

*working standard solution.* Prepare a solution containing 0.25 milligram per milliliter of dactinomycin in mobile phase.

(2) *Preparation of sample solution.* Prepare the sample solution as described in the individual monograph for the drug being tested.

(e) *Procedure.* Use the equipment, mobile phase, operating conditions, and working standard and sample solutions described in paragraphs (a), (b), (c), and (d) of this section, and proceed as directed in paragraph (e)(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 two or more replicate injections of 10 microliters each of the working standard solution. Allow an elution time sufficient to obtain satisfactory separation of expected components after each injection. Record the peak responses and, calculate the relative standard deviation as described for system suitability tests in the U.S.P. XX General Chapter 621 chromatography. Proceed as directed in paragraph (e)(2) of this section if the minimum performance requirement for the relative standard deviation is not more than 1.0 percent. If the minimum performance requirement is not met, adjustment must be made to the system to obtain satisfactory operation before proceeding as described in paragraph (e)(2) of this section.

(2) *Determination of the chromatogram.* Inject 10 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 10 microliters of the sample solution into the chromatograph and repeat the procedure described for the working standard solution.

(f) *Calculations.* Calculate the dactinomycin content as described in the individual monograph for the drug being tested.

[49 FR 24017, June 11, 1984, as amended at 50 FR 5749, Feb. 12, 1985]

**§ 436.332 High-pressure liquid chromatographic assay for moxalactam.**

(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 30-centimeter column having an inside diameter of 4.0 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 to 10 micrometers in diameter, U.S.P. XX;

(5) A suitable recorder of at least 25.4 centimeter deflection;

(6) A suitable integrator.

(b) *Mobile phase.* Mix 0.01M ammonium acetate:methanol (19:1). 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 just prior to its introduction into the chromatograph pumping system.

(c) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 0.5 milliliter per minute. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale.

(d) *Preparation of working standard solution.* Transfer the contents of an ampoule of working standard to a tared weighing bottle. Place the unstoppered weighing bottle in a desiccator containing a saturated aqueous solution of potassium carbonate to provide an atmosphere of 42 percent relative humidity. Allow the moisture content of the working standard to equilibrate for 16 hours. Determine the moisture content as described in § 436.201 of this chapter. Equilibrated standard material must be kept in a closed weighing bottle and used within 36 hours of equilibration. Dissolve approximately 50 milligrams of the working standard, accurately weighed and corrected for moisture, with sufficient distilled water to obtain a solution containing 0.5 milligram of

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.