

proceed as directed in paragraph (f)(1) of this section.

(f) *Procedure*—(1) *Cephadrine content*. Using the equipment, reagents, mobile phase, and operating conditions as listed in paragraphs (a), (b), (c), and (d) of this section, inject 10 microliters of the cephradine working standard solution into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of the expected components. After separation of the working standard solution has been completed, inject 10 microliters of the sample solution prepared as described in paragraph (e)(3)(i) of this section into the chromatograph and repeat the procedure described for the working standard solution. The elution order is void volume, cephalixin, and cephradine. If the sample is packaged for dispensing, repeat the procedure for each sample solution prepared as described in paragraphs (e)(3)(ii) (a) and (b) of this section.

(2) *Cephalixin content*. Proceed as directed in paragraph (f)(1) of this section, except:

(i) Use a detector sensitivity setting that gives a peak height for the cephalixin in the cephalixin working standard that is about 75 percent of full scale; and

(ii) Use the cephalixin working standard in lieu of the cephradine working standard.

(g) *Calculations*. (1) Calculate the micrograms of cephradine per milligram of sample as follows:

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

where:

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

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

$P_s$ =Cephradine activity in the cephradine working standard solution in micrograms per milliliter;

$C_u$ =Milligrams of sample per milliliter of sample solution; and

$m$ = Percent moisture content of the sample.

(2) Calculate the cephradine content of the vial as follows:

$$\text{Milligrams of cefoperazone per vial} = \frac{A_u \times P_s \times d}{A_s \times 1,000}$$

where:

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

$A_s$ =Area of the cephradine peak in the Chromatogram of the cephradine working standard;

$P_s$ =Cephradine activity in the cephradine working standard solution in micrograms per milliliter;

$C_s$ =Milligrams of the standard per milliliter; and

$d$ =Dilution factor of the sample.

(3) Calculate the percent cephalixin content of the sample as follows:

$$\text{Percent cephalixin} = \frac{A_a \times W_b \times P_b \times 10}{A_b \times W_u \times (100 - m)}$$

where:

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

$A_b$ =Area of the cephalixin peak in the chromatogram of the cephalixin working standard;

$W_b$ =Milligrams of cephalixin per milliliter of cephalixin working standard solution;

$W_u$ =Milligrams of cephradine per milliliter of sample solution;

$P_b$ =Micrograms of cephalixin per milligram of cephalixin working standard; and

$m$ =Percent moisture content of the sample.

[49 FR 47483, Dec. 5, 1984]

**§ 436.338 High-pressure liquid chromatographic assay for cefoperazone.**

(a) *Equipment*. A suitable high-pressure liquid chromatograph equipped with:

(1) A low dead volume cell 8 to 20 microliters;

(2) A light path length of 1 centimeter;

(3) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(4) A suitable recorder of at least 25.4 centimeter deflection;

(5) A suitable integrator;

(6) A 30-centimeter column having an inside diameter of 4.0 millimeters and

packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 to 10 micrometers in diameter, United States Pharmacopeia XX.

(b) *Mobile phase.* Mix 1.2 milliliters 1M triethylammonium acetate, 2.8 milliliters 1M acetic acid, and 120 milliliters acetonitrile in a one liter flask and dilute to volume with distilled water. Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatographic pumping system.

(c) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 2.0 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale.

(d) *Preparation of working standard solution.* Dissolve approximately 40 milligrams of working standard, accurately weighed, with mobile phase to obtain a solution containing 0.16 milligram of cefoperazone activity per milliliter.

(e) *Preparation of sample solutions—(1) Product not packaged for dispensing (micrograms of cefoperazone per milligram).* Dissolve an accurately weighed portion of the sample with sufficient mobile phase to obtain a solution containing 0.16 milligram of cefoperazone activity per milliliter. Using this sample solution, proceed as directed in paragraph (f) of this section.

(2) *Product packaged for dispensing.* Determine both micrograms of cefoperazone per milligram of the sample and milligrams of cefoperazone per container. Use separate containers for preparation of each sample solution as described in paragraph (e)(2)(i) and (ii) of this section.

(i) *Micrograms of cefoperazone per milligram.* Dissolve and accurately weighed portion of the sample with sufficient mobile phase to obtain a solution containing 0.16 milligram of cefoperazone activity per milliliter. Using this sample solution, proceed as directed in paragraph (f) of this section.

(ii) *Milligrams of cefoperazone per container.* Reconstitute the sample as directed in the labeling. Then using a

suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Further dilute and aliquot of this solution with mobile phase to a concentration of 0.16 milligram of cefoperazone activity per milliliter. Using this sample solution, proceed as directed in paragraph (f) of this section.

