

(19) *Cefpodoxime proxetil*—(i) *Dissolution fluid: 0.04 molar glycine buffer, pH 3.0*—(A) *Stock solution.* Dissolve 54.5 grams of glycine (aminoacetic acid) and 42.6 grams of sodium chloride in about 500 milliliters of deionized water in a 1-liter volumetric flask. Add cautiously, and with swirling, 14.2 milliliters of concentrated hydrochloric acid. Cool to room temperature. Dilute to volume with deionized water and mix. Check the pH of the solution obtained by diluting 50 milliliters of the stock solution to 900 milliliters with deionized water. The pH should be 3.0 ± 0.1 . If necessary, adjust the pH of the stock solution with 50 percent sodium hydroxide or concentrated hydrochloric acid. Recheck that the pH of the working solution is 3.0 ± 0.1 .

(B) *Working solution.* Dilute 50 milliliters of stock solution to 900 milliliters with deionized water.

(ii) *Preparation of the working standard solutions.* Accurately weigh approximately 28 milligrams for the 100-milligram tablets and 56 milligrams for the 200-milligram tablets of the cefpodoxime proxetil working standard and dissolve in 10 milliliters of methanol. Dilute to 200 milliliters with dissolution fluid. Prepare fresh daily.

(iii) *Sample solutions.* Filter the sample solutions through a 0.45-micron filter before use. Use the sample solution as it is removed from the dissolution vessel without further dilution.

(iv) *Procedure.* Using a suitable spectrophotometer and water as the blank, determine the absorbance of each standard and sample solution at the absorbance peak at approximately 259 nanometers. Determine the exact position of the absorption peak for the particular instrument used.

(v) *Calculations.* Determine the percent of label dissolved as follows:

$$\text{Percent dissolved} = (A_{sam}/A_{std}) \times (C_s/L) \times V \times P \times F1$$

where:

A_{sam} = Absorbance of the sample at 259 nanometers;

A_{std} = Absorbance of the working standard solution at 259 nanometers;

C_s = Concentration of the working standard preparation in milligrams per milliliter;

L = Tablet strength, in milligrams per tablet;

P = Purity of the reference standard in percent;

V = Volume of dissolution fluid used in milliliters (900); and

$F1$ = 0.7666 (conversion factor to free acid equivalents).

(d) *Evaluation.* Use the dissolution acceptance table and interpretation in the United States Pharmacopeia XXI.

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EDITORIAL NOTE: For FEDERAL REGISTER citations affecting § 430.215, see the List of CFR Sections Affected appearing in the Finding Aids section of this volume.

§ 436.216 High-performance liquid chromatographic assay.

(a) *Equipment.* 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, flow rate, and calculations specified in the monograph for the drug being tested. Use a detector sensitivity setting that gives a peak height for the working standard solution that is at least 50 percent of scale with typical chart speed of not less than 2.5 millimeters per minute. Use the equipment described in paragraph (a) of this section. Use the reagents, working standard solution, and sample solution 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 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 described for the system suitability test in paragraph (c) of this section.

(c) *System suitability test.* Select the system suitability requirements specified in the monograph for the drug being tested. Then, using the equipment and procedure described in this section, test the chromatographic system for assay as follows:

(1) *Trailing factor or asymmetry factor.* Calculate either the trailing factor (T), from distances measured along the horizontal line at 5 percent of the peak height above the baseline or the asymmetry factor (A_s) measured at a point 10 percent of the peak height from the baseline; whichever is required in the appropriate monograph, 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.

$$A_s = \frac{a+b}{2a}$$

where:

a =Horizontal distance from point of ascent to point of maximum peak height; and
 b =Horizontal distance from the point of maximum peak height to point of descent.

(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.

Calculate the absolute efficiency of the column, (reduced plate height) (h),

$$h_r = \frac{(L)(10,000)}{(n)(d_p)}$$

where:

L =Length of column in centimeters;
 n =Number of theoretical plates; and
 d_p

(3) *Resolution.* Calculate the resolution (R) as follows:

$$R = \frac{2(t_{Rj} - t_{Ri})}{w_i + w_j}$$

where:

t_{Rj} =Retention time of a solute eluting after i (t_{Rj} is larger than t_{Ri});

t_{Ri} =Retention time for any solute;

w_i =Width of peak at baseline for any solute; and

w_j =Width of peak at baseline for any solute eluting after i .

(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 of individual measurements of X_i .

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, except alternate injections of the working standard solution with injections of the sample solution.

(5) *Capacity factor.* Calculate the capacity factor (k), if required in the monograph as follows:

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

where:

t_r =Retention time of solute; and

t_m =Retention time of solvent or unretained substance, calculated as follows:

$$t_m = \frac{(3.1416)(D^2)(L)(0.75)}{4F}$$

where:

D =Column diameter in centimeters;

L =Column length in centimeters;

0.75 =Average total column porosity; and

F =Flow rate in milliliters per minute.

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