

5.1.23

AOAC Official Method 985.51 Furazolidone in Feeds and Premixes

Liquid Chromatographic Method

First Action 1985

Final Action 1988

(Applicable to premixes containing 2–22% furazolidone and to feeds containing 0.005–0.05% furazolidone.)

Note: Furazolidone solutions are light-sensitive. Protect extracts and standards from direct sun and artificial light.)

A. Principle

Unground premix is extracted with DMF and concentration of extract is adjusted with 5% tetraethylammonium bromide (TEAB) to ca 55 g furazolidone/mL for LC. Complete feed is extracted with H₂O–acetone on continuous extraction apparatus, solvent is evaporated, and residue is dissolved in DMF. 5% TEAB is added to separate fat. Extract is cooled and clarified for LC.

B. Apparatus

(a) *Liquid chromatograph.*—Instrument capable of maintaining constant pulseless flow of mobile phase at 0.5–1.5 mL/min. Operating conditions: flow rate 1.5 mL/min; loop injection volume 20 L; detector sensitivity 0.32 AUFS or adjusted to produce working standard peak response 60–80% full scale; detector wavelength 365 nm (settings from 390 to 405 nm may be used to improve selectivity for very low level test samples if chromatographic column does not adequately resolve interfering peaks).

(b) *Chromatographic column.*—Any reversed-phase column, C18 or C8 with particle size 10 m that will produce single, sharp furazolidone peak with peak skew <1.4. Guard column may be used.

(c) *Continuous extraction apparatus.*—Goldfish (Labconco Corp., 8811 Prospect Ave, Kansas City, MO 64132, USA; No. 3001), or equivalent.

(d) *Extraction thimbles.*—Whatman, single thickness, 19 90 mm (Scientific Products, Inc.; No. E6480-4).

(e) *Sample clarification filter.*—13 mm glass fiber prefilter pads (Gelman No. 66073, available from Fisher Scientific Co., No. 09-731A) inserted in 0.5 in. id 5 mL syringe barrel (Pharmseal Scientific Products; No. S9504-5) or any filtration device designed for clarification of aqueous chromatographic samples.

C. Reagents

(a) *Extractions.*—DMF, reagent grade, for premixes. Acetone (reagent grade)–H₂O (93 + 7) for complete feeds.

(b) *Diluent.*—5% TEAB (w/v) (Eastman Kodak Co.; No. 1516) in distilled, deionized water. Keep in refrigerator. For correct final concentrations, warm to room temperature before pipetting.

(c) *Furazolidone standard solutions.*—(1) *Stock solution.*—Ca 1.1 mg/mL. Accurately weigh 0.110 ± 0.005 g furazolidone standard and record exact weight to nearest 0.1 mg (W_s). Transfer into 100 mL volumetric flask, dissolve and dilute to volume with DMF (sonication aids dissolution). Solution is stable if stored in dark. (2) *Intermediate solution.*—Ca 110 g/mL. Dilute 10.0 mL stock solution to 100 mL with DMF. Solution is stable if stored in dark. (3) *Working standard solution.*—Ca 55 g/mL. Mix 10.0 mL intermediate solution with equal volume 5% TEAB. (Mix equal volumes. Do not dilute to volume.) Let solution cool to room temperature. Prepare daily. Dilution of standard (D_s) = 2000.

Table 985.51. Test portion sizes, dilutions, and total test portion dilutions for assay of furazolidone in premixes

Label claim, %	Test portion weight, g	Dilutions with DMF, mL	Total test portion dilution (D_u), mL
2.2	1.00	None	400
3.3	1.00	30/50	666.7
11.0	1.00	20/100	2000
22.0	1.00	10/100	4000

(d) *Mobile phase.*—CH₃CN (LC quality)–2% CH₃COOH in distilled, deionized H₂O (20 + 80), or as adjusted to give capacity factor (k) of ca 2.5 for furazolidone.

D. Extraction

(a) *Complete feeds.*—Determine approximate test portion weight to contain ca 550 g furazolidone by using formula, test portion weight, g = 0.055/% guarantee. Accurately weigh (to nearest 0.01 g) calculated weight of ground, mixed test sample (±5%) into extraction thimble (W_u = actual test portion weight). Press cotton plug down onto top of feed to prevent channeling. Add 45–50 mL acetone–H₂O (93 + 7) extractant and 2 or 3 boiling chips to extraction beaker. Extract 8–18 h on extraction apparatus at “Hi” setting. Evaporate solvent on steam bath (stream of air directed into beakers or blowing across beakers, as with partially closed hood door, hastens evaporation). If any H₂O is evident in beaker after initial evaporation, add ca 25 mL acetone, swirl to mix and re-evaporate (repeat as necessary to remove all H₂O). Remove from steam bath as soon as evaporation is complete. Add 5.00 mL DMF, heat on steam bath just until bottom of beaker is hot (15–30 s) and swirl, washing sides, to dissolve residue (generally all residue dissolves in warm DMF). Add 5.00 mL 5% TEAB, mix, pour solution into 15 mL centrifuge tube, and let cool. Centrifuge 5–10 min at 2000 rpm (2500 g). Using disposable pipet attached to aspirator and trap, remove fat layer floating on supernate. Dilution for complete feed test portions (D_u) = 10.

(b) *Premixes.*—Accurately weigh (±5%) amount unground test portion indicated in Table 985.51 into 500 mL glass-stoppered Erlenmeyer (W_u = actual test portion weight). Add by pipet 200.0 mL DMF, stopper, and shake flask 30 min. Either let suspended material settle or centrifuge or filter portion of extract. Dilute with DMF to ca 110 g furazolidone/mL. To 5.00 mL 5% TEAB solution add 5.00 mL diluted extract, mix, and let solution cool to room temperature. Clarify as for complete feeds, (a). (Total test portion dilution = D_u ; Table 985.51.)

E. Determination

(a) *Complete feeds.*—Make several injections of furazolidone working standard solution, adjusting mobile phase strength to give k ca 2.5 and peak height 60–80% full scale [$k = (t_1 - t_0)/t_0$, where t_0 = distance from injection to first perturbation of standard chromatogram]. Make 2 or more injections of standard to ensure 1–2% repeatability of peak responses. Bracket each 2 test portion injections by standard injections. Use average peak height P_s (or average peak area) of standards bracketing each pair of test portions to calculate furazolidone concentration in test samples (test portion peak response = P_u). If no drift in standard peak heights is evident throughout run, then use average for all standard injections in calculations.

(b) *Premixes*.—Determine furazolidone as for complete feeds
(a). Dilution (D_u) is shown in Table **985.51**.

F. Calculations

$$\text{Furazolidone, \%} = \frac{P_u W_s D_u}{P_s W_u D_s} 100$$

$$\text{Furazolidone, mg/kg} = \frac{P_u W_s D_u}{P_s W_u D_s} 10^6$$

where P_u and P_s = peak response of test portion (unknown) and standard, respectively; W_u and W_s = g test portion and standard,

respectively; and D_u and D_s = mL total dilutions of test portion and standards, respectively. Determine total dilutions as in following example: If extraction of 1 g test portion in 200 mL solvent is followed by serial dilutions of 20/100 and 5/10, then total dilution is 1 g/200 mL \times 20/100 \times 5/10 = 1 g/2000 mL, and D_u = 2000 mL.

Reference: *JAOAC* **68**, 1033(1985).

CAS-67-45-8 (furazolidone)

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