

$$A_s = \frac{a+b}{2a}$$

where:

a=Horizontal distance from point of ascent to point of maximum peak height; and
b=Horizontal distance from the point of maximum peak height to point of descent.

The asymmetry factor (*A_s*) is satisfactory if it is not more than 1.5.

(B) *Efficiency of the column.* From the number of theoretical plates (*n*) calculated as described in § 436.216(c)(2) of this chapter, calculate the reduced plate height (*h_r*) as follows:

$$h_r = \frac{(L)(10,000)}{(n)(d_p)}$$

where:

L=Length of the column in centimeters;
n=Number of theoretical plates; and
d_p=Average diameter of the particles in the analytical column packing in micrometers.

The absolute efficiency (*h_r*) is satisfactory if it is not more than 20.0, equivalent to 1,500 theoretical plates for a 30-centimeter column of 10 micrometer particles.

(C) *Resolution factor.* The resolution factor (*R_s*) between the peak for mupirocin and its nearest eluting peak produced from its acid degradation is satisfactory if it is not less than 2.0. The chromatogram of the resolution test solution should show a significantly reduced mupirocin peak immediately preceded by a peak due to mupirocin degradation products. This degradation peak may appear as a single peak or be partially resolved showing a shoulder or two overlapping peaks.

(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.

If the system suitability parameters have been met, then proceed as described in § 436.216(b) of this chapter.

(iv) *Calculations.* Calculate the micrograms of mupirocin per milligram of sample as follows:

$$\text{Micrograms of mupirocin per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

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

A_s=Area of the mupirocin peak in the chromatogram of the mupirocin working standard;

P_s=Mupirocin activity in the mupirocin working standard solution in micrograms per milliliter;

C_u=Milligrams of mupirocin sample per milliliter of sample solution;

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 a saturated aqueous solution.

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

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

[55 FR 2641, Jan. 26, 1990; 55 FR 11110, Mar. 26, 1990; 55 FR 14378, Apr. 17, 1990]

§ 455.50 Calcium novobiocin.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Calcium novobiocin is the calcium salt of a kind of novobiocin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 840 micrograms per milligram, expressed in terms of novobiocin on an anhydrous basis.

(ii) [Reserved]

(iii) Its loss on drying is not more than 10 percent.

(iv) Its pH in a saturated aqueous suspension containing 25 milligrams per milliliter is not less than 6.5 and not more than 8.5.

(v) Its specific rotation in an acidmethyl alcohol solution at 25° C. is not less than -50° and not more than -58°.

(vi) It demonstrates a positive color identity test.

(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, loss on drying, pH, specific rotation, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 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 5 milliliters of absolute ethyl alcohol and then dilute with sufficient 0.1M potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of 1,000 micrograms (estimated) per milliliter. Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 0.5 microgram of novobiocin 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 aqueous suspension prepared by suspending 25 milligrams of calcium novobiocin per milliliter.

(5) *Specific rotation.* Proceed as directed in § 455.51a(b)(8).

(6) *Identity.* Proceed as directed in § 455.51(b)(7).

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

[39 FR 19166, May 30, 1974, as amended at 41 FR 10886, Mar. 15, 1976; 43 FR 9801, Mar. 10, 1978; 50 FR 19921, May 13, 1985]

§ 455.51 Sodium novobiocin.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Sodium novobiocin is the monosodium salt of a kind of novobiocin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 850 micrograms of novobiocin per milligram, calculated on an anhydrous basis.

(ii) [Reserved]

(iii) Its loss on drying is not more than 6.0 percent.

(iv) Its pH in a solution containing 25 milligrams per milliliter is not less than 6.5 and not more than 8.5.

(v) Its residue on ignition is not less than 10.5 percent and not more than 12.0 percent, calculated on an anhydrous basis.

(vi) Its specific rotation in an acidmethyl alcohol solution at 25° C. is not less than -50° and not more than -58°.

(vii) It demonstrates a positive color identity test.

(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, specific rotation, identity and crystallinity.

(ii) Samples required on the batch; 10 packages, each containing approximately 600 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 0.1M potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 0.5 microgram of novobiocin 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 solution containing 25 milligrams of sodium novobiocin per milliliter.

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

(6) *Specific rotation.* Accurately weigh approximately 1.25 grams of the sample in a 25-milliliter glass-stoppered volumetric flask. Prepare an acid-methyl