

5.2.03

AOAC Official Method 966.30 Griseofulvin in Feeds Spectrophotometric Method First Action 1966 Final Action 1975

(Applicable to concentrations 10 mg/oz.)

A. Reagents

- (a) *Activated alumina*.—Alcoa grade F-20.
- (b) *Solvent mixture*.—Petroleum ether–CHCl₃ (65 + 35).
- (c) *Griseofulvin standard solution*.—10 g/mL. Accurately weigh ca 25 mg USP Griseofulvin Reference Standard into 250 mL volumetric flask; dissolve and dilute to volume with solvent mixture. Dilute 10 mL of this solution to 100 mL in volumetric flask.

B. Apparatus

- (a) *Chromatographic tube*.—20–400 mm, with fritted disk and stopcock.
- (b) *High-speed blender*.—Waring-type, or equivalent, 1 L capacity.
- (c) *Spectrophotometer*.—Capable of accurate readings at 290 and 320 nm.

C. Extraction

Accurately weigh ca 14 g ground test portion into fat-free thimble and extract in Soxhlet apparatus 2 h with 100 mL CHCl₃. Evaporate extract to 10 mL on steam bath, dilute with 100 mL petroleum ether, and chromatograph.

D. Preparation of Chromatographic Column

Place 50 mL solvent mixture, A(b), in tube and add 45 mL activated alumina portionwise, with tapping to ensure uniform

packing. Place small glass wool pad on top of alumina and drain solvent to just below top of pad.

E. Determination

Add petroleum ether–CHCl₃ test extract to column. As last of extract passes through glass wool pad, rinse flask with solvent mixture, add to column, and begin elution with solvent mixture. Adjust liquid head to give flow rate of 15–20 mL/min. Start collecting 25 mL fractions when green eluate first appears (discard yellow and almost colorless eluates which precede). When A of fractions at 290 nm exceeds A at 320 nm, stop fractionating, and collect next 700 mL eluate. Dilute eluate to volume in 1 L volumetric flask with solvent mixture. Determine A of this solution and of griseofulvin standard solution at 290 and 320 nm against solvent mixture blank.

$$\text{Griseofulvin/oz, mg} = \frac{(A_{290} - A_{320})(W)(10)(28.35)}{(A_{290} - A_{320})(25)(\text{g test portion})}$$

where A refers to test portion eluate, A to standard solution, and W = mg reference standard griseofulvin used to prepare standard solution.

Reference: *JAOAC* **49**, 494(1966).

CAS-126-07-8 (griseofulvin)

Revised: March 2002