

(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 with a concentration 100 milligrams per milliliter.

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

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

(7) *Identity.* Proceed as directed in paragraph (b)(1)(i) of this section.

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

#### § 455.40 Mupirocin.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Mupirocin is nonanoic acid, 9-[[3-methyl-1-oxo-4-[tetrahydro-3,4-dihydroxy-5-[[3-(2-hydroxy-1-methylpropyl)oxiranyl]methyl]-2H-pyran-2-yl]-2-butenyl]oxy]-, [2S-[2 $\alpha$ (E), 3B, 4B, 5 $\alpha$ [2R\*, 3R\*(1R\*, 2R\*)]]]-. It is a white to off-white crystalline solid. It is so purified and dried that:

(i) Its potency is not less than 920 micrograms per milligram on an anhydrous basis.

(ii) Its moisture content is not more than 1.0 percent.

(iii) The pH of a saturated aqueous solution of mupirocin is not less than 3.5 and not more than 4.0.

(iv) It is crystalline.

(v) It gives a positive identity test for mupirocin.

(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 potency, moisture, pH, crystallinity, and identity.

(ii) Samples, if required by the Center for Drug Evaluation and Research: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay—(1) Potency.* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 229 nanometers, a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing

material such as an octadecylsilane, a flow rate of not more than 2.0 milliliters per minute, and a known injection volume of between 10 and 20 microliters. Use the resolution test solution to determine resolution in lieu of the working standard solution. Reagents, working standard and sample solutions, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Reagents—(A) Acetonitrile.* Distilled in glass. Ultraviolet grade.

(B) *Phosphate buffer, pH 6.3.* Prepare a 0.05M sodium monobasic phosphate solution and adjust to pH 6.3 with 1.0N sodium hydroxide.

(C) *Mobile phase.* To 750 milliliters of 0.05M, pH 6.3 phosphate buffer, add 250 milliliters of acetonitrile. 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 working standard, sample, and resolution, test solutions—(A) Working standard solution.* Accurately weigh approximately 11 milligrams of the mupirocin working standard into a 100-milliliter volumetric flask. Dissolve the standard in about 20 milliliters of acetonitrile and dilute to volume with pH 6.3 phosphate buffer. Mix well.

(B) *Sample solution.* Transfer approximately 11 milligrams of sample, accurately weighed, to a 100-milliliter volumetric flask. Dissolve the sample in about 20 milliliters of acetonitrile and dilute to volume with pH 6.3 phosphate buffer. Mix well.

(C) *Resolution test solution.* Acidify approximately 10 milliliters of the working standard solution with 6N hydrochloric acid to pH 2.0. Allow to stand at room temperature for about 2 hours. Neutralize this solution. Use this solution to determine the resolution requirement for the chromatographic system.

(iii) *System suitability requirements—(A) Asymmetry factor.* Calculate the asymmetry factor ( $A_s$ ), measured at a point 5 percent of the peak height from the baseline as follows:

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

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<sub>s</sub>*) 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<sub>r</sub>*) 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<sub>p</sub>*=Average diameter of the particles in the analytical column packing in micrometers.

The absolute efficiency (*h<sub>r</sub>*) 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<sub>s</sub>*) 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<sub>R</sub>* 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<sub>u</sub>*=Area of the mupirocin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

*A<sub>s</sub>*=Area of the mupirocin peak in the chromatogram of the mupirocin working standard;

*P<sub>s</sub>*=Mupirocin activity in the mupirocin working standard solution in micrograms per milliliter;

*C<sub>u</sub>*=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.