

nanometers, using suitable spectrophotometer cells with a 1-centimeter light path. Determine the exact position of the absorbance peak for the particular instrument used.

(3) *Calculation.* Determine the percentage of griseofulvin dissolved as follows:

$$\text{Percent griseofulvin dissolved} = \frac{A_u \times W_s \times V \times 10}{A_s \times P}$$

where:

$A_u$ =Absorbance of the sample solution minus the absorbance of the sample-blank solution;

$W_s$ =Weight of the working standard in milligrams;

$V$ =Volume of the dissolution medium in liters;

$A_s$ =Absorbance of the standard solution minus the absorbance of the standard-blank solution;

$P$ =Labeled potency of the sample in milligrams of griseofulvin per tablet.

(e) *Evaluation.* The tablet passes the solubility characteristic test if it dissolves to the extent of not less than 50 percent at 60 minutes. If the tablet fails to meet this requirement, repeat the test on five additional tablets. The batch passes the solubility characteristic test if not less than 5 of 6 tablets meet the requirement.

[40 FR 41522, Sept. 8, 1975; 40 FR 45426, Oct. 2, 1975]

#### § 436.318 Continuous flow thin layer chromatography identity test.

(a) *Equipment*—(1) *Chromatography tank.* 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 × 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 × 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 chloroform, redistilled methanol

and concentrated ammonium hydroxide in volumetric proportions of 25:60:30, respectively.

(2) *Spray solution.* Dissolve 1 gram of ninhydrin in 100 milliliters of *n*-butanol and add 1 milliliter of pyridine.

(c) *Preparation of spotting solutions.* Prepare solutions of the sample and working standard, each containing 6 milligrams of antibiotic to be tested 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 3 microliters each of the standard solution and the sample solution. In addition, prepare one spot composed of 3 microliters of the sample solution and 3 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 20x3 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 5.5 hours. Allow the plate to air dry and then heat it for 15 minutes at 110° C in an oven. Remove the plate from the oven and immediately spray it with the spray solution. The compound appears as a pink spot.

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

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