

**§ 436.319 Thin layer chromatography identity test for bacitracin and bacitracin zinc.**

(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 20- by 20-centimeter thin layer chromatography plate coated with silica gel G or equivalent to a thickness of 250 micrometers. Activate the plate by heating for 20 minutes at 110° C. Allow to cool to room temperature and use immediately.

(b) *Reagents*—(1) *Developing solvent*. Mix *n*-butanol, water, pyridine, glacial acetic acid, and ethyl alcohol in volumetric proportions of 60:10:6:15:5, respectively.

(2) *Spray solution*. Dissolve 1 gram of ninhydrin in a mixture of 1 milliliter of pyridine and sufficient *n*-butanol to make 100 milliliters.

(c) *Preparation of spotting solutions*. Prepare solutions of the sample and working standard, each containing 6.0 milligrams of bacitracin per milliliter in 1 percent disodium ethylenediamine tetraacetic acid in 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 at least 30 minutes. Prepare a plate as follows: On a line 2.0 centimeters from the base of the silica gel plate, and at intervals of 2.0 centimeters, spot approximately 1.0 microliter of the standard solution to points 1 and 3. When these spots are dry, apply approximately 1.0 microliter of sample solution to points 2 and 3. After all spots are thoroughly dry, place the base of the silica gel plate directly into the glass trough in the chromatography tank. Cover and seal the tank. Allow the solvent front to travel approximately 13 centimeters from the starting line. Remove the plate from the tank, and allow it to air dry. After the plate is dry, spray lightly with the spray solution. The plate may take 1 hour or more to develop at room tem-

perature. The development may be speeded up by warming the plate in a 110° C oven.

(e) *Evaluation*. The sample and standard should have spots of corresponding  $R_f$  value (approximately 0.26) and standard and sample combined should appear as a single spot of corresponding  $R_f$  value.

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**§ 436.320 Ferric chloride colorimetric assay.**

(a) *Reagents*. (1) 1*N* hydrochloric acid.

(2) 0.01*N* hydrochloric acid.

(3) Ferric chloride stock solution. Quickly weigh (very hygroscopic) 5.0 grams of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  into a 100-milliliter beaker. Add approximately 10 milliliters of 1*N* hydrochloric acid and stir to dissolve. Quantitatively transfer to a 50-milliliter glass-stoppered amber volumetric flask and make up to volume with water.

(4) Ferric chloride working reagent. Pipette 10.0 milliliters of ferric chloride stock solution into a 2-liter volumetric flask, add 20 milliliters 1*N* hydrochloric acid, and bring to volume with water. Check the pH; it should be between 2.0 and 2.1.

(b) *Standard solution*. Accurately weigh approximately 50 milligrams of the working standard of the antibiotic to be tested and dissolve with 25 milliliters of 0.1*N* hydrochloric acid. Quantitatively transfer to a 250-milliliter volumetric flask and dilute to volume with distilled water. Keep in a glass-stoppered flask and store under refrigeration. Discard solution after 7 days.

(c) *Sample solution*. Accurately weigh approximately 50 milligrams of the sample and dissolve with 25 milliliters of 0.1*N* hydrochloric acid. Quantitatively transfer to a 250-milliliter volumetric flask and dilute to volume with distilled water.

(d) *Procedure*. Pipette exactly 10.0 milliliters of the standard solution and of the sample solution into separate test tubes. To each tube add exactly 10 milliliters of ferric chloride working reagent, mix, and allow to stand 15 minutes. Determine the absorbance of each solution at 490 nanometers in a suitable spectrophotometer against a blank prepared from 10.0 milliliters of