

5.1.40

AOAC Official Method 967.38 Piperazine in Feeds Spectrophotometric Method First Action 1967 Final Action

A. Principle

Piperazine or piperazine salt is quantitatively extracted from feed into slightly acidic aqueous solution. Diluted filtrate is reacted with equal volume benzoquinone solution at 80 °C. Colored complex formed is determined spectrophotometrically at 490 nm.

Applicable to determination of (50–5000 mg/kg) piperazine, usually present as one of its salts, in animal feeds. Amines give similar color reaction. Alkalies produce increased color; pH adjustment in method overcomes interference of this kind.

B. Apparatus

(a) *Water bath*.—Approximately 25 cm (10 in.) diameter with 15–20 cm (6–8 in.) depth H₂O. Thermostatically controlled at 80 ± 0.1 °C. (Viscosity bath is satisfactory.)

(b) *Test tubes*.—Pyrex, 15 × 125 mm, with rubber stoppers, and rack capable of supporting tubes when immersed in water bath.

C. Reagents

(a) *Quinone solution*.—Dissolve 0.5 g *p*-benzoquinone in 2.5 mL CH₃COOH and little alcohol in dry 100 mL volumetric flask and dilute to volume with alcohol. Keep solution in ice bath or refrigerator. Prepare fresh daily. (*Note: p*-Benzoquinone is lachrymator; avoid breathing vapor and contact with skin and clothing.) If blanks are high or variable, purify *p*-benzoquinone by steam distillation in hood.

(b) *Piperazine standard solutions*.—(1) *Stock solution*.—Dissolve exactly 185 mg pure piperazine 2HCl (Vétoquinol Prolab, Inc., 700 rue St-Henri, Princeville (Quebec) G6L 4X1, Canada, Tel: 819-364-3073, Fax: 819-364-7895; www.vetoquinol.qc.ca) (equivalent to 100 mg piperazine) in H₂O and dilute to 250 mL. (2) *Working solution*.—20 g/mL. Dilute 25.0 mL stock solution to volume in 500 mL volumetric flask.

D. Preparation of Standard Curve

Using working solution, add, by microburet or pipets, 20, 40, 60, 80, and 100 g piperazine equivalent and intermediate values, if required, into test tubes. Dilute to 5 mL in each test tube with H₂O. Include H₂O blank with each determination.

Add 5 mL quinone reagent to each standard and blank. Stopper tubes and mix by inverting. Remove stoppers and immerse in water bath at 80 ± 0.1 °C exactly 10 min. (Bath temperature can be varied; use same temperature for test portions and standards.) Immediately immerse tubes in ice bath 3 min. Let stand at room temperature 20 min, but 40 min. Read *A* at 490 nm in 1.0 cm cells, using reagent blank to zero instrument. Plot *A* of each standard against g piperazine.

E. Determination

Weigh 10.00 g ground test portion into 500 mL (16 oz) wide-mouth, screw-cap bottle. Add exactly 200 mL H₂O from graduate and adjust to pH 4–5 (0.15 mL H₂SO₄ [1 + 2] is usually enough for 10 g feed). Cap or stopper bottle and place in wrist-action shaker