

milligrams of the sample, ceforanide for injection, into a 100-milliliter volumetric flask. Dissolve and dilute to volume with distilled water. Transfer 2.0 milliliters of the sample solution into a 10-milliliter volumetric flask, add 2.0 milliliters of THAM solution and 3.0 milliliters of 2,4-dinitrofluorobenzene solution. Cap tightly and mix well. Place the flask in a 50° C water bath for 30 minutes. Remove from water bath, allow the flask to cool to room temperature, and dilute to volume with methanol. Mix well.

(f) *Procedure.* Use the equipment, reagents, mobile phase, operating conditions, and standard and sample solutions described in paragraphs (a), (b), (c), (d), and (e) of this section, and proceed as directed in paragraph (f)(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 three replicate injections of 20 microliters each of the standard solution. Allow an elution time sufficient to obtain satisfactory separation of the expected components after each injection. Record the peak responses and calculate the resolution factor, tailing factor, efficiency of the column, coefficient of variation, and capacity factor as described for system suitability tests in the U.S.P. XX General Chapter 621 chromatography. Proceed as directed in paragraph (f)(2) of this section if the following minimum performance requirements have been met:

(i) *Resolution factor.* The resolution factor between the peak for derivatized *L*-lysine and from the peak for the dinitrofluorobenzene derivatizing reagent is satisfactory if it is not less than 4.5;

(ii) *Tailing factor.* The tailing factor is satisfactory if it is not more than 1.3;

(iii) *Efficiency of the column.* The efficiency of the column is satisfactory if it is greater than 1,500 theoretical plates;

(iv) *Coefficient of variation.* The coefficient of variation of at least three replicate injections is satisfactory if it is not more than 1.5 percent; and

(v) *Capacity factor.* The capacity factor is satisfactory if it is not less than 4 and not more than 6.

If the minimum performance requirements are not met, adjustments must be made to the system to obtain satisfactory operation before proceeding as described in paragraph (f)(2) of this section.

(2) *Determination of the chromatogram.* Inject 20 microliters of the standard solution into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of the expected components. After separation of the standard solution is completed, inject 20 microliters of the sample solution into the chromatograph and repeat the procedure described for the standard solution.

(g) *Calculations.* Calculate the percent of *L*-lysine per milligram of ceforanide for injection as follows:

$$\text{Percent of } L\text{-lysine} = \frac{A_u \times P_s}{A_s \times C_u} \times 10$$

where:

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

$A_s$  = Area of the *L*-lysine peak in the chromatogram of the *L*-lysine standard;

$P_s$  = *L*-lysine content in the *L*-lysine standard solution in micrograms per milliliter; and

$C_u$  = Milligrams of sample per milliliter of sample solution.

[49 FR 25846, June 25, 1984; 49 FR 34347, Aug. 30, 1984; 49 FR 40006, Oct. 12, 1984]

#### § 436.350 High-performance liquid chromatographic assay for cefonicid.

(a) *Apparatus.* A suitable high-performance liquid chromatograph equipped with:

(1) A suitable detection system specified in the monograph for the drug being tested;

(2) A suitable recording device of at least 25-centimeter deflection;

(3) A suitable chromatographic data managing system; and

(4) An analytical column, 3 to 30 centimeters long, packed with a material as defined in the monograph for the drug being tested; and if specified in

that monograph, the inlet of this column may be connected to a guard column 3 to 5 centimeters in length, packed with the same material of 40 to 60 micrometers particle size.

(b) *Procedure.* Perform the assay and calculate the drug content using the temperature, instrumental conditions, and calculations specified in the monograph for the drug being tested with a flow rate not to exceed 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 typical chart speed of not less than 2.5 millimeters per minute. Use the apparatus described in paragraph (a) of this section; and the reagents and working standard and sample solutions described in the monograph for the drug being tested. Equilibrate and condition the column by passage of 10 to 15 void volumes of mobile phase followed by five replicate injections of the same volume (between 10 and 20 microliters) of the working standard solution. Allow an operating time sufficiently long to obtain satisfactory separation and elution of the expected components after each injection. Record the peak responses and calculate the prescribed system suitability requirements as follows:

(c) *System suitability test.* Using the apparatus and procedure described in this section, test the chromatographic system for assay as follows:

(1) *Tailing factor.* Calculate the tailing factor ( $T$ ), from distances measured along the horizontal line at 5 percent of the peak height above the baseline, as follows:

$$T = \frac{W_{0.05}}{2f}$$

where:

$W_{0.05}$  = Width of peak at 5 percent height; and  
 $f$  = Horizontal distance from point of ascent to a point coincident with maximum peak height.

(2) *Efficiency of the column.* Calculate the number of theoretical plates ( $n$ ) of the column as follows:

$$n = 5.545 \left[ \frac{t_R}{W_h} \right]^2$$

where:

$n$  = Efficiency, as number of theoretical plates for column;

$t_R$  = Retention time of solute; and

$w_h$  = Peak width at half-height.

(3) *Resolution factor.* Calculate the resolution factor ( $R$ ), between desacetyl cefonicid and cefonicid, as follows:

$$R = \frac{2(t_2 - t_1)}{w_1 + w_2}$$

where:

$t_1$  = Retention time of desacetyl cefonicid;

$t_2$  = Retention time of cefonicid; and

$w_1$  and  $w_2$  = Widths of the bases of the corresponding peaks obtained by extrapolating the relatively straight sides of the peaks to the baseline.

(4) *Coefficient of variation (relative standard deviation).* Calculate the coefficient of variation ( $S_R$  in percent) as follows:

$$S_R = \frac{100}{\bar{X}} \left[ \frac{\sum_{i=1}^n (X_i - \bar{X})^2}{N-1} \right]^{1/2}$$

where:

$\bar{X}$  is the mean of  $N$  individual measurements of  $X$ .

If the complete operating system meets the system suitability requirements of the monograph for the drug being tested, proceed as described in paragraph (b) of this section, using the sample solution in lieu of the working standard solution.

[49 FR 34347, Aug. 30, 1984, as amended at 49 FR 44460, Nov. 7, 1984; 50 FR 29209, July 18, 1985]

#### § 436.351 High-performance liquid chromatographic assay for amoxicillin and clavulanic acid.

(a) *Apparatus.* A suitable high-performance liquid chromatograph equipped with:

(1) A suitable detection system specified in the monograph for the drug being tested;

(2) A suitable recording device of at least 25-centimeter deflection;

(3) A suitable chromatographic data managing system; and