

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).

(2) *Mobile phase.* Mix distilled water:glacial acetic acid:acetonitrile (800:10:190). 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. The minimum between peaks must be no more than 2 millimeters above the baseline.

(d) *Preparation of working standard and sample solutions.* Use the working standard and sample solutions prepared as described in the individual monographs for the drug being tested.

(e) *Procedure.* Using the equipment, reagents, and operating conditions as described 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.

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

[49 FR 47827, Dec. 7, 1984]

§ 436.348 High-pressure liquid chromatographic assay for ceforanide.

(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. A particular column used for analysis of ceforanide should not be used for the analysis of other drugs.—

(b) *Mobile phase.* Mix 18.0 milliliters of 10 percent aqueous tetrabutylammonium hydroxide and 8.56 milliliters of 11N potassium hydroxide. Add the mixture to approximately 700 milliliters of distilled water. Add 200 milliliters of reagent grade methanol. Adjust the pH of the mixture to pH 7.0 with concentrated phosphoric acid and dilute to 1,000 milliliters with distilled water. Prepare fresh daily. Filter the mobile phase through a suitable glass fiber filter or equivalent which 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 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.* Prepare a solution containing 1,000 micrograms of ceforanide activity per milliliter in mobile phase. Inject working standard solution within 5 minutes after dissolution.

(2) *Preparation of sample solution.* Prepare the sample solution as directed in the individual monograph for the drug being tested. Inject sample solution within 5 minutes after dissolution.

(e) *Procedure.* Use the equipment, mobile phase, 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