

(b) Calculate the ceftriaxone anhydrous free acid content of the container as follows:

$$\frac{\text{Milligrams of ceftriaxone anhydrous}}{\text{free acid per container}} = \frac{A_u \times P_s \times d}{A_s \times 1,000}$$

where:

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

$A_s$ =Area of the ceftriaxone peak in the chromatogram of the ceftriaxone working standard;

$P_s$ =Ceftriaxone activity in the ceftriaxone working standard solution in micrograms of anhydrous free acid per milliliter; and

$d$ =Dilution factor of the sample.

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens*. Proceed as directed in § 436.32(h) of this chapter, using a solution containing 20 milligrams of ceftriaxone 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 an aqueous solution containing 100 milligrams per milliliter.

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

(7) *Identity*. Proceed as directed in § 436.211 of this chapter, using a potassium bromide disc containing 1.3 milligrams of ceftriaxone sodium in 300 milligrams of potassium bromide, prepared as described in paragraph (b)(1) of that section.

[50 FR 9999, Mar. 13, 1985; 50 FR 11690, Mar. 25, 1985; 50 FR 18243, Apr. 30, 1985, as amended at 55 FR 11583, Mar. 29, 1990]

**§ 442.58a Sterile cefotiam dihydrochloride.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Cefotiam dihydrochloride is 5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[2-amino-4-thiazolyl]acetyl]-amino-3[[1-[2-(dimethylamino)ethyl]-1H-tetrazol-5-yl]-thio]methyl]-8-oxo-, dihydrochloride, (6*R*-trans)-. It is so purified and dried that:

(i) Its potency is not less than 790 and not more than 925 micrograms of cefotiam per milligram on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) Its moisture content is not more than 7.0 percent.

(v) It passes the identity test.

(vi) 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 potency, sterility, pyrogens, moisture, identity, and crystallinity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(B) For sterility testing: One package containing approximately 6 grams of a composite sample.

(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 254 nanometers, a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl hydrocarbon bonded silicas, a flow rate not to exceed 2.0 milliliters per minute, and a known injection volume of between 10 and 20 microliters. Mobile phase, working standard and sample solutions, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Mobile phase*. Dissolve 13.1 grams of ammonium sulfate in 850 milliliters of water. Adjust the pH to 6.5 with dilute aqueous ammonia. Add 150 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.* Dissolve approximately 100 milligrams of the cefotiam working standard, accurately weighed, in water and dilute to 100 milliliters. Further dilute with mobile phase to obtain a solution containing 50 micrograms of cefotiam activity per milliliter.

(B) *Sample solution.* Dissolve approximately 100 milligrams of the sample, accurately weighed, in water and dilute to 100 milliliters. Further dilute with mobile phase to obtain a solution containing 50 micrograms of cefotiam activity per milliliter (estimated).

(C) *Resolution test solution.* Dissolve an accurately weighed portion of cefotiam working standard in water to obtain a solution containing approximately 1.0 milligram of cefotiam activity per milliliter. Heat this solution at 95 °C for 15 minutes. This procedure allows cefotiam lactone to be produced. Dilute 1.0 milliliter of this solution to 100 milliliters with mobile phase.

(iii) *System suitability requirements—*

(A) *Tailing factor.* The tailing factor (*T*) for the cefotiam peak is satisfactory if it is not more than 1.76 at 5 percent of peak height.

(B) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 1985 theoretical plates for the cefotiam peak.

(C) *Resolution factor.* The resolution factor (*R*) between the peak for cefotiam and the peak for cefotiam lactone (generated in situ) is satisfactory if it is not less than 4.0.

(D) *Coefficient of variation.* The coefficient of variation (*S<sub>R</sub>* in percent) of 5 replicate injections is satisfactory if it is not more than 1.0 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 cefotiam per milligram of sample as follows:

$$\begin{array}{l} \text{Micrograms of} \\ \text{cefotiam} \\ \text{per milligram} \end{array} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

*A<sub>u</sub>*=Area of the cefotiam 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 cefotiam peak in the chro-

matogram of the cefotiam working standard;

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

*C<sub>u</sub>*=Milligrams of the sample per milliliter of sample solution; and

*m*=Percent moisture content of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(g) of this chapter, using a solution containing 40 milligrams per milliliter.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter, using the sample preparation described in paragraph (d)(4) of that section and the titration procedure described in paragraph (e)(3) of that section, except:

(i) In lieu of 3 milliliters of anhydrous methanol solution, inject 20 milliliters of a formamide:methanol solution (2:1) into the container and shake to dissolve the contents (prior to use in preparation of the formamide:methanol solution, dry 500 grams of formamide over 20 grams of anhydrous sodium sulfate for 24 hours);

(ii) Rinse the syringe, needle, and immediate container with two separate 5-milliliter portions of anhydrous methanol, in lieu of one 3-milliliter portion of anhydrous methanol; and

(iii) In paragraph (e)(3) of that section, add a sufficient volume of the formamide:methanol solution (2:1) to cover the electrodes in the dry titrating vessel, in lieu of 20 milliliters of solvent A before starting the titration.

(5) *Identity.* Using a solution containing 20 micrograms per milliliter of water and a suitable spectrophotometer, record the ultraviolet absorption spectrum from 220 to 310 nanometers. The spectrum compares qualitatively to that of the cefotiam working standard similarly tested.

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

[54 FR 20785, May 15, 1989]

**§ 442.60 Cefpiramide.**

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Cefpiramide is (6*R*, 7*R*)-7-