

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.