

5.1.24

AOAC Official Method 960.63 Furazolidone, Nitrofurazone, or Bifuran in Feeds

Colorimetric Method
First Action 1960
Final Action 1961

A. Reagents

(a) *Phenylhydrazine hydrochloride solution*.—Dissolve 0.5 g phenylhydrazine HCl in 50 mL H₂O. Prepare fresh daily. Mix equal volume of this solution with HCl.

(b) *Furazolidone standard solutions*.—(1) *Stock solution*.—0.55 mg/mL. Weigh 55 mg furazolidone standard into 100 mL volumetric flask, dilute to volume with dimethylformamide (DMF), and mix. Solution is stable several months when protected from light. (2) *Working solution*.—Prepare working standard corresponding to label declaration. For feeds containing 0.011% furazolidone, pipet 2 mL stock solution into 100 mL volumetric flask, add 48 mL DMF, and dilute to volume with H₂O. For feeds containing 0.00275% furazolidone, pipet 0.5 mL stock solution into 100 mL volumetric flask, add 49.5 mL DMF, and dilute to volume with H₂O.

(c) *Nitrofurazone standard solutions*.—(1) *Stock solution*.—0.56 mg/mL. Weigh 56 mg nitrofurazone standard into 100 mL volumetric flask, dilute to volume with DMF, and mix. Solution is stable several months when protected from light. (2) *Working solution*.—Prepare working standard corresponding to label declaration. For feeds containing 0.0056% nitrofurazone, pipet 1 mL stock solution into 100 mL volumetric flask, add 49 mL DMF, and dilute to volume with H₂O. For feeds containing 0.0112% nitrofurazone, pipet 2 mL stock solution into 100 mL volumetric flask, add 48 mL DMF, and dilute to volume with H₂O.

(d) *Bifuran standard solutions*.—(1) *Stock solution*.—0.1285 mg/mL. Pipet 20 mL nitrofurazone stock solution and 3 mL furazolidone stock solution into 100 mL volumetric flask and dilute to volume with DMF. (2) *Working solution*.—For feeds containing 0.0064% total nitrofurans, prepare working standard by pipetting 5 mL bifuran stock solution into 100 mL volumetric flask, adding 45 mL DMF, and diluting to volume with H₂O.

(e) *Adsorbent*.—To 100 parts alumina, **961.24B(b)** (see 5.1.08), in screw cap bottle, add 4 parts Mg(OH)₂, shake until thoroughly mixed, then add 5 parts H₂O, and mix until all lumps disappear. Store in tightly sealed container.

B. Determination

Weigh 10 g test portion of ground feed into 125 mL Erlenmeyer, add exactly 50 mL DMF, stopper loosely, and place in boiling water bath 5 min. Mechanically shake 10 min and filter through rapid paper. To 25 mL filtrate add 25 mL H₂O and mix.

Prepare ca 20 mm diameter adsorption column, containing adsorbent, to height of 5 cm. Pass the 50% DMF sample solution through column, discarding first 3 mL eluate. (If column flow stops, break up gummy film at top of adsorbent, using long thin glass rod.) Pipet 5 mL aliquots of eluate into each of 2 numbered test tubes. Protect one tube from light. To other tube, add 3 drops freshly prepared 2% (w/v) solution of sodium hydrosulfite and let stand 20 min, shaking at ca 5 min intervals. Treat 5 mL aliquots of working standard solution in exactly same manner.

Pipet 5 mL phenylhydrazine HCl solution into each of the numbered test tubes containing test portions and standards. Mix and place tubes in 70 C water bath 25 min; cool in 15 C water bath 5 min. Add exactly 10 mL toluene to each tube, stopper, and shake vigorously 40 times. Centrifuge or filter toluene solution directly into absorption cell through cotton wad inserted in stem of small funnel. Read *A* of solutions at 440 nm.

$$\text{Furazolidone, \%} = \frac{(A_{\text{test portion}} - A_{\text{reduced test portion}}) \cdot 0.011(\text{or } 0.00275)}{A_{\text{standard}} - A_{\text{reduced standard}}}$$

$$\text{Total nitrofurans (bifuran), \%} = \frac{(A_{\text{test portion}} - A_{\text{reduced test portion}}) \cdot 0.0064}{A_{\text{standard}} - A_{\text{reduced standard}}}$$

$$\text{Nitrofurazone, \%} = \frac{(A_{\text{test portion}} - A_{\text{reduced test portion}}) \cdot 0.0056(\text{or } 0.011)}{A_{\text{standard}} - A_{\text{reduced standard}}}$$

References: *JAOAC* **40**, 463(1957); **41**, 333(1958);
43, 310(1960); **44**, 30(1961); **52**, 233(1969).

CAS-67-45-8 (furazolidone)
CAS-59-87-0 (nitrofurazone)

Revised: March 2002