

The asymmetry factor (A_s) is satisfactory if it is not less than 0.8 and not more than 1.8.

(2) *Efficiency of the column.* From the number of theoretical plates (n) calculated as described in § 436.216(c)(2) calculate the reduced plate height (h_r) for the vancomycin B peak as follows:

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

where:

L =Length of the column in centimeters;

n =Number of theoretical plates; and

d_p =Average diameter of the particles in the column in micrometers.

The absolute efficiency (h_a) is satisfactory if it is not more than 40 for the vancomycin B peak in the resolution solution.

(3) *Resolution.* The resolution (R) between the vancomycin B peak and the peak for resolution compound 1 is not less than 3.0. Resolution compound 2 is eluted between 3 and 6 minutes after the start of the period when the percentage of mobile phase B is increasing from 0 percent to 100 percent.

(4) *Coefficient of variation (relative standard deviation).* The coefficient of variation (S_R in percent) of five replicate injections of the resolution solution is calculated as described in § 436.216(c)(4) is satisfactory if it is not more than 2.0 percent.

(5) *Capacity factor (k).* Calculate the capacity factor (k) for vancomycin B 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.

The capacity factor (k) for vancomycin B is satisfactory if it is not less than 2.6 and not more than 3.3.

When the system suitability requirements have been met, then proceed as described in

paragraph (f) of this section. Alternate chromatographic conditions are acceptable provided that the system suitability parameters are met. However, the sample preparation described in paragraph (e)(2) of this section should not be changed.

(h) *Calculations.* (1) Calculate the percentage of vancomycin B in the specimen as follows:

$$\text{Percentage of vancomycin B} = \frac{A_B}{A_{Total}} \times 100 \text{ percent}$$

where:

A_B =Area of the vancomycin B peak in the dilute (0.4 milligram per milliliter) sample solution; and

A_{Total} =Area of the vancomycin B peak in the dilute (0.4 milligram per milliliter) solution+[Area of the total related substances peaks (exclude the area of the vancomycin B peak) in the concentrated solution (10 milligrams per milliliter) divided by 25].

(2) Calculate the percentage of each other peak as follows:

$$\text{Percentage of related substance (i)} = \frac{[A_{i/25}]}{A_{Total}} \times 100 \text{ percent}$$

where:

A_i =Area of any given peak, other than the main peak in the concentrated solution (10 milligrams per milliliter); and

A_{Total} =Area of the vancomycin B peak in the dilute (0.4 milligram per milliliter) solution+[Area of the total related substances peaks (exclude the area of the vancomycin B peak) in the concentrated solution (10 milligrams per milliliter) divided by 25].

[54 FR 20383, May 11, 1989; 54 FR 25849, June 20, 1989]

§ 436.367 Thin-layer chromatographic identity test for cephalixin hydrochloride.

(a) *Equipment*—(1) *Chromatography tank.* Use a rectangular tank approximately 23 × 23 × 9 centimeters, with a glass solvent trough in the bottom and a tight-fitting cover. Line the inside walls of the tank with Whatman #3 MM chromatographic paper or equivalent.

(2) *Plates.* Use 20 × 20 centimeter thin layer chromatographic plates coated with silica gel 60F-254 or equivalent to a thickness of 250 microns.

(b) *Developing solvent.* Mix ethylacetate, acetonitrile, water and glacial acetic acid in volumetric proportions of 42:14:18:14, respectively.

(c) *Preparation of the spotting solutions.* Prepare a solution of the sample containing 25 milligrams per milliliter of cephalexin hydrochloride in water. Prepare a solution of cephalexin monohydrate reference material at a concentration of 25 milligrams per milliliter. Add water and 0.1*N* hydrochloric acid in a dropwise mode until the material is completely dissolved.

(d) *Procedure.* Pour the developing solvent into the glass trough at the bottom of the chromatography tank. Cover and seal the tank. Allow it to equilibrate for 1 hour. Prepare a plate as follows: On a line 2 centimeters from the base of the plate, and at intervals of 2 centimeters, spot approximately 5 microliters of the standard solution to points 1 and 3 and approximately 5 microliters of the sample solution to point 2. After all spots are thoroughly dry, place the plate directly into the glass trough of the chromatography tank. Cover and seal the tank. Allow the solvent front to travel approximately 15 centimeters from the starting line. Remove the plate from the tank and allow it to air dry.

(e) *Evaluation.* View the dry plate under ultraviolet light (254 nanometers). Measure the distance the solvent front traveled from the starting line and the distance the spots are from the starting line. Calculate the R_f value by dividing the latter by the former. The sample and standard should have spots of corresponding R_f values of approximately 0.35.

[54 FR 48860, Nov. 28, 1989; 54 FR 51816, Dec. 18, 1989]

§ 436.368 Thin layer chromatographic identity test for cefprozil.

(a) *Equipment*—(1) *Chromatography tank.* Use a glass rectangular tank approximately 23 x 23 x 9 centimeters lined with filter paper and equipped with a tight-fitting cover.

(2) *Plates.* Use 20 x 20 centimeter thin layer chromatography plates coated with silica gel GF to a thickness of 250 microns.

(b) *Reagents*—(1) *Diluent.* Mix 0.1*N* HCl and acetone in volumetric proportions of 1:4.

(2) *Developing solvent.* Mix n-butanol, glacial acetic acid and water in volumetric proportions of 60:20:20.

(3) *Detection reagent.* Iodine vapor.

(c) *Assay solutions*—(1) *Reference standard solution.* Dissolve 50 milligrams of cefprozil (Z) reference standard in 10 milliliters of diluent.

(2) *Sample solution.* Place an amount of sample containing approximately 50 milligrams of cefprozil in a 20-milliliter glass stoppered vial. Add 10 milliliters of diluent. Shake for 5 minutes and allow the solids to settle.

(d) *Procedure.* Pour a suitable quantity of the developing solvent into a glass, chromatographic tank lined with filter paper and allow to equilibrate for 1 hour. On a line 2 centimeters from the bottom edge of the plate, spot 10 microliters each of the reference solution and sample solution. Draw a line indicating the distance to which the developing solvent must travel at a point 12 centimeters from the bottom edge of the plate. Place the plate in the tank and allow the solvent to migrate to the finishing line. Remove the plate and air dry in a fume hood. Place the dried plate in a chamber containing iodine vapors.

(e) *Evaluation.* Measure the distance the solvent front traveled from the starting line, and the distance the spots are from the starting line. Divide the latter by the former to calculate the R_f value. The identity test is positive if the sample solution produces a yellow spot at the same R_f value and has the same appearance as the spot obtained for the reference solution. The R_f value for cefprozil (Z) is approximately 0.45. Cefprozil (E), has an R_f value of approximately 0.47. Cefprozil (Z) is "absent" if the above test is performed and no spots, which correspond to those from the reference solution, are obtained for the sample.

[58 FR 26660, May 4, 1993]

§ 436.369 Thin layer chromatography test for free *N*-isobutylpiperidone content in rifabutin.

(a) *Equipment*—(1) *Chromatography tank.* A rectangular tank, approximately 23 X 23 X 9 centimeters, with a glass solvent trough on the bottom and a tight-fitting cover.

(2) *Iodine vapor chamber.* A rectangular tank, approximately 23 X 23 X 9 centimeters, with a suitable cover, containing iodine crystals.