

(2) *Plates.* Use 20 by 20 centimeter thin layer chromatography plates coated with silica gel G or equivalent, to a thickness of 250 microns.

(b) *Reagents*—(1) *Developing solvent.* Mix *n*-butanol, glacial acetic acid, and water in volumetric proportions of 4:2:1, respectively.

(2) *Spray solution.* Prepare a one-percent solution of ninhydrin in ethanol.

(c) *Preparation of spotting solutions.* Prepare solutions of the sample and working standard, each containing 1 milligram of mitomycin per milliliter, in water.

(d) *Procedure.* Pour the developing solvent into the solvent 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 30 minutes. Prepare a plate as follows: Apply spotting solutions on a line 2.5 centimeters from the base of the silica gel plate and at points 2.0 centimeters apart. Apply approximately 2 microliters of the working standard solution to points 1 and 3. When these spots are dry, apply approximately 2 microliters of sample solution to points 2 and 3. After all spots are thoroughly dry, place the silica gel plate into the trough in the chromatography tank. Cover and seal the tank tightly. Allow the solvent front to travel about 10 centimeters from the starting line. Remove the plate and allow it to air dry. After the plate is dry, spray lightly with the spray solution. Heat the plate in an oven at 110° C. for 10–15 minutes. Mitomycin appears as a pink spot.

(e) *Evaluation.* The sample and standard should have spots of corresponding  $R_f$  value (approximately 0.51), and standard and sample combined should appear as a single spot of corresponding  $R_f$  value.

[39 FR 18944, May 30, 1974, as amended at 49 FR 2242, Jan. 19, 1984]

#### § 436.311 Thin layer chromatography identity test for amoxicillin.

Using the sample solution prepared as described in the section for the antibiotic drug to be tested, proceed as described in paragraphs (a) through (e) of this section.

(a) *Equipment*—(1) *Chromatography tank.* A rectangular tank, approxi-

mately 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's 3MM chromatographic paper (0.33 millimeters) or equivalent.

(2) *Plates.* Use 20- by 20-centimeter thin layer chromatography plates coated with Silica Gel G or equivalent to a thickness of 250 microns.

(b) *Reagents*—(1) *Developing solvent.* Mix methyl alcohol, chloroform, pyridine, and distilled water in volumetric proportions of 90:80:10:30, respectively.

(2) *Spray solution.* Dissolve 300 milligrams of ninhydrin in 100 milliliters of ethyl alcohol.

(c) *Preparation of working standard.* Weigh an amount of the amoxicillin working standard equivalent to 200 milligrams of amoxicillin into a 50-milliliter volumetric flask and bring to volume with 0.1*N* hydrochloric acid.

(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 at least 2 hours. Spot duplicate plates by applying approximately 5 microliters each of standard and sample solutions on a line 1.5 centimeters from the base of the plate and at intervals of not less than 2.0 centimeters. All solutions must be spotted within 10 minutes of preparation. Place spotted plate in a desiccator until solvent has evaporated from spots. Place the plate into the glass trough at the bottom of the chromatography tank. Cover the tank. Allow the solvent to reach the 15-centimeter scored mark, remove the plate from the tank and dry with a current of warm air until there is no detectable solvent odor. Apply the ninhydrin spray solution to the plate—do not saturate—and place immediately into an oven maintained at 110° C for 15 minutes.

(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. Amoxicillin has an  $R_f$  value of about 0.53. The sample and standard

should have spots of corresponding  $R_f$  values.

[39 FR 34032, Sept. 23, 1974; 48 FR 11427, Mar. 18, 1983, as amended at 49 FR 2242, Jan. 19, 1984]

**§ 436.312 Atomic absorption method for determining the zinc content of zinc bacitracin.**

(a) *Equipment.* An atomic absorbance spectrophotometer equipped with a zinc hollow-cathode discharge lamp, an air-acetylene flame, a nebulizer-burner system for introducing the sample solution into the flame, an optical dispersing device (such as a monochromator) for isolating a resonance line of zinc from others produced by the emission source, and a suitable radiation detector and recorder.

(b) *Preparation of working standard and sample solutions—(1) Working standard solutions.* Prepare a standard stock solution containing 10 milligrams of zinc per milliliter as follows: Weigh 3.11 grams of zinc oxide into a 250-milliliter volumetric flask, add 80 milliliters of 1N HCl, warm to dissolve, cool to room temperature, and dilute to volume with water. Dilute aliquots of this standard stock solution with 0.001N HCl to obtain three working standard solutions containing respectively 0.5, 1.5, and 2.5 micrograms of zinc per milliliter.

(2) *Sample solution.* Accurately weigh approximately 200 milligrams of the sample into a 100-milliliter volumetric flask. Dissolve and dilute to volume with 0.01N HCl. Transfer a 2.0-milliliter aliquot of this solution to a 200-milliliter volumetric flask and dilute to volume with 0.001N HCl.

(c) *Procedure.* Using 0.001N HCl as the blank, adjust the absorbance of the instrument to zero at a detection wavelength of 213.8 nanometers. Determine the absorbance of each standard solution and the sample solution at 213.8 nanometers.

(d) *Calculations.* Plot the absorbance versus the concentration of each of the working standard solutions. Draw a straight response line of best fit through these points. Read the concentration of zinc in micrograms per milliliter corresponding to the absorbance of the sample solution. Calculate

the percent zinc in the sample as follows:

$$\text{Percent zinc} = \frac{C \times 100,000}{\text{Milligrams of sample} \times (100 - m)}$$

where:

$C$ =Concentration of zinc in the sample solution in micrograms per milliliter;  
 $m$ =Percent moisture in the sample.

[40 FR 15088, Apr. 4, 1975]

**§ 436.316 Determination of penicillin G content.**

(a) *Reagents.* The reagents are freshly prepared every three days and are of such quality that when used in this procedure with an authentic sample of penicillin G, not less than 97 percent of penicillin G is recovered.

(1) *Amyl acetate (iso-amyl acetate) solution.* Saturate the amyl acetate (boiling range 138.5° C—141.5° C) with the *N*-ethylpiperidine salt of penicillin G by adding 2 milligrams of the salt for each 1.0 milliliter of the solvent. Cool this solution to 0° C—8° C and filter it through a sintered-glass filter immediately before use.

(2) *Acetone solution.* Saturate reagent grade acetone with the *N*-ethylpiperidine salt of penicillin G using 3 milligrams of salt for each 1 milliliter of acetone. Cool this solution to 0° C—8° C and filter it through a sintered-glass filter immediately before use.

(3) *N-ethylpiperidine solution.* *N*-ethylpiperidine (boiling range 129.5° C—131.0° C) should be stored in brown bottles in a refrigerator. Dilute 1.0 milliliter of this reagent with 4.0 milliliters of amyl acetate. Saturate this solution with the *N*-ethylpiperidine salt of penicillin G, using about 3 milligrams of the salt for each 1.0 milliliter of solution. Cool this solution to 0° C—8° C and filter it through a sintered-glass filter immediately before use.

(4) *Phosphoric acid solution.* Prepare by dissolving 1.0 milliliter of reagent grade phosphoric acid (85 percent) in 4.0 milliliters of water. Cool to 0° C—8° C and shake before using.

(5) *Silica gel.* Use dry silica gel (mesh size 6-16, Tyler standard). Place about