

0.5 gram of the silica gel in a micro filter funnel (approximately 10-millimeter diameter) having a fritted-glass disc of medium porosity.

(b) *Procedure.* Accurately weigh from 60 to 70 milligrams of the sample to be tested, except if penicillin G procaine is to be tested weigh 90 to 100 milligrams of sample, into a glass test tube or glass vial of approximately 10-milliliter capacity. Add 2.0 milliliters of water to dissolve or suspend (procaine) the penicillin and cool to 0° C—5° C. Add 2.0 milliliters of amyl acetate solution and 0.5 milliliter of phosphoric acid solution, stopper and shake the container vigorously for approximately 15 seconds. For penicillin G procaine, add a second 0.5-milliliter portion of phosphoric acid solution and shake vigorously. Centrifuge to obtain a clear separation of the two layers (approximately 20 seconds). If any penicillin procaine remains undissolved, add a third 0.5-milliliter portion of phosphoric acid solution, shake the container vigorously, and centrifuge. After centrifuging, remove as much of the amyl acetate layer as possible, usually about 1.7 milliliters to 1.8 milliliters, with a suitable hypodermic needle and syringe and place the portion removed into the filter funnel containing silica gel, described in paragraph (a)(5) of

this section. Allow the amyl acetate to remain in contact with the silica gel for exactly 20 seconds, then apply suction and collect the filtrate in a small test tube placed in a suction flash surrounded by cracked ice. Pipet a 1.0-milliliter aliquot of the amyl acetate filtrate into a tared flat-bottom glass tube (approximately 15 x 50 millimeters) containing 1.0 milliliter of acetone solution and 0.5 milliliter of *N*-ethylpiperidine solution. The time elapsing between acidification and the addition of the filtrate to the above reagents should not be more than 3 minutes. Place the glass tube containing the mixture into a large weighing bottle, stopper the bottle and allow to stand for not less than 2 hours in a refrigerator at 0° C—8° C. Remove the liquid from the precipitate by means of a tared micro filter stick and wash with a total of 1.0 milliliter of acetone solution adding the latter by means of a hypodermic syringe equipped with a fine needle. Place the filter stick inside the glass tube, dry under vacuum at room temperature for not less than 1 hour, and weigh. (The *N*-ethylpiperidine penicillin G residues can be saved for saturating reagents).

(c) *Calculations.* Calculate the percent penicillin G content as follows:

$$\text{Percent penicillin G content} = \frac{\text{Milligrams } N\text{-ethylpiperidine penicillin precipitate} \times 149.4}{\text{Weight of sample in milligrams}}$$

[42 FR 59857, Nov. 22, 1977]

§ 436.317 Solubility characteristic test for griseofulvin (ultramicrosize) tablets.

(a) *Apparatus*—(1) *Vessel.* A cylindrical glass tank. The approximate dimensions are 40 centimeters in diameter and at least 23 centimeters in height.

(2) *Heating system.* A 1,500-watt immersion heating element connected to a partial immersion, contact thermometer and an appropriate control relay.

(3) *Circulating system components.* The circulating system consists of three different circulating devices:

(i) Circulating pump of a centrifugal, immersion type. Tubing approximately 1 centimeter outside diameter and 46 centimeters in length is attached to the pump outlet producing a flow rate of approximately 1,600 milliliters per minute when operated as described.

(ii) A "4-element stirrer" consisting of a motor and a shaft approximately 45 centimeters long and 8 millimeters in diameter. The motor rotates the vertical shaft in a clockwise direction at approximately 180 revolutions per minute. There are 4 elements or sets of stirring blades on the shaft. One set, located at the bottom of the shaft, is a 3-bladed element of 2.5 centimeters

overall radius with circular blades, 1.8 centimeters in diameter and 1 to 2 millimeters in thickness, pitched at an angle of approximately 45 degrees from the horizontal plane, so that fluid is propelled downward when the shaft is rotated in a clockwise direction. The three remaining sets of stirring blades have 4 blades each, symmetrically positioned about the shaft. Each set of blades is 3.2 centimeters in overall radius. Each blade is rectangular in shape, 2.4 centimeters in length, 1.2 centimeters in height, and 1 to 2 millimeters in thickness. The four sets of blades are located at 5 centimeter intervals on the shaft, the top three being fixed in a staggered configuration.

(iii) A rotating basket device consisting of a motor capable of constant speed of 100 ± 5 revolutions per minute in a clockwise direction, a shaft, and a cylindrical basket. The shaft and the basket are fabricated from Type 316 stainless steel. The shaft is 6 millimeters in diameter and approximately 30 centimeters in length. It must run true on the motor axis so that the basket rotates smoothly and without perceptible wobble. The basket consists of two parts, one of which, the top, is attached to the shaft. It is of solid metal except for a 2-millimeter round vent, and is fitted with three spring clips that allow the removal of the lower part, or the basket proper, to admit the test sample. The detachable part of the basket is fabricated of welded seam stainless steel, 40 mesh woven wire cloth, formed into a cylinder 3.66 centimeters high and 2.5 centimeters in diameter, with a narrow rim of sheet metal around the top.

(4) *Circulating system configuration.* All three circulating devices are located in one half of the tank. In clockwise order they are the circulating pump, the rotating basket, and the 4-element stirrer. There is a distance of 12 to 13 centimeters between each of the three devices. The rotating basket shaft and the stirring shaft are located 9 to 10 centimeters from the tank wall. The 4-element stirrer is positioned 1 to 1.5 centimeters from the bottom of the tank. The rotating basket is fixed at 7 to 8 centimeters from the bottom. The circulating pump intake is located ap-

proximately 3 centimeters from the top of the fluid in the tank and 5 to 6 centimeters from the wall of the tank. The pump's outlet hose is held by a clamp so that hose makes a clockwise arc around the inside wall of the tank, descending to a point near the bottom of the tank and 5 to 6 centimeters from the wall, which is 180 degrees from the pump inlet.

(b) *Dissolution medium.* Distilled water.

(c) *Procedure.* Place 24 liters of dissolution medium into the vessel and maintain the temperature at $37 \pm 0.5^\circ \text{C}$ by means of the heater, circulating pump, and the 4-element stirrer. Withdraw a 25-milliliter portion of the dissolution medium as a sample-blank solution. Place one tablet into the basket, and lower it into its proper position in the tank. Rotate the basket at 100 ± 5 revolutions per minute in a clockwise direction. After 60 minutes, withdraw a second 25-milliliter portion as the sample solution. Filter the sample-blank solution and the sample solution through water-washed glass wool, or an equivalent filter, discarding the first 10 to 15 milliliters of each filtrate. Determine the amount of griseofulvin dissolved as directed in paragraph (d)(2) of this section.

(d) *Griseofulvin assay—(1) Preparation of standard solution and standard-blank solution.* Accurately weigh approximately 50 milligrams of griseofulvin working standard and place into a 100-milliliter volumetric flask. Dissolve and dilute to volume with methyl alcohol. Transfer 2.0 milliliters of this solution to a 200-milliliter volumetric flask and dilute to volume with distilled water. This is the standard solution. Transfer a 2.0-milliliter portion of methyl alcohol to a 200-milliliter volumetric flask and dilute to volume with distilled water. This is the standard-blank solution. Filter the standard-blank solution and the standard solution through water-washed glass wool, or an equivalent filter, discarding the first 10 to 15 milliliters of each filtrate.

(2) *Procedure.* Using a suitable spectrophotometer and distilled water as the blank, determine the absorbance of the four filtered solutions at the absorbance peak at approximately 295

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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