

§ 436.330 Thin layer chromatographic identity test for bacampicillin.

(a) *Equipment*—(1) *Chromatography tank*. Use a rectangular tank approximately 23 × 23 × 9 centimeters, with a glass solvent trough on the bottom and a tight-fitting cover, lined with Whatman's 3MM chromatographic paper (0.3 millimeter) or equivalent.

(2) *Plates*. Use 20 × 20 centimeter thin layer chromatography plates coated with Silica Gel 60F 254 or equivalent to a thickness of 250 microns.

(b) *Reagents*—(1) *Developing solvent*. Mix methylene chloride, chloroform, and 95 percent ethyl alcohol in volumetric proportions of 100:10:10, respectively.

(2) *Spray solution*. Dissolve 1 gram of ninhydrin in 100 milliliters of *n*-butanol and add 1 milliliter of pyridine.

(c) *Spotting solutions*—(1) *Preparation of working standard solution*. Dissolve and dilute a weighed amount of the bacampicillin hydrochloride working standard with sufficient 95 percent ethyl alcohol to obtain a solution containing 2 milligrams per milliliter.

(2) *Preparation of sample solution*. Dissolve and dilute a weighed amount of the sample with sufficient 95 percent ethyl alcohol to obtain a solution containing 2 milligrams per milliliter. Proceed as described in paragraphs (d) and (e) of this section.

(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 one hour. Prepare a plate as follows: On a line 2.5 centimeters from the base of the thin layer chromatography plate and at intervals of 2.0 centimeters, spot 5 microliters of the working standard solution to positions 1 and 3. When these spots are dry, apply 5 microliters of the sample solution to points 2 and 3. After all the spots are thoroughly dry, place the plate into the trough in the bottom of the tank. Cover and tightly seal the tank, allow the solvent front to travel about 15 centimeters from the starting line (about 30 minutes) and then remove the plate from the tank. Air dry the plate. Visualize the spots by spraying with spray solution and heating in an oven at 100° C for approximately 10 minutes.

(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. Bacampicillin appears as a purple spot at an R_f value of approximately 0.52. The test is satisfactory if the R_f value of the sample compares with that of the working standard. The combined spot should appear as a single spot of corresponding R_f value.

[46 FR 25602, May 8, 1981, as amended at 49 FR 2242, Jan. 19, 1984]

§ 436.331 High-pressure liquid chromatographic assay for dactinomycin.

(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; and

(6) 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 micrometers to 10 micrometers in diameter, U.S.P. XX.

(b) *Mobile phase*. Mix acetonitrile (high-pressure liquid chromatography grade): water (60:40). 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 2.5 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale. The minimum between peaks must be no more than 2 millimeters above the initial baseline.

(d) *Preparation of working standard and sample solutions*—(1) *Preparation of*

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