

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.

(4) A suitable recorder of at least 25.4 centimeter deflection;

(5) A suitable integrator;

(6) A 30-centimeter column having an inside diameter of 4.6 millimeters and packed with a suitable reverse phase packing such as: Waters Associates, Micro-Bondapak C18.¹

(b) *Reagents.* (1) Solvent mixture: Water: acetonitrile (69:31).

(2) Mobile phase: Water: acetonitrile (69:31) adjusted to pH 2 with phosphoric acid. 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.

(3) Internal standard solution: Prepare a 2.0-milligram-per-milliliter solution of 2-naphthalenesulfonic acid in the solvent mixture.

(c) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 1.5 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the reference 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) *Procedure.* Use the standard and sample solutions prepared as directed in the individual monographs for the drug being tested. Use the equipment, reagents, and operating conditions listed in paragraphs (a), (b), and (c) of this section. Inject 5 microliters of the standard solution into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of expected components (ordinarily this time is 20 minutes). After separation of the standard solution has been completed, inject 5 microliters of the sample solution into the chromatograph and repeat the procedure described for the standard solution. The elution order is: Void volume, internal standard, doxorubicin, dihydrodaunomycin, daunomycin, adriamycinone, dihydrodaunomycinone, bromodaunomycin, daunomycinone, and bis-anhydrodaunomycinone.

(e) *Calculations.* Calculate the anthracycline content as directed in

the individual monograph for the drug being tested.

[43 FR 44836, Dec. 29, 1978]

§ 436.323 Continuous flow thin layer chromatography identity test for cefamandole nafate.

(a) *Equipment*—(1) *Chromatography tank.* Use a rectangular tank approximately 23 centimeters long, 23 centimeters high, and 9 centimeters wide equipped with a glass solvent trough in the bottom.

(2) *Plates.* Use a 20 x 20 centimeter thin-layer chromatography plate coated with silica gel G or equivalent to a thickness of 250 micrometers.

(3) *Cover.* A stainless steel cover with a slot measuring 21 x 0.6 centimeters, cut in the front edge.

(4) *Supporting platform.* A platform that can be placed in the bottom of the chromatography tank so that the solvent trough is elevated about 3.75 centimeters.

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

(2) *Spray solution.* Mix starch iodide solution, glacial acetic acid, and 0.1 *N* iodine test solution, U.S.P. in volumetric proportions of 50:3:1. Prepare the starch iodide solution by mixing starch iodide paste test solution, U.S.P. and water in volumetric proportions of 1:1.

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

(d) *Procedure.* Prepare a plate as follows: On a line 2 centimeters from the base of the silica gel plate, and at intervals of 1 centimeter, spot 5 microliters each of the standard solution and the sample solution. In addition, prepare one spot composed of 5 microliters of the sample solution and 5 microliters of the standard solution. Place the supporting platform in the bottom of the tank and place the solvent trough on it, near the front of the tank. Place a piece of Whatman #3 MM filter paper or equivalent, measuring 20 x 3 centimeters and folded in half, lengthwise, over the front edge of the tank to form a cushion and drying