

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

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

where:

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

R_s =Area of the clindamycin palmitate hydrochloride standard peak/Area of internal standard peak;

W_s =Weight of the clindamycin palmitate hydrochloride working standard in milligrams;

W_u =Weight of the sample in milligrams;

f =Micrograms of clindamycin activity per milligram of clindamycin palmitate hydrochloride working standard.

§ 436.304 Clindamycin phosphate vapor phase chromatography.

(a) *Equipment.* Gas chromatograph equipped with an electronic integrator and with a flame ionization detector that has a sensitivity of at least 1×10^{-10} amperes: Hewlett-Packard 7600⁴ or equivalent.

(b) *Reagents.* (1) Trifluoroacetic anhydride.

(2) Intestinal alkaline phosphatase.

(3) pH 9.0 borate buffer: Transfer 3.1 grams of boric acid into a 1-liter volumetric flask containing 500 milliliters of water, mix, and add 21 milliliters of 1.0*N* sodium hydroxide and 10 milliliters of 0.1*M* magnesium chloride. Dilute to volume with water and mix well.

(4) Internal standard: Prepare a chloroform solution containing approximately 0.45 milligram hexacosane per milliliter.

(5) Anhydrous sodium carbonate.

(c) *Typical conditions.* (1) Column: 2 feet \times 3 millimeters ID, glass, with 1 percent SE-30 on Diatoport S (80/100 mesh), or equivalent.

(2) Temperatures: Column, 180° C., detector, 215° C., injection port, ambient temperature.

(3) Carrier gas: Helium approximately 60 milliliters per minute.

(4) Detector: Hydrogen flame—hydrogen flow at 40 milliliters per minute. Air flow at 400 milliliters per minute.

(5) Sensitivity: 1×10^{-9} amperes.

(d) *Preparation of clindamycin phosphate sample solution.* Accurately weigh approximately 12 milligrams of the clindamycin phosphate sample into a 50-milliliter glass-stoppered centrifuge tube. Pipet 25 milliliters of the pH 9.0 borate buffer into the centrifuge tube. Add 10 milliliters chloroform and shake vigorously for 15 minutes. Centrifuge the resulting mixture and pipet a 20-milliliter aliquot of the aqueous phase into a 35-milliliter centrifuge tube. Add a weighed amount of intestinal alkaline phosphatase equivalent to 50 units of activity⁵ and allow the solution to stand until the enzyme has completely dissolved. Place the tube into a water bath at 37° C. \pm 2° C. for 2.5 hours. After the 2.5-hour hydrolysis, allow the solution to cool and proceed as directed in paragraph (f) of this section.

(e) *Preparation of the clindamycin hydrochloride standard solution.* Accurately weigh approximately 9 milligrams of the clindamycin hydrochloride working standard into a 35-milliliter glass-stoppered centrifuge tube and dissolve in 20 milliliters of pH 9.0 borate buffer. Proceed as directed in paragraph (f) of this section.

(f) *Procedure.* Add 10 milliliters of the internal standard solution to each sample and standard solution. Shake the centrifuge tubes vigorously for 30 minutes and centrifuge. Remove the aqueous layer and discard. Shake the tubes again; mix in an ultrasonic mixer for 2 minutes, then centrifuge. No emulsion should be present at this stage. Remove the remaining aqueous layer by suction and transfer a 3-milliliter aliquot of the chloroform layer to a 1-dram tablet vial containing approximately 1 gram of anhydrous sodium sulfate. Swirl the vial to dry the chloroform and transfer a 1-milliliter aliquot to another 1-dram tablet vial. Using a 0.25-milliliter pipet, add 0.25 milliliter of trifluoroacetic anhydride to

⁵Defined such that 50 units hydrolyzes at least 20 micromoles of a clindamycin phosphate authentic sample under the assay conditions described in this section.

⁴See footnote 4 to § 436.303(a).

each of the vials and place into a water bath at 45° C.±2° C. for 30 minutes. Remove the vials from the bath, add about 10 granules of anhydrous sodium carbonate to each vial, and allow to stand for approximately 30 minutes. Centrifuge the vials for approximately 10 minutes at 5,000 r.p.m. Inject 2 microliters of each of the resulting solutions into the gas chromatograph. Use the conditions and materials listed in paragraphs (a), (b), and (c) of this section. The elution order is: Epiclindamycin (if present), clindamycin B (if present), clindamycin, and internal standard. Calculate the clindamycin content as directed in paragraph (g) of this section.

(g) *Calculations.* Calculate the clindamycin content of the sample as follows:

Micrograms of clindamycin per milligram=

$$\frac{R_u \times W_s \times f}{R_s \times W_u}$$

where:

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

R_s =Area of the clindamycin standard peak/Area of internal standard peak;

W_s =Weight of the clindamycin working standard in milligrams;

W_u =Weight of the sample in milligrams;

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

[39 FR 18944, May 30, 1974, as amended at 41 FR 24704, June 18, 1976]

§ 436.305 Thin layer chromatographic identity test for hetacillin.

(a) *Equipment*—(1) *Chromatography tank.* A rectangular tank, approximately 9 × 9 × 3.5 inches with a glass solvent trough on the bottom.

(2) *Plates.* Use 20 × 20 centimeter thin layer chromatography plates coated with Silica Gel G or equivalent to a thickness of 250 microns.

(b) *Developing solvent.* Mix 650 milliliters acetone with 100 milliliters distilled water, 100 milliliters benzene, and 25 milliliters acetic acid.

(c) *Spray solution.* Dissolve 300 milligrams of ninhydrin in 100 milliliters of ethanol.

(d) *Preparation of spotting solutions*—(1) *Sample solution.* Use the sample solution prepared as described in the section for the particular product to be tested.

(2) *Reference solutions.* Prepare a solution containing 10 milligrams of an authentic hetacillin sample per milliliter in a 4:1 solution of acetone and 0.1N hydrochloric acid, and a solution of ampicillin standard at 1 mg/ml in the same solvent.

(e) *Procedure.* Spot a plate as follows: Apply approximately 10 microliters of the sample solution, 1 μ l, of the reference hetacillin solution, and 1 μ l, of the ampicillin reference solution on a line 1.5 centimeters from the base of the silica gel plate and at intervals of not less than 2.0 centimeters. Pour developing solvent into the glass trough in the bottom of the chromatography tank. After all spots are thoroughly dry, place the silica gel plate directly into the glass trough of the chromatography tank. Cover and seal the tank. Allow the solvent front to travel about 11.5 centimeters from the bottom of the plate, remove the plate from the tank, and allow to air dry. Apply the spray solution (do not saturate) and place immediately into an oven maintained at 90° C. Heat 15 minutes.

(f) *Evaluation.* Measure the distance the solvent front traveled from the starting line and the distance the spots are from the starting line. Calculate the R_f value by dividing the latter by the former. The sample and standard should have spots of corresponding R_f values.

[39 FR 18944, May 30, 1974, as amended at 45 FR 16472, Mar. 14, 1980]

§ 436.306 Lincomycin gas liquid chromatography.

(a) *Equipment.* Gas chromatograph equipped with a flame ionization detector; Barber-Colman 5000 or equivalent.

(b) *Reagents.* (1) Pyridine, reagent grade, kept over potassium hydroxide.

(2) Methanol, reagent grade, anhydrous.

(3) Ethanol, absolute, reagent grade.

(4) *Internal standard:* Prepare a solution containing 2 milligrams of tetraphenylcyclopentadienone per milliliter in pyridine.