

moxalactam per milliliter. Use the prepared solution immediately.

(e) *Preparation of sample solution.* Mix contents of vial thoroughly. Dissolve an accurately weighed portion of approximately 50 milligrams of sample with distilled water to obtain a concentration of 0.5 milligram per milliliter (estimated); also, 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. Further dilute an aliquot of this solution with distilled water to obtain a concentration of 0.5 milligram per milliliter (estimated). Use the prepared solution immediately.

(f) *Procedure.* Using the equipment, reagents, and operating conditions as listed in paragraphs (a), (b), and (c) of this section, inject 5 microliters of the 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 5 microliters of the sample solution into the chromatograph and repeat the procedure described for the working standard solution.

(g) *Calculations.* (1) Calculate the moxalactam content in micrograms per milligram as follows:

$$\text{Micrograms of moxalactam per milligram of sample} = \frac{R_u \times W_s \times P}{R_s \times W_u}$$

where:

R_u =Sum of the areas of the moxalactam sample R-isomer and the S-isomer peaks;

R_s =Sum of the areas of the moxalactam working standard R-isomer and the S-isomer peaks;

W_u =Weight of the sample in milligrams;

W_s =Weight of the moxalactam working standard in milligrams;

P =Potency of the moxalactam working standard in micrograms per milligram, corrected for moisture.

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

$$\text{Milligrams of moxalactam per vial} = \frac{R_u \times W_s \times P \times d}{R_s \times 100,000}$$

where:

R_u =Sum of the areas of the moxalactam R-isomer and the S-isomer peaks;

R_s =Sum of the areas of the moxalactam working standard R-isomer and the S-isomer peaks;

W_s =Weight of the moxalactam working standard in milligrams;

P =Potency of the moxalactam working standard in micrograms per milligram, corrected for moisture;

d =Dilution factor.

(3) Calculate the ratio of R-isomer to S-isomer as follows:

$$\text{Ratio of R-isomer to S-isomer} = \frac{\text{Area of the R-isomer peak}}{\text{Area of the S-isomer peak}}$$

[46 FR 61069, Dec. 15, 1981]

§ 436.333 Thin layer chromatographic identity test for moxalactam.

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

(2) *Plates.* Use a 20x20 centimeter thin layer chromatography plate coated with silica gel G or equivalent to a thickness of 250 micrometers.

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

(c) *Preparation of spotting solutions.* Prepare solutions of the sample and working standard, each containing 10 milligrams per milliliter of moxalactam in distilled water.

(d) *Procedure.* Pour the developing solvent into the glass trough on the bottom of the tank and onto the paper lining the walls of the 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 silica gel plate, and at intervals of 2 centimeters, spot 10 microliters each of the standard solution and the sample solution. After all spots are thoroughly dry, place the silica gel plate directly into the glass trough. Cover and seal the tank. Allow the solvent front to travel about 15 centimeters from the starting line. Remove the plate from the tank and air dry. Expose the plate to iodine vapors for 40 minutes. Immediately circumscribe all spots using a suitable marker.

(e) *Evaluation.* 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 and intensity.

[46 FR 61070, Dec. 15, 1981, as amended at 49 FR 2242, Jan. 19, 1984]

§ 436.334 High-pressure liquid chromatographic assay for piperacillin.

(a) *Equipment.* A 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 25-centimeter column having an inside diameter of 4.6 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 to 10 micrometers in diameter (United States Pharmacopeia XX).

(b) *Reagents.* (1) 0.2M monobasic sodium phosphate: Dissolve 27.60 grams of monobasic sodium phosphate with sufficient water to make 1,000 milliliters.

(2) 10 percent tetrabutylammonium hydroxide in water.

(3) Ampicillin-piperacillin solution: Dissolve and dilute 25 milligrams of ampicillin and 5 milligrams of piperacillin monohydrate with sufficient mobile phase to obtain 100 milliliters, and mix.

(c) *Mobile phase.* Methanol:water:0.2M monobasic sodium phosphate:10 percent tetrabutylammonium hydroxide (450:447:100:3) adjusted to pH 5.5±0.02 with phosphoric acid. The concentration of reagents may be varied to obtain acceptable operation of the system. De-gas the mobile phase just prior to its introduction into the chromatograph pumping system.

(d) *Preparation of working standard and sample solutions—*(1) *Working standard solution.* Place approximately 20 milligrams of the working standard, accurately weighed, into a 50-milliliter volumetric flask. Add 25 to 30 milliliters of mobile phase. Shake until dissolved. Dilute to volume with mobile phase.

(2) *Sample solution—*(i) *Micrograms per milligram.* Place approximately 20 milligrams of the sample, accurately weighed, into a 50-milliliter volumetric flask. Add 25 to 30 milliliters of mobile phase. Shake until dissolved. Dilute to volume with mobile phase.

(ii) *Milligrams per vial.* Reconstitute as directed in the labeling. Withdraw the total contents and dilute with mobile phase to a concentration of 0.4 milligram of piperacillin per milliliter.

(e) *Procedure.* Use the equipment, reagents, mobile phase, and working standard and sample solutions described in paragraphs (a), (b), (c), and (d) of this section and proceed as directed in paragraph (e) of this section.

(1) *Systems suitability test.* Chromatograph three replicate samples of ampicillin-piperacillin solution as directed in paragraph (e)(2) of this section. Allow an elution time sufficient to obtain satisfactory separation of expected components after each injection. Record the peak responses and calculate the resolution factor as described for system suitability tests in the United States Pharmacopeia XX General Chapter 621 for gas chromatography. The resolution factor between ampicillin and piperacillin is not