

## 5.1.52

### AOAC Official Method 963.35 Sulfaquinoxaline in Feeds

Spectrophotometric Method  
First Action 1963  
Final Action 1988

#### Method I

(Applicable only to nonpelleted feeds containing arsanilic acid. In absence of arsanilic acid, use F.)

#### A. Principle

Sulfaquinoxaline is extracted from feed with DMF and separated from interfering substances by column chromatography on alumina. Isolated sulfaquinoxaline is acidified, diazotized, and coupled in presence of Zr, and colored complex is extracted with butyl alcohol and measured at 550 nm. Arsanilic acid remains in final aqueous solution and can be measured at 540 nm and compared with standard treated similarly.

#### B. Reagents

See 969.53A(b)–(d) (see 5.1.04) and in addition:

(a) *Alkaline salt solution*.—Dissolve 2.0 g NaOH and 100.0 g NaCl in 500 mL H<sub>2</sub>O.

(b) *Zirconium solution*.—Dissolve 5.0 g zirconyl chloride, ZrOCl<sub>2</sub>·8H<sub>2</sub>O (Fisher Scientific Co.), in 100 mL H<sub>2</sub>O.

(c) *Sulfaquinoxaline standard solutions*.—(1) *Stock solution*.—Weigh 40.0 mg USP Sulfaquinoxaline Reference Standard and dissolve in 50.0 mL DMF. Solution is stable at least 1 month if kept tightly stoppered and protected from light. (2) *Intermediate solution*.—80 g/mL. Dilute 5 mL stock solution to 50 mL with DMF. (3) *Working solution*.—8 mg/mL. Dilute 5 mL intermediate solution to 50 mL with DMF. Prepare from freshly prepared intermediate solution just before use.

(d) *Butanol mixture*.—Mix 100 mL *n*-hexane with 400 mL *n*-butyl alcohol.

#### C. Preparation of Test Extract

Weigh 4.00 g ground test portion into 100 mL volumetric flask. Add 50.0 mL DMF, stopper, and agitate by magnetic stirrer or mechanical shaker 60 min. Transfer mixture to 50 mL centrifuge tube and centrifuge 5 min at 2500 rpm.

#### D. Chromatography

(a) *Preparation of column*.—Constrict end of 50–60 cm length of 9–11 mm id glass tubing by rotating in hot flame until opening is 4–5 mm. Insert small plug of Pyrex glass wool in lower end and compress with glass rod to thickness of 2–3 mm. Transfer 5.0 g alumina, 961.24B(b) (see 5.1.08), to dry tube and pack by gentle tapping while applying vacuum.

(b) *Separation*.—Pipet 10 mL clear extract onto column and let pass through by gravity. Do not let column run dry; keep 5 mm head of liquid. Wash inner walls with two 5.0 mL portions CHCl<sub>3</sub>. Let final washing drain until no further liquid appears at tip. Discard effluent and washings. Attach column tip to vacuum and draw air through until alumina is dry, indicated by tube returning to room temperature. Elute column by gravity with 25 mL alkaline salt solution, collecting eluate in 25 mL volumetric flask. Add 1.0 mL HCl to eluate, dilute to volume with H<sub>2</sub>O, and mix well.

Prepare reagent blank by transferring 10 mL DMF onto fresh column and proceeding as for test extract. Prepare standard by

transferring 10.0 mL sulfaquinoxaline working standard solution onto fresh column and proceeding as for test extract.

#### E. Determination

Transfer 10 mL aliquots of each eluate to separate centrifuge tubes. Add 2.0 mL Zr solution and mix. Add 1.0 mL 0.1% NaNO<sub>2</sub> solution, mix, and let stand 2 min. Add 1.0 mL 0.5% ammonium sulfamate solution, mix, and let stand 2 min. Add 1.0 mL coupling reagent, 969.53A(d) (see 5.1.04), mix, and let stand 10 min. Add 2.0 g NaCl and 10.0 mL butyl alcohol mixture, stopper, and shake vigorously until NaCl dissolves. Centrifuge, carefully transfer portion of clear, colored top solvent layer to 1 cm cell, and read *A* at 550 nm against butyl alcohol mixture. Correct for reagent blank.

$$\text{Sulfaquinoxaline, \%} = 0.04 \frac{A/A}{W}$$

$$\text{Sulfaquinoxaline, mg/kg} = 400 \frac{A/A}{W}$$

where *A* and *A* refer to test extract and standard solution (blank corrected), respectively, and *W* = g test portion.

#### Method II Final Action 1960

(Applicable in absence of arsanilic acid.)

#### F. Determination

Weigh 5 g ground test portion into 250 mL volumetric flask, add 150 mL H<sub>2</sub>O and 5 mL 0.5M NaOH, and place in boiling water bath 15 min. Remove, cool, dilute to volume with H<sub>2</sub>O, mix, and let settle. Transfer 50 mL supernatant to 100 mL volumetric flask, add 3 mL HCl, and dilute to volume. Mix, and filter through 18.5 cm Whatman No. 2 paper (or equivalent), discarding first 15 mL filtrate if turbid.

To 10 mL filtrate in each of two 50 mL beakers add 2 mL freshly prepared 0.1% NaNO<sub>2</sub> solution and let stand 3 min. Add 2 mL 0.5% ammonium sulfamate solution and let stand 2 min. Add 1 mL coupling reagent, 969.53A(d) (see 5.1.04), to first beaker and 1 mL H<sub>2</sub>O to second beaker. Mix thoroughly after adding each reagent. After 10 min, read *A* in spectrophotometer at 545 nm. Subtract *A* of feed blank from test extract *A* and determine amount of sulfaquinoxaline from standard curve. Divide by 1000 to obtain percent sulfaquinoxaline. Multiply by 10 to obtain mg/kg.

Prepare standard curve as follows: Dissolve 0.250 g pure sulfaquinoxaline in 5 mL 0.5M NaOH and 50 mL H<sub>2</sub>O in 500 mL volumetric flask, and dilute to volume with H<sub>2</sub>O. Pipet 5 mL aliquot of this solution into 100 mL volumetric flask and dilute to volume with H<sub>2</sub>O. Pipet 2, 4, 6, 8, and 10 mL portions of this diluted solution (equivalent to 50, 100, 150, 200, and 250 mg sulfaquinoxaline, respectively) into separate 100 mL volumetric flasks, add 3 mL HCl to each flask, and dilute to volume with H<sub>2</sub>O. Treat 10 mL aliquots of these final dilutions as in second paragraph. Determine *A* at 545 nm against H<sub>2</sub>O blank, and plot *A* against mg sulfaquinoxaline.

References: *JAOAC* 33, 156(1950); 38, 229(1955);

39, 307(1956); 56, 758(1973); 59, 399(1976);

62, 423(1979).

CAS-59-40-5 (sulfaquinoxaline)

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