

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 244¹ 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;

¹Available from Waters Associates, Inc., Maple St., Milford, Mass. 01757.