

(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

wick for the plate. Place the plate in the solvent trough with the coated side toward the front of the tank and leaning against the filter paper at the top. Pour the developing solvent into the trough and bottom of the tank. Cover the tank. The plate should extend approximately 1 centimeter beyond the top of the tank and through the slot in the cover. Seal all the openings in the tank with masking tape, except where the plate leans against the filter paper. Remove the plate from the tank after 4 hours. Allow the plate to air dry and then heat it in an oven for 15 minutes at 110° C. Remove the plate from the oven and immediately spray it with the spray solution. The compound appears as a white spot on a purple background.

(e) *Evaluation.* The sample and standard should have traveled the same distance from the origin, and the combined standard and sample should appear as a single spot that has traveled the same distance as the sample and standard individually.

[44 FR 20664, Apr. 6, 1979]

§ 436.324 Polarographic analysis of cefamandole.

(a) *Equipment*—(1) *Polarograph.* Use a polarograph equipped with a dropping mercury indicating electrode, a platinum auxiliary electrode, and a saturated calomel reference electrode, such as Princeton Applied Research Model 174¹ or equivalent.

(2) *X-Y plotter.* Use a suitable X-Y plotter, such as Houston Omnigraphic Model 2200-3-3² or equivalent.

(3) *Nitrogen.* Use a nitrogen tank equipped with a pressure-reducing regulator and a filter to remove traces of oxygen, such as an oxisorb filter¹ or equivalent.

(b) *Reagent.* pH 2.3 Buffer: Dissolve 3.6 grams of dibasic sodium phosphate, 39.4 grams of citric acid, and 70.8 grams of potassium chloride in sufficient distilled water to make 1 liter.

(c) *Operating conditions*—(1) Operating mode: Differential pulse.

(2) Scan range: -0.3 volt to -1.05 volts.

(3) Scan rate: -2 millivolts per second.

(4) Sensitivity: 10 to 20 microamperes or equivalent to keep peak on scale.

(5) Mercury drop time: 1 second per drop.

(6) Modulation amplitude: 25 millivolts.

(7) Display direction: +

(8) Damping: None.

(d) *Preparation of sample and working standard solutions.* Use the cefamandole lithium working standard. Accurately weigh approximately 12 milligrams of sample or working standard into a 50-milliliter volumetric flask. Dissolve the sample or working standard in 4 milliliters of distilled water. Immediately prior to polarography, add 30 milliliters of pH 2.3 buffer, dilute to volume with distilled water, and mix.

(e) *Procedure.* Transfer a portion of the sample or working standard solution to the polarographic cell. Pass a stream of nitrogen through the solution for 5 minutes to remove the dissolved oxygen. After 5 minutes, disperse the nitrogen above the sample. Start the mercury dropping from the mercury dropping electrode, and, using the operating conditions described in paragraph (c) of this section, record the polarogram. Compare the polarogram of the sample to that of the working standard.

(f) *Calculations.* Calculate the potency of cefamandole as follows:

$$\text{Micrograms of cefamandole per milligram} = \frac{A \times \text{Milligrams of working standard} \times \text{Potency of working standard in micrograms per milligram}}{B \times \text{Milligrams of sample}}$$

where:

A=The peak height of the sample;

¹Available from Princeton Applied Research Corporation, P.O. Box 2565, Princeton, NJ 08540.

²Available from Houston Instrument, 8500 Cameron Road, Austin, TX 78753.