

§ 436.310

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make appropriate dilutions with chloroform to obtain a readable value.

(6) *Calculations*—(i) *Percent anhydrotetracyclines*. Calculate the percent anhydrotetracyclines as follows:

$$\text{Number of milligrams of anhydrotetracyclines in each fraction containing anhydrotetracyclines} = \frac{A \times b \times c}{20.28}$$

where:

*A*=Absorbance of the sample solution at 438 nanometers;

*b*=Volume of fraction in milliliters;

*c*=Dilution factor of the fraction (for example, if 2 milliliters of the fraction are diluted to 10 milliliters for reading, *c* will be 5).

20.28=Absorptivity (1 milligram per milliliter, 1 centimeter) of anhydrotetracyclines in chloroform at 438 nanometers.

Total weight of anhydrotetracyclines in the sample=Sum of weights of anhydrotetracyclines in the fractions labeled anhydrotetracyclines × Number of milliliters in the sample solution

Percent anhydrotetracyclines in tetracycline, tetracycline hydrochloride,

tetracycline phosphate complex bulk packaged for repacking or for use in the manufacture of another drug =

$$100 \times \frac{\text{Total weight of anhydrotetracyclines in the sample}}{\text{Weight of the sample}}$$

Percent anhydrotetracyclines in dosage forms=

$$100 \times \frac{\text{Total weight of anhydrotetracyclines in the sample}}{\text{Tetracycline content of the sample}}$$

(ii) *Percent 4-epianhydrotetracycline*. Calculate the percent 4-epianhydrotetracycline as follows:

$$\text{Number of milligrams of 4-epianhydrotetracycline in each fraction labeled 4-epianhydrotetracycline} = \frac{A \times b \times c}{20.08}$$

where:

*A*=Absorbance of the sample solution at 438 nanometers;

*b*=Volume of the fraction in milliliters;

*c*=Dilution factor of the fraction (for example, if 2 milliliters of the fraction are diluted to 10 milliliters for reading, *c* will be 5);

20.08=Absorptivity (1 milligram per milliliter, 1 centimeter) of 4-epianhydrotetracycline in chloroform at 438 nanometers.

Total weight of 4-epianhydrotetracycline in the sample=Sum of weights of 4-epianhydrotetracycline in the fractions labeled 4-epianhydrotetracycline × Number of milliliters in the sample solution

Percent 4-epianhydrotetracycline in tetracycline, tetracycline hydrochloride, tetracycline phosphate complex bulk packaged for repacking or for use in the manufacture of another drug =

$$100 \times \frac{\text{Total weight of 4-epianhydrotetracycline in the sample}}{\text{Weight of the sample}}$$

Percent 4-epianhydrotetracycline in dosage forms=

$$100 \times \frac{\text{Total weight of 4-epianhydrotetracycline in the sample}}{\text{Tetracycline content of the sample}}$$

[39 FR 18944, May 30, 1974, as amended at 40 FR 22251, May 22, 1975; 43 FR 11153, Mar. 17, 1978]

**§ 436.310 Thin layer chromatography identity test for mitomycin.**

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

(2) *Plates.* Use 20 by 20 centimeter thin layer chromatography plates coated with silica gel G or equivalent, to a thickness of 250 microns.

(b) *Reagents*—(1) *Developing solvent.* Mix *n*-butanol, glacial acetic acid, and water in volumetric proportions of 4:2:1, respectively.

(2) *Spray solution.* Prepare a one-percent solution of ninhydrin in ethanol.

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

(d) *Procedure.* Pour the developing solvent into the solvent trough on the bottom of the tank and onto the paper lining the walls of the tank. Cover and seal the tank. Allow it to equilibrate for 30 minutes. Prepare a plate as follows: Apply spotting solutions on a line 2.5 centimeters from the base of the silica gel plate and at points 2.0 centimeters apart. Apply approximately 2 microliters of the working standard solution to points 1 and 3. When these spots are dry, apply approximately 2 microliters of sample solution to points 2 and 3. After all spots are thoroughly dry, place the silica gel plate into the trough in the chromatography tank. Cover and seal the tank tightly. Allow the solvent front to travel about 10 centimeters from the starting line. Remove the plate and allow it to air dry. After the plate is dry, spray lightly with the spray solution. Heat the plate in an oven at 110° C. for 10–15 minutes. Mitomycin appears as a pink spot.

(e) *Evaluation.* The sample and standard should have spots of corresponding  $R_f$  value (approximately 0.51), and standard and sample combined should appear as a single spot of corresponding  $R_f$  value.

[39 FR 18944, May 30, 1974, as amended at 49 FR 2242, Jan. 19, 1984]

#### § 436.311 Thin layer chromatography identity test for amoxicillin.

Using the sample solution prepared as described in the section for the antibiotic drug to be tested, proceed as described in paragraphs (a) through (e) of this section.

(a) *Equipment*—(1) *Chromatography tank.* A rectangular tank, approxi-

mately 23 centimeters long, 23 centimeters high, and 9 centimeters wide, equipped with a glass solvent trough in the bottom and a tight-fitting cover for the top. Line the inside walls of the tank with Whatman's 3MM chromatographic paper (0.33 millimeters) or equivalent.

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

(b) *Reagents*—(1) *Developing solvent.* Mix methyl alcohol, chloroform, pyridine, and distilled water in volumetric proportions of 90:80:10:30, respectively.

(2) *Spray solution.* Dissolve 300 milligrams of ninhydrin in 100 milliliters of ethyl alcohol.

(c) *Preparation of working standard.* Weigh an amount of the amoxicillin working standard equivalent to 200 milligrams of amoxicillin into a 50-milliliter volumetric flask and bring to volume with 0.1N hydrochloric acid.

(d) *Procedure.* Pour the developing solvent into the glass trough on the bottom of the tank and onto the paper lining the walls of the tank. Cover and seal the tank. Allow it to equilibrate for at least 2 hours. Spot duplicate plates by applying approximately 5 microliters each of standard and sample solutions on a line 1.5 centimeters from the base of the plate and at intervals of not less than 2.0 centimeters. All solutions must be spotted within 10 minutes of preparation. Place spotted plate in a desiccator until solvent has evaporated from spots. Place the plate into the glass trough at the bottom of the chromatography tank. Cover the tank. Allow the solvent to reach the 15-centimeter scored mark, remove the plate from the tank and dry with a current of warm air until there is no detectable solvent odor. Apply the ninhydrin spray solution to the plate—do not saturate—and place immediately into an oven maintained at 110° C for 15 minutes.

(e) *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. Amoxicillin has an  $R_f$  value of about 0.53. The sample and standard