

5.1.21

AOAC Official Method 970.85 Dimetridazole in Feeds Spectrophotometric Method First Action 1970 Final Action 1988

A. Principle

Dimetridazole is extracted from feeds with methanol, separated from interfering substances by two alumina chromatographic steps, and determined spectrophotometrically at its UV wavelength maximum. Nihydrzone, furazolidone, zoalene, 2-chloro-4-nitrobenzamide, tylosin, and large amounts procaine (from procaine penicillin) interfere.

B. Apparatus and Reagents

(a) *Spectrophotometer*.—For use in UV.

(b) *Chromatographic tubes*.—13 150 and 15 250 mm, constricted at bottom to hold glass wool plug and 6 mm od delivery tube.

(c) *Aluminum oxide*.—Suitable for chromatography, **961.24B(b)** (see 5.1.08). To determine suitability of alumina, perform determination on feed that does not contain dimetridazole or other imidazole drugs. If feed appears to contain >0.004% dimetridazole, use another batch of alumina.

(d) *1,2-Dimethyl-5-nitroimidazole (dimetridazole) standard solutions*.—(1) *Stock solution*.—0.1 mg/mL. Weigh 100 mg dimetridazole standard into 100 mL volumetric flask. Dissolve in H₂O by shaking frequently ca 20 min. Dilute to volume with H₂O and mix. Pipet 20 mL into 200 mL volumetric flask, dilute to volume with H₂O, and mix. (2) *Working solutions*.—Pipet 5, 10, 20, 30, and 40 mL stock solution into separate 100 mL volumetric flasks. Add 5.0 mL 3M HCl to each, immediately dilute to volume with H₂O, and mix. Pipet 5 mL each solution and 5 mL 0.10M NaOH into separate 50 mL Erlenmeyers. Stopper and mix. These solutions contain 2.5, 5, 10, 15, and 20 g dimetridazole/mL.

C. Preparation of Standard Curve

Proceed as in F, using working standard solutions and blank prepared by mixing 5 mL 0.15M HCl with 5 mL 0.10M NaOH.

Read *A* against blank for recording or manual spectrophotometers. Construct standard curve by plotting *A* against g dimetridazole/mL.

D. Preparation of Test Portion

Weigh ground test portion containing 0.5–2.0 mg dimetridazole (usually 5 g) into 100 mL volumetric flask. Add 70–75 mL methanol and place in 60 C constant temperature bath 30 min. Make certain that H₂O level covers flask to ca 3 mm below methanol level. Swirl flask 2 or 3 times during first 5 min to heat evenly. Cool to room temperature, dilute to volume with methanol, and mix. Let stand 5–10 min to let coarse feed particles settle.

E. Chromatography

Place small glass wool plug in bottom of 250 15 mm chromatographic tube and add 8 cm layer alumina; pack column

tightly to prevent streaking. (If streaks enter effluent, positive bias is introduced.) Decant methanolic extract onto column so that settled feed particles are not disturbed. Collect ca 30 mL eluate in 50 mL volumetric flask. Stopper until ready for use.

(Note: Dimetridazole sublimes at temperatures >70 C; manner of solvent removal is critical.) For feed containing 0.015% dimetridazole, pipet 15 mL effluent (4 mL if feed contains 0.06%; 3 mL if feed contains 0.10%) into 125 mL suction or round-bottom flask and evaporate under reduced pressure from H₂O aspirator. If 15 mL is taken, use hot plate (low heat) or water bath to reduce to 3–4 mL. Shake to prevent bumping. When volume approaches 3–4 mL remove flask from heat and remove last 3–4 mL only with heat from palm of hand. Continue shaking to prevent bumping. Do not attempt to attain complete dryness because part of the 2–3 drops of oily residue is dimetridazole.

Wash down walls of flask, beginning at base of neck, with 5.0 mL 0.10M NaOH. Swirl to wash walls. Let stand 5 min and add 5.0 mL 0.15M HCl. Swirl to mix and wash flask walls. Stopper until ready for chromatography.

F. Determination

Prepare second alumina column by inserting small glass wool plug into bottom of 150 13 mm chromatographic tube, add 4 cm layer alumina, and tap gently to pack column lightly. Pour entire 10 mL solution onto column and let pass through by gravity. Collect effluent in 50 mL Erlenmeyer. Force out liquid adhering to column by applying air pressure with rubber bulb. Swirl flask to mix. Stopper until ready to read. Pass blank solution of 5 mL 0.15M HCl and 5 mL 0.10M NaOH through separate 4 cm alumina column as above.

(a) *Using recording spectrophotometer*.—Fill matched pair silica cells with reagent blank and with test solution (always use same cell for blank) and scan from 330 to 310 nm. Read *A* at peak and obtain concentration of solution in g/mL from standard curve.

$$\text{Dimetridazole, \%} = \frac{(\text{g/mL from standard curve}) \text{ dilution factor (DF)} \ 100}{\text{g test portion} \ 10^6}$$

$$\text{Dimetridazole, mg/kg} = \text{g/mL} \ \text{DF/g test portion}$$

where dilution factor = 66.67 for feeds containing 150 mg/kg (0.015%); 250.0, 600 mg/kg (0.06%); and 333.3, 1000 mg/kg (0.1%).

(b) *Using manual spectrophotometer*.—Locate peak *A* of test solution (ca 318 nm), using matched pair silica cells, and set wavelength at peak. Read *A* of test portion and blank solutions and correct test portion for blank. Obtain concentration of solution in g/mL from standard curve, and calculate mg/kg (%) in feed as above.

References: *JAOAC* **48**, 301(1965); **53**, 646(1970).

CAS-551-92-8 (dimetridazole)