

**§ 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.

[42 FR 27228, May 27, 1977]

**§ 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

0.01N hydrochloric acid and 10.0 milliliters of ferric chloride working reagent.

(e) *Estimation of potency.* Calculate the potency as follows:

$$\text{Micrograms of antibiotic per milligram} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{Milligrams of standard}}{\text{Milligrams of sample}} \times \text{Potency of standards in micrograms per milligram}$$

[43 FR 11154, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978]

**§ 436.321 Griseofulvin gas liquid chromatography.**

(a) *Equipment.* Gas chromatograph equipped with an electronic integrator and with a flame ionization detector: Hewlett Packard 7600 or equivalent.

(b) *Reagents.* (1) Chloroform, reagent grade.

(2) Internal standard solution: Prepare a solution containing 1.0 milligram of tetraphenylcyclopentadienone per milliliter in chloroform.

(c) *Typical conditions*—(1) *Column.* 1.2 meters by 4 millimeters ID, glass, packed with 1 percent OV-17 on Gas Chrom Q (100/120 mesh), or equivalent.

(2) *Temperatures.* Column 245° C; detector 260° C; injection port 260° C.

(3) *Carrier gas.* Helium approximately 60 millimeters per minute and 40 pounds per square inch (1.7 kilograms per square centimeter).

(4) *Detector.* Hydrogen flame ionization-hydrogen at 12 pounds per square inch (0.5 kilogram per square centimeter), air at 34 pounds per square inch (1.43 kilograms per square centimeter).

(5) *Sensitivity.* Adjusted to obtain peak heights greater than 50 percent full scale deflection.

(d) *Preparation of griseofulvin sample and working standard solutions.* Accurately weigh approximately 40 milligrams of both the sample and the working standard into separate 25-milliliter volumetric flasks. Add sufficient internal standard solution to dissolve the contents of each flask with vigorous mixing and then dilute to volume with internal standard solution and mix. Proceed as directed in paragraph (e) of this section.

(e) *Procedure.* Inject 1.0 microliter of this solution into the gas chro-

matograph. Use the typical conditions and materials listed in paragraphs (a), (b), and (c) of this section. The resolution of the peaks should be complete. The griseofulvin peak will elute before the internal standard peak. Calculate the griseofulvin content as directed in paragraph (f) of this section.

(f) *Calculations.* Calculate the griseofulvin content of the sample as follows:

$$\text{Micrograms of griseofulvin per milligram} = \frac{R_u \times W_s \times f}{R_s \times W_u}$$

where:

$R_u$ =Area of the griseofulvin sample peak (at a retention time equal to that observed for the griseofulvin standard)/Area of the internal standard peak;

$R_s$ =Area of the griseofulvin working standard peak/Area of the internal standard peak;

$W_s$ =Weight of the griseofulvin working standard in milligrams;

$W_u$ =Weight of the sample in milligrams;

$f$ =Potency of the griseofulvin working standard in micrograms per milligram.

[44 FR 20660, Apr. 6, 1979]

**§ 436.322 High-pressure liquid chromatographic assay for anthracycline antibiotics.**

(a) *Equipment.* A suitable high-pressure liquid chromatograph, such as a Waters Associates Model 244<sup>1</sup> or equivalent 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;

<sup>1</sup>Available from Waters Associates, Inc., Maple St., Milford, Mass. 01757.