(f) *Procedure.* Using the equipment, reagents, and operating conditions as listed in paragraphs (a), (b), and (c) of this section, inject 10 microliters of the working standard solution into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of the expected components. After separation of the working standard solution has been completed, inject 10 microliters of the sample solution prepared as described in paragraph (e)(1) of this section into the chromatograph and repeat the procedure described for the working standard solution. If the sample is packaged for dispensing, repeat the procedure for each sample solution prepared as described in paragraphs (e)(2)(i) and (ii) of this section.

(g) *Calculations—(1)* Calculate the micrograms of cefoperazone per milligram of sample as follows:

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

where:

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

$A_s$ =Area of the cefoperazone working standard peak;

$P_s$ =Cefoperazone activity in the cefoperazone working standard solution in micrograms per milliliter;

$C_u$ =Milligrams of sample per milliliter of sample solution; and

$m$ = Percent moisture content of the sample.

(2) Calculate the cefoperazone content of the vial as follows:

$$\frac{\text{Milligrams of cefoperazone per vial}}{\text{cefoperazone per vial}} = \frac{A_u \times P_s \times d}{A_s \times 1,000}$$

where:

$A_s$ =Area of the cefoperazone sample peak (at a retention time equal to that observed for the standard);

$A_r$ =Area of the cefoperazone working standard peak;

$P_s$ =Cefoperazone activity in the cefoperazone working standard solution in micrograms per milliliter; and

$d$ =Dilution factor of the sample.

[48 FR 789, Jan. 7, 1983; 48 FR 7439, Feb. 22, 1983; 48 FR 28250, June 21, 1983]

**§ 436.339 High-pressure liquid chromatographic assay for bleomycin fractions.**

(a) *Equipment.* A high-pressure liquid chromatograph equipped with:

- (1) Two solvent pumps;
- (2) A solvent programmer;
- (3) A low dead volume cell 8 to 20 microliters;
- (4) A light path length of 1 centimeter;
- (5) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;
- (6) A suitable recorder;
- (7) A suitable integrator; and
- (8) A suitable-sized column approximately 25 centimeters in length having an inside diameter of 4.6 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 to 10 micrometers in diameter, USP XX.

(b) *Reagents*—(1) *0.005M 1-pentanesulfonic acid in 0.5 percent acetic acid adjusted to pH 4.3 with concentrated ammonium hydroxide.* Filter and degas before using.

(2) *Methanol, spectrophotometric grade.* Filter and degas before using.

(3) *Mobile phase.* Adjust the solvent programmer for linear gradient development starting with a mixture of 0.005M 1-pentanesulfonic acid:methanol (9:1) and ending with a mixture of 0.005M 1-pentanesulfonic acid:methanol (6:4) in 1 hour at a flow rate of 1.2 milliliters per minute. Minor flow rate and gradient changes can be made as necessary depending on column and instrument conditions. Disodium ethylenediaminetetraacetic acid USP at a concentration of 0.005M may be added to the mobile phase if necessary for satisfactory performance.

(c) *Preparation of sample solution.* Reconstitute the vial with 6 milliliters of deaerated water.

(d) *Procedure.* Using the equipment and reagents listed in paragraphs (a) and (b) of this section, start pumping the mobile solvent at the initial conditions. Inject 10 microliters of the sample solution into the chromatograph and begin the linear gradient pumping program. After the final mobile phase conditions are reached (1 hour) continue to pump the solvent mixture for an additional 20 minutes or until the demethylbleomycin A<sub>2</sub> is eluted. The elution order is void volume, bleomycinic acid, bleomycin A<sub>2</sub>, bleomycin A<sub>5</sub>, bleomycin B<sub>2</sub>, bleomycin B<sub>4</sub>, and demethylbleomycin A<sub>2</sub>.

(e) *Calculations.* Calculate the percentage of each bleomycin by comparing its peak area contribution to that of the total response of all the bleomycins.

[48 FR 51912, Nov. 15, 1983]

**§ 436.340 High-pressure liquid chromatographic assay for tetracycline hydrochloride content and 4-epitetracycline hydrochloride content.**

(a) *Equipment.* A suitable high-pressure liquid chromatograph equipped with:

- (1) A low dead volume cell 8 to 20 microliters;
- (2) A light path length of 1 centimeter;
- (3) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;
- (4) A suitable recorder of at least 25.4-centimeter deflection;
- (5) A suitable integrator; and
- (6) A 30-centimeter column having an inside diameter of 4.0 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles.

(b) *Mobile phase.* Dissolve 0.55 gram of monobasic ammonium phosphate in 900 milliliters of water. Adjust the pH to 1.8 with concentrated phosphoric acid and dilute to 1 liter with water. Mix 800 milliliters of this solution with 200 milliliters of methanol. Filter the mobile