

as directed in paragraph (b)(1) of that section.

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

[39 FR 19040, May 30, 1974, as amended at 40 FR 23725, June 2, 1975; 50 FR 19919, May 13, 1985]

§ 442.40 Cephadrine.

(a) *Requirements of certification—(1) Standards of identity, strength, quality, and purity*. Cephadrine is (6*R*, 7*R*)-7-[(*R*)-2-amino-2-(1,4-cyclohexadien-1-yl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylic acid. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms and not more than 1,050 micrograms of cephradine per milligram on an anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not more than 6.0 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 3.5 and not more than 6.0.

(v) Its cephalixin content is not more than 5 percent on an anhydrous basis.

(vi) It passes the 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 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, cephalixin content, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 500 milligrams.

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

(i) *Microbiological agar diffusion assay*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution containing 1.0 milligram of cephradine per milliliter (estimated). Further dilute

an aliquot of the stock solution with solution 1 to the reference concentration of 10 micrograms of cephradine per milliliter (estimated).

(ii) *Hydroxylamine colorimetric assay for cephradine—(a) Typical equipment*. Use automated equipment capable of performing the following functions: Introduction of sample into reaction vessels, addition of reagents to the samples to form reaction mixtures, incubation of the reaction mixtures, colorimetric determination of the reaction product at 480 nanometers using a 1-centimeter tubular flow cuvette, and documentation of the results with a strip chart recorder. A suitable system is the Auto Analyzer II equipment consisting of a Solid or Liquid Sampler II, a twenty channel Pump III, a colorimeter equipped with a 1-centimeter tubular flow cuvette and light filters producing incident light at 480 nanometers, and a strip chart recorder with scale expander.

(b) *Reagents—(1) Hydroxylamine hydrochloride solution*. Dissolve 20 grams of hydroxylamine hydrochloride and 5 milliliters of emulsifying stock solution (prepared to contain 100 milligrams of polyoxyethylene fatty alcohol ether, such as Brij-35 or equivalent, per 100 milliliters distilled water) in sufficient distilled water to make 1 liter.

(2) *Buffer*. Dissolve 173 grams of sodium hydroxide and 20.6 grams of sodium acetate in sufficient distilled water to make 1 liter. Dilute 75 milliliters of this solution with distilled water to 500 milliliters.

(3) *3.3*N* Sulfuric acid*. Dilute 91 milliliters of concentrated sulfuric acid to 1 liter with distilled water.

(4) *Ferric nitrate solution*. Dissolve 300 grams of ferric nitrate nonahydrate (9H₂O) in a mixture of 2.8 milliliters of concentrated sulfuric acid and sufficient distilled water to make 1 liter.

(c) *Preparation of working standard solutions*. Dissolve and dilute an accurately weighed portion of the cephradine working standard in sufficient distilled water to obtain a concentration of 1 milligram of cephradine per milliliter.

(d) *Preparation of sample solutions*. Dissolve an accurately weighed portion of the sample in distilled water and

