solution. Mix well and heat in a water bath at 37° C. for 10 minutes. The development of a blue color is a positive test for the presence of novobiocin. To 2 milliliters of the blue solution, add 2 milliliters of *N*-butyl alcohol and shake well. A green color should develop in the butyl alcohol layer. To the other 2-milliliter portion of the blue solution, add 2 milliliters of benzene (c.p.), and shake well. A pink color should develop in the benzene layer.

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

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§ 455.70 Rifampin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality,*

and purity. Rifampin is a red-brown powder. It is 3-(4-methylpiperazinyliminomethyl) rifamycin SV. It is very slightly soluble in water, soluble in ethyl acetate and in methyl alcohol, and freely soluble in chloroform. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) [Reserved]

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

(iv) Its pH is not less than 4.0 and not more than 6.0 in a 1 percent aqueous suspension.

(v) When calculated on the anhydrous basis, its absorptivity at 475 nanometers is 100±4 percent of that of the rifampin working standard, similarly treated.

(vi) It passes the identity test.

(vii) 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 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, 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 § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient methyl alcohol to give a stock solution containing 1.0 milligram of rifampin per milliliter (estimated). Further dilute an aliquot of the stock solution with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 5.0 micrograms of rifampin per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying*. Proceed as directed in § 436.200(b) of this chapter, except dry the sample for 4 hours.

(4) *pH*. Proceed as directed in § 436.202 of this chapter, using a 1 percent aqueous suspension.

(5) *Absorptivity*. Determine the absorbance of the sample and standard

solutions in the following manner: Dissolve approximately 100 milligrams each of the sample and standard in a 100-milliliter volumetric flask containing 50 milliliters of absolute methyl alcohol, and dilute to volume with absolute methyl alcohol. Transfer a 2-milliliter aliquot to a 100-milliliter volumetric flask, and dilute to volume with 1 percent potassium phosphate buffer,

pH 6.0, as listed in § 436.101(a)(1) of this chapter. Using a suitable spectrophotometer equipped with a 1-centimeter cell, immediately determine the absorption of each solution at 475 nanometers with the blank containing the same proportion of solution 1 and methyl alcohol as the sample and standard solutions. Calculate the absorptivity as follows:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{milligrams standard} \times (100 - m_1)}{\text{Absorbance of standard} \times \text{milligrams sample} \times (100 - m_2)} \times 100$$

where:

m_1 =percent moisture in standard;
 m_2 =percent moisture in sample.

(6) *Identity*. Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(3) of that section, except use a 4 percent solution of the sample in chloroform and 0.1-millimeter matched absorption cells.

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

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§ 455.80a Sterile spectinomycin hydrochloride.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Sterile spectinomycin hydrochloride is the pentahydrated dihydrochloride salt of decahydro-4a, 7, 9-trihydroxy-2-methyl-6,8-bis(methylamino)4H-pyrano[2,3-b][1,4]benzodioxin-4-one. It is so purified and dried that:

(i) Its spectinomycin content is not less than 603 micrograms per milligram. If it is packaged for dispensing, its spectinomycin content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of spectinomycin that it is represented to contain.

(ii) Its microbiological activity is not less than 603 micrograms of spectinomycin per milligram.

(iii) It is sterile.

(iv) It is nonpyrogenic.

(v) [Reserved]

(vi) It contains no depressor substances.

(vii) Its moisture content is not less than 16 percent nor more than 20 percent.

(viii) Its pH is an aqueous solution containing 10 milligrams per milliliter is not less than 3.8 nor more than 5.6. If it is packaged for dispensing, when reconstituted as directed in the labeling, its pH is not less than 4.0 nor more than 7.0.

(ix) It passes the identity test.

(x) Its residue on ignition is less than 1 percent.

(xi) 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 spectinomycin content, microbiological activity, sterility, pyrogens, depressor substances, moisture, pH, identity, residue on ignition, and crystallinity.

(ii) Samples required:

(a) If the batch is packaged for repackaging or for use in the manufacture of another drug:

(1) For all tests except sterility: eight packages, each containing approximately 300 milligrams and two containing not less than 3 grams.