

## 5.1.15

### AOAC Official Method 967.34 Buquinolate in Feeds

#### Fluorometric Method

First Action 1967

Final Action 1972

#### A. Principle

Buquinolate is extracted from feed with  $\text{CHCl}_3$ , concentrated to small volume, and separated from interfering substances by TLC utilizing 2 solvent systems. Buquinolate is eluted from substrate and determined fluorometrically.

#### B. Reagents

(a) *Alcohol, 80%*.—Dilute 84.3 mL alcohol to 100 mL with  $\text{H}_2\text{O}$ .

(b) *Developing solvent*.—Mix  $\text{CHCl}_3$  with alcohol (10 + 1). Prepare fresh daily.

(c) *Buquinolate standard solutions*.—(1) *Stock solution*.—0.5 mg/mL. Dissolve 50.0 mg Buquinolate Reference Standard in  $\text{CHCl}_3$  to make 100 mL. Warm mixture on steam bath as necessary. Solution is stable 1 month if protected from evaporation. (2) *Working solution*.—100  $\mu\text{g/mL}$ . Pipet 5 mL stock solution into 25 mL volumetric flask, dilute to volume with  $\text{CHCl}_3$ , and mix well. Prepare fresh daily.

#### C. Apparatus

(a) *Developing tanks*.—Line developing tanks (for plates 20 cm) with Whatman 3 MM paper. Add 100 mL  $\text{CHCl}_3$  to one tank; add 100 mL developing solvent, **B(b)**, to second tank. Prepare each tank fresh daily.

(b) *Plates for TLC*.—Clean plates thoroughly with alkyl benzene sulfonate-type detergent and brush; rinse plates with  $\text{H}_2\text{O}$  and then with acetone. Let plates air dry. Slurry 60 g silica gel G (Brinkmann No. 68-00-261-3) with 120 mL  $\text{H}_2\text{O}$ . Pour into suitable applicator and spread 0.500 mm layer on 20  $\times$  20 cm plates. Air dry 15–30 min; then dry 2 h at 110  $^\circ\text{C}$ . Cool and store plates in desiccator until used.

(c) *Fluorometer*.—Use either spectrophotofluorometer or filter fluorometer. (Suitable filters are: excitation, PTR Optics type UV-7E [UV Spectrum Filter, No. 59-07-9, PTR Optics Corp., 145 Newton St, Waltham, MA 02154, USA]; emission, Kopp Glass Co. filters C7380 and C5840.)

#### D. Determination

Accurately weigh test portion of ground feed containing 1.25 mg buquinolate into 250 mL glass-stoppered Erlenmeyer. Pipet 100 mL  $\text{CHCl}_3$  into test portion flask. Shake mechanically 1 h. Filter extract through Whatman No. 54 paper on Büchner with mild vacuum. (Take care to prevent solvent loss by evaporation.) Transfer exactly 80 mL extract to 150 mL beaker and evaporate almost to dryness on steam bath. Take up residue in small portion  $\text{CHCl}_3$  and transfer to 10 mL volumetric flask with small portions  $\text{CHCl}_3$ . Dilute to volume with  $\text{CHCl}_3$  and mix well.

Apply 250  $\mu\text{L}$  extract and 250  $\mu\text{L}$  working standard solution to TLC plate. Place spots ca 25 mm from bottom of plate and 40 mm apart. *Do not touch pipet to plate*. Develop plate in  $\text{CHCl}_3$  developing tank, **C(a)**, until solvent front nearly reaches top of plate (ca 1 h). Observe plate under short wavelength UV light: Buquinolate remains at origin; feed background migrates. Transfer air-dried (5–10 min) plate to tank containing developing solvent, **B(b)**. Let plate develop until solvent front advances 12 cm. Air dry 5–10 min. Examine plates under short wavelength UV light. Buquinolate migrates from origin ( $R_f$ , 0.4–0.6). With spatula, outline each buquinolate spot plus blank spot of equivalent area and  $R_f$ . Remove adsorbent from around buquinolate spots and discard. Quantitatively transfer each spot to separate glass-stoppered 25 mL Erlenmeyers. Pipet 10 mL 80% alcohol, **B(a)**, into each flask, shake mechanically 20 min, and centrifuge.

Determine intensity of fluorescent radiation ( $I$ ) of test portion, standard, and blank in 10  $\times$  10 mm silica cells, at excitation and emission wavelengths of 265 and 375 nm, respectively.

$$\text{Buquinolate, \%} = \frac{I_T}{I_S} \frac{I_B}{I_B} \cdot 0.125 / \text{g test sample}$$

where T = test portion; B = blank; S = standard.

Reference: *JAOAC* **50**, 264(1967).

CAS-5486-03-3 (buquinolate)