

azlocillin. The penicilloate and penilloate of azlocillin as well as ampicillin appear as additional spots with R_f values of approximately 0.15, 0.3, and 0.25, reectively.

[47 FR 53348, Nov. 26, 1982]

§ 436.337 High-pressure liquid chromatographic assay for cephradine.

(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 8 millimeters;

(3) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(4) A suitable recorder that is compatible with the detector output;

(5) A suitable integrator (optional); and

(6) A 25-centimeter column having an inside diameter of 4.6 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 10 micrometers in diameter, U.S.P. XX.

(b) *Reagents.* (1) 4 percent glacial acetic acid.

(2) 3.86 percent sodium acetate.

(c) *Mobile phase.* 4 percent glacial acetic acid:3.86 percent sodium acetate:methanol:distilled water (3:15:200:782). 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 prior to its introduction into the chromatograph pumping system. The distilled water:methanol ratio may be varied to obtain acceptable operation of the system.

(d) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 1.2 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the cephradine in the cephradine working standard that is about 75 percent of full scale.

(e) *Preparation of working standard and sample solutions—(1) Preparation of cephradine working standard solution.* Place an accurately weighed portion of the cephradine working standard into a

suitably sized container. Add 5.0 milliliters of distilled water and place in an ultrasonic bath to facilitate dissolution. Dilute with a sufficient amount of mobile phase to obtain a solution containing 0.8 milligram of cephradine activity per milliliter.

(2) *Preparation of cephalixin working standard solution.* Dissolve an accurately weighed portion of the cephalixin working standard with mobile phase to obtain a solution containing 0.02 milligram of cephalixin activity per milliliter. Place in an ultrasonic bath to facilitate dissolution.

(3) *Preparation of sample solutions—(i) Product not packaged for dispensing (micrograms of cephradine per milligram).* Dissolve an accurately weighed portion of the sample with mobile phase to obtain a solution containing 0.8 milligram per milliliter. Place in an ultrasonic bath to facilitate dissolution. Using this sample solution, proceed as directed in paragraph (f)(1) of this section.

(ii) *Product packaged for dispensing.* Determine both micrograms of cephradine per milligram of the sample and milligrams of cephradine per container. Use separate containers for preparation of each sample solution as described in paragraphs (e)(3)(i) (a) and (b) of this section.

(a) *Micrograms of cephradine per milligram.* Dissolve an accurately weighed portion of the sample with mobile phase to obtain a solution containing 0.8 milligram per milliliter. Place in an ultrasonic bath to facilitate dissolution. Using this sample solution, proceed as directed in paragraph (f)(1) of this section.

(b) *Milligrams of cephradine per container.* Reconstitute the sample as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the solution thus obtained with mobile phase to obtain a solution containing 0.8 milligram per milliliter. Using this sample solution,

proceed as directed in paragraph (f)(1) of this section.

(f) *Procedure*—(1) *Cephadrine content*. Using the equipment, reagents, mobile phase, and operating conditions as listed in paragraphs (a), (b), (c), and (d) of this section, inject 10 microliters of the cephradine working standard solution into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of the expected components. After separation of the working standard solution has been completed, inject 10 microliters of the sample solution prepared as described in paragraph (e)(3)(i) of this section into the chromatograph and repeat the procedure described for the working standard solution. The elution order is void volume, cephalixin, and cephradine. If the sample is packaged for dispensing, repeat the procedure for each sample solution prepared as described in paragraphs (e)(3)(ii) (a) and (b) of this section.

(2) *Cephalixin content*. Proceed as directed in paragraph (f)(1) of this section, except:

(i) Use a detector sensitivity setting that gives a peak height for the cephalixin in the cephalixin working standard that is about 75 percent of full scale; and

(ii) Use the cephalixin working standard in lieu of the cephradine working standard.

(g) *Calculations*. (1) Calculate the micrograms of cephradine per milligram of sample as follows:

$$\frac{\text{Micrograms of cefoperazone per milligram}}{\text{milligram}} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u =Area of the cephradine peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s =Area of the cephradine peak in the chromatogram of the cephradine working standard;

P_s =Cephradine activity in the cephradine 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.

(2) Calculate the cephradine content of the vial as follows:

$$\text{Milligrams of cefoperazone per vial} = \frac{A_u \times P_s \times d}{A_s \times 1,000}$$

where:

A_u =Area of the cephradine peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s =Area of the cephradine peak in the Chromatogram of the cephradine working standard;

P_s =Cephradine activity in the cephradine working standard solution in micrograms per milliliter;

C_s =Milligrams of the standard per milliliter; and

d =Dilution factor of the sample.

(3) Calculate the percent cephalixin content of the sample as follows:

$$\text{Percent cephalixin} = \frac{A_a \times W_b \times P_b \times 10}{A_b \times W_u \times (100 - m)}$$

where:

A_a =Area of the cephalixin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_b =Area of the cephalixin peak in the chromatogram of the cephalixin working standard;

W_b =Milligrams of cephalixin per milliliter of cephalixin working standard solution;

W_u =Milligrams of cephradine per milliliter of sample solution;

P_b =Micrograms of cephalixin per milligram of cephalixin working standard; and

m =Percent moisture content of the sample.

[49 FR 47483, Dec. 5, 1984]

§ 436.338 High-pressure liquid chromatographic assay for cefoperazone.

(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;

(6) A 30-centimeter column having an inside diameter of 4.0 millimeters and