

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 ceftizoxime per milligram of sample as follows:

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

where:

R_u =Area of the ceftizoxime 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 ceftizoxime peak in the chromatogram of the ceftizoxime working standard/Area of internal standard peak;

P_s =Ceftizoxime activity in the ceftizoxime 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.

$$\frac{\text{Milligrams of ceftizoxime per vial}}{R_s \times 1,000} = \frac{R_u \times P_s \times d}{R_s \times 1,000}$$

where:

R_u =Area of the ceftizoxime 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 ceftizoxime peak in the chromatogram of the ceftizoxime working standard/Area of internal standard peak;

P_s =Ceftizoxime activity in the ceftizoxime working standard solution in micrograms per milliliter; and

d =Dilution factor of the sample.

[48 FR 46270, Oct. 12, 1983; 48 FR 49656, Oct. 27, 1983]

§ 436.346 High-pressure liquid chromatographic assay for cyclosporine.

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

(1) A suitable pump capable of reproducibly delivering a liquid to a pressure of 4,500 pounds per square inch and a flow rate of at least 5 milliliters per minute;

(2) A suitable ultraviolet detection system operating at a wavelength of 210 nanometers;

(3) A suitable recorder;

(4) A suitable integrator;

(5) An oven or water bath capable of maintaining the column at an operating temperature of 70° C;

(6) A steel capillary tube, 1 meter in length, having an inside diameter of 0.25 millimeter. This tube is inserted between the injection system and the chromatographic column and is equilibrated to 70° C; and

(7) A sample injection valve on which the loop determines the sample size.

(b) *Columns*. The chromatographic column is packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing materials that exhibit some degree of polarity such as the hydrocarbon bonded silicas with dimethyl, trimethyl, or octyl groups. Connect a saturation column gravity packed with similarly bonded silica particles 40 to 60 microns in diameter to the inlet of the analytical column.

(c) *Mobile phase*. Mix acetonitrile, water, methanol, and *o*-phosphoric acid (900:525:75:0.075 by volume). Degas by passing through a 0.5-micrometer filter with vacuum and ultrasonicate for no less than 2 minutes before use. The mobile phase may be sparged perceptibly with helium through a 2-micrometer metal filter for the duration of the analysis. Adjust the ratio of acetonitrile to aqueous buffer as necessary to obtain satisfactory retention of the peaks.

(d) *Operating conditions*. Perform the assay at a constant operating temperature of 70° C 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 with a typical chart speed of 2.5 millimeters per minute. Obtain chromatograms for performance parameters at a chart speed of not less than 25 millimeters per minute to allow a more accurate measurement of peak geometry.

(e) *Preparation of working standard and sample solutions*. Prepare the working standard and sample solutions as directed in the individual monographs for cyclosporine.

(f) *Systems suitability*. Equilibrate and condition the column by passage of about 10 to 15 void volumes of mobile phase followed by about 5 injections of not less than 10 microliters each of working standard solution. Proceed

with the analysis when the following minimum performance requirements have been met or exceeded.

(1) *Capacity ratio factor*. Calculate the capacity ratio (k) of the cyclosporine peak as follows:

$$k = \frac{t - t_m}{t_m}$$

where:

t =Retention time of solute; and
 t_m =Retention time of solvent or unretained substance.

The capacity ratio is satisfactory if it is not less than 3 or not more than 10.

(2) *Coefficient of variation*. The coefficient of variation of at least five replicate injections is less than 1 percent.

(3) *Efficiency*. Calculate the efficiency (n) as follows:

$$n = 5.545 \left(\frac{t}{W_{0.5}} \right)^2$$

where:

t =Retention time of solute; and
 $W_{0.5}$ =Peak width at half height. Both t and $W_{0.5}$ must be measured in the same units.

The efficiency is satisfactory if it is greater than 1,500 theoretical plates when assaying cyclosporine and greater than 700 theoretical plates when assaying finished dosage forms.

(4) *Asymmetry factor*. Calculate the asymmetry factor (A_s) as follows:

$$A_s = \frac{W_{0.1}}{2f}$$

where:

$W_{0.1}$ =Horizontal distance measured from a point on the cyclosporine peak ascent 10 percent above the baseline to an intercept with the cyclosporine peak descent; and

f =Horizontal distance from point of 10 percent ascent above the baseline of the cyclosporine peak to point of maximum peak height.

The asymmetry factor is satisfactory if it is not more than 1.5.

(5) *Resolution*. Calculate the resolution (R_s) as follows:

$$R_s = \frac{2(t_j - t_i)}{(W_i + W_j)}$$

where:

t =Retention time of solute; and the subscripts i and j designate two different peaks and where t_j is larger than t_i ; and
 W =Width of peak at baseline as determined by extrapolating the relative straight sides to the baseline. Both t and W must be measured in the same units.

Resolution between the cyclosporine peak and any other peak must be at least 1.1.

(g) *Procedure*. Using the equipment, columns, mobile phase, operating conditions and the working standard and sample solutions listed in paragraphs (a), (b), (c), (d), and (e) of this section, inject 20 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 solution has been completed, inject 20 microliters of the sample solution into the chromatograph and repeat the procedure described for the working standard solution.

(h) *Calculations*. Calculate the cyclosporine content of cyclosporine and its dosage forms as directed in the individual monographs.

[49 FR 22631, May 31, 1984; 49 FR 27489, July 5, 1984]

§ 436.347 High-pressure liquid chromatographic assay for cefoxitin.

(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, 5 micrometers to 10 micrometers in diameter, U.S.P. XX.

(b) *Reagents*—(1) *One percent potassium phosphate buffer, pH 6.0*. Prepare as described in § 436.101(a)(1).