

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

(5) A suitable integrator; and

(6) A 25-centimeter column having an inside diameter of 4.6 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 micrometers to 10 micrometers in diameter, U.S.P. XX.

(b) *Reagents*—(1) 0.01M phosphoric acid.

(2) *Mobile phase*. Mix acetonitrile (high-pressure liquid chromatography grade):0.01M phosphoric acid (350:650). 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 chromatograph 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 working standard that is at least 50 percent of scale.

(d) *Preparation of working standard and sample solutions*—(1) *Preparation of working standard solution*. Place approximately 5 milligrams of the plicamycin working standard, accurately weighed, into a 50-milliliter, amber volumetric flask and dilute to volume with mobile phase and mix.

(2) *Preparation of sample solution*. Prepare the sample solution as described in the individual monograph for the drug being tested.

(e) *Procedure*. Use the equipment, reagents, operating conditions, and working standard and sample solutions described in paragraphs (a), (b), (c), and (d) of this section, and proceed as directed in paragraph (e)(1) of this section.

(1) *System suitability test*. Equilibrate and condition the column by passage of about 10 to 15 void volumes of mobile phase followed by two or more replicate injections of the working standard solution. Allow an elution time sufficient to obtain satisfactory separation of expected components after each injection. Record the peak responses and calculate the relative standard deviation as described for system suitability tests in the U.S.P. XX General

Chapter 621 chromatography. Proceed as directed in paragraph (e)(2) of this section if the minimum performance requirement for the relative standard deviation is not more than 2.0 percent. If the minimum performance requirement is not met, adjustment must be made to the system to obtain satisfactory operation before proceeding as described in paragraph (e)(2) of this section.

(2) *Determination of the chromatogram*. Inject 10 microliters of the working standard solution into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of expected components. After separation of the working standard has been completed, inject 10 microliters of the sample solution into the chromatograph and repeat the procedure described for the working standard solution.

(f) *Calculations*. Calculate the plicamycin content as described in the individual monograph for the drug being tested.

[49 FR 24017, June 11, 1984, as amended at 50 FR 5749, Feb. 12, 1985]

§ 436.342 High-pressure liquid chromatographic assay for cefazolin.

(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; and

(5) 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, USP XX.

(b) *Reagents*—(1) *Buffer solution, pH 3.6*. Transfer 0.9 gram of sodium phosphate, dibasic USP and 1.298 grams of citric acid USP to a 1-liter volumetric flask. Dissolve and dilute to volume with distilled water and mix.

(2) *Buffer solution, pH 7.0*. Transfer 5.68 grams of sodium phosphate, dibasic

USP and 3.63 grams of potassium phosphate monobasic to a 1-liter volumetric flask. Dissolve and dilute to volume with distilled water and mix.

(3) *Mobile phase.* Mix buffer solution, pH 3.6; acetonitrile (9:1). Filter 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 chromatograph pumping system.

(4) *Internal standard solution.* Transfer 1.2 grams of salicylic acid to a 200-milliliter volumetric flask. Dissolve in 10 milliliters of methyl alcohol, dilute to volume with buffer solution, pH 7.0, and mix.

(c) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 2 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. The minimum between peaks must be no more than 2 millimeters above the initial baseline.

(d) *Preparation of working standard and sample solutions—(1) Working standard solution.* Place approximately 50 milligrams of cefazolin working standard, accurately weighed, into a 50-milliliter volumetric flask. Dissolve and dilute to volume with buffer solution, pH 7.0, and mix. Transfer 4.0 milliliters of this solution to a 200-milliliter volumetric flask, add 5.0 milliliters of internal standard solution, dilute to volume with buffer solution, pH 7.0, and mix.

(2) *Sample solution.* Place approximately 50 milligrams of the sample, accurately weighed, into a 50-milliliter volumetric flask. Dissolve and dilute to volume with buffer solution, pH 7.0, and mix. Transfer 4.0 milliliters of this solution to a 200-milliliter volumetric flask, add 5.0 milliliters of internal standard solution, dilute to volume with buffer solution, pH 7.0, and mix.

(e) *Procedure.* Using the equipment, mobile phase, and operating conditions listed in paragraphs (a), (b), and (c) of this section, inject 10 microliters of the working standard solution prepared as directed in paragraph (d)(1) of this section into the chromatograph. After separation of the working standard solution has been completed, inject 10

microliters of the sample solution prepared as described in paragraph (d)(2) of this section into the chromatograph and repeat the procedure described for the working standard solution. Allow an elution time sufficient to obtain satisfactory separation of the expected components. The elution order is void volume, salicylic acid and cefazolin.

(f) *Calculation.* Calculate the micrograms of cefazolin per milligram of sample as follows:

$$\text{Micrograms of cefazolin per milligram} = \frac{R_u \times P_s \times 100}{R_s \times C_u \times (100 - m)}$$

where:

R_u = Area of the cefazolin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard) /Area of internal standard peak;

R_s = Area of the cefazolin peak in the chromatogram of the cefazolin working standard/Area of internal standard peak;

P_s = Cefazolin activity in the cefazolin 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.

[48 FR 33478, July 22, 1983; 48 FR 34947, Aug. 2, 1983]

§ 436.343 High-pressure liquid chromatographic assay for cefuroxime.

(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 15-centimeter column having an inside diameter of 4.6 millimeters and packed with hexyl silane chemically bonded to porous silica or ceramic microparticles, 5 micrometers in diameter.

(b) *Reagents—(1) Acetate buffer, pH 3.4.* Place 50 milliliters of 0.1M sodium acetate into a 1,000-milliliter volumetric flask and dilute to volume with 0.1M acetic acid. Mix.