

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 A₂ is eluted. The elution order is void volume, bleomycinic acid, bleomycin A₂, bleomycin A₅, bleomycin B₂, bleomycin B₄, and demethylbleomycin A₂.

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

[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

phase through a suitable glass fiber filter that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatography pumping system.

(c) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 1.0 milliliter per minute. Use a detector sensitivity setting that gives a peak height for the 4-epitetracycline peak that is at least 50 percent of scale.

(d) *Preparation of working standard and sample solutions—(1) Working standard solution.* Accurately weigh approximately 18 milligrams of the tetracycline hydrochloride working standard into a 50-milliliter volumetric flask. Into the same flask, accurately weigh approximately 38 milligrams of the 4-epitetracycline working standard. Dissolve and dilute to volume with a methanol:water mixture (7:18).

(2) *Sample solution.* Reconstitute the sample as directed in the labeling,

Transfer 10.0 milliliters of the reconstituted sample into a 50-milliliter volumetric flask and dilute to volume with a methanol:water mixture (7:18).

(e) *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 separation of the expected components. After separation of the working standard solution has been completed, inject 10 microliters of the sample solution into the chromatograph and repeat the procedure described for the working standard solution. The elution order is 4-epitetracycline followed by tetracycline.

(f) *Calculations.* Calculate the tetracycline hydrochloride and 4-epitetracycline hydrochloride content as follows:

$$\begin{aligned} \text{Milligrams of tetracycline hydrochloride} & \\ \text{per milliliter of sample} & = \frac{A_1[(W_1 \times B) + (W_e \times C)]}{A_2 \times 1,000} \end{aligned}$$

$$\begin{aligned} \text{Milligrams of 4-epitetracycline hydrochloride} & \\ \text{per milliliter of sample} & = \frac{A_3[(W_e \times D) + (W_t \times E)]}{A_4 \times 1,000} \end{aligned}$$

where:

A_1 =Area of the tetracycline sample peak (at a retention time equal to that observed for tetracycline in the tetracycline working standard);

A_2 =Area of the tetracycline peak in the tetracycline working standard;

A_3 =Area of the 4-epitetracycline sample peak (at a retention time equal to that observed for the 4-epitetracycline peak in the 4-epitetracycline working standard);

A_4 =Area of the 4-epitetracycline peak in the 4-epitetracycline working standard;

W_t =Milligrams of the tetracycline working standard;

W_e =Milligrams of the 4-epitetracycline working standard;

B =Percent tetracycline hydrochloride in the tetracycline working standard;

C =Percent tetracycline hydrochloride in the 4-epitetracycline working standard;

D =Percent 4-epitetracycline hydrochloride

in the 4-epitetracycline working standard; and

E =Percent 4-epitetracycline hydrochloride in the tetracycline working standard.

[48 FR 51290, Nov. 8, 1983]

§ 436.341 High-pressure liquid chromatographic assay for plicamycin.

(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 280 nanometers;