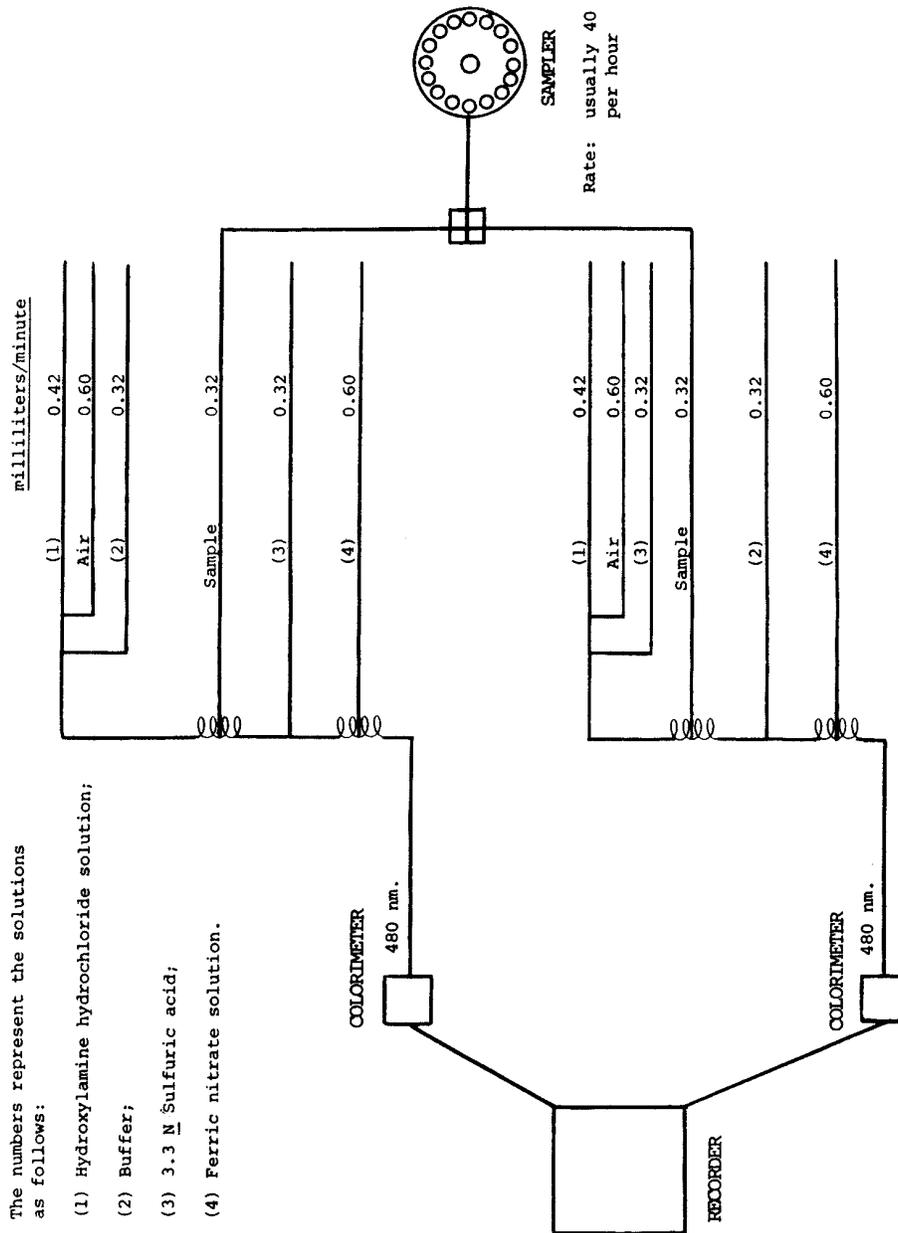
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further dilute to 1 milligram of cephadrine per milliliter (estimated).

(e) *Procedure.* Use the standard and sample solutions prepared as indicated in paragraph (b)(1)(ii) (c) and (d) of this section respectively. The arrangement

of the apparatus and flow of samples and reagents are shown in the manifold diagram set forth in this paragraph (b)(1)(ii)(e). The sampler rate is usually 40 per hour, but may be varied.



(f) Calculate the potency of the sample in micrograms per milligram as follows:

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

where:

- A_u =Absorbance of sample solution;
- P_s =Potency of working standard solution in micrograms, per milliliter;
- A_s =Absorbance of working standard solution;
- W_u =Milligrams of sample per milliliter of sample solution;
- m =Percent moisture in sample.

(iii) *High-pressure liquid chromatographic assay.* Proceed as directed in §436.337 of this chapter, preparing the sample as described in paragraph (e)(3)(i) of that section.

(2) [Reserved]

(3) *Moisture.* Proceed as directed in §436.201 of this chapter.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Cephalexin content.* Proceed as directed in §436.337 of this chapter.

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

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

[40 FR 26270, June 23, 1975, as amended at 45 FR 16474, Mar. 14, 1980; 46 FR 25608, May 8, 1981; 48 FR 51293, Nov. 8, 1983; 49 FR 47485, Dec. 5, 1984; 50 FR 19919, May 13, 1985]

§ 442.40a Sterile cephradine.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Cephradine is 7-[D-2 - amino -2- (1,4 - cyclohexadien - 1 -yl) acetamido] - 3 - methyl - 8 - oxo - 5-thia - 1- azabicyclo[4.2.0]oct -2 - ene-2-carboxylic acid. It is so purified and dried that:

(i) Its potency is not less than 900 and not more than 1,050 micrograms of cephradine per milligram on the anhydrous basis. If it is packaged for dispensing, its cephradine content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of cephradine that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its moisture content is not more than 6.0 percent.

(vi) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 3.5 and not more than 6.0.

(vii) Its cephalixin content is not more than 5 percent on an anhydrous basis.

(viii) It passes the identity test.

(ix) 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, pH, cephalixin content, identity, and crystallinity.

(ii) Samples required:

(a) If the batch is packaged for re-packing or for manufacturing use:

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

(2) For sterility testing: 1 package containing approximately 6 grams of a composite sample.

(b) If the batch is packaged for dispensing:

(1) For all tests except sterility: A minimum of 10 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

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

(i) *Microbiological agar diffusion assay.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution containing 1.0 milligram of cephradine per milliliter (estimated); also, if it is packaged for dispensing, reconstitute the sample as directed in the labeling, except use distilled water in lieu of reconstituting fluid. Then using a suitable hypodermic needle and syringe, remove an accurately measured representative portion from each container. Dilute with solution 1 to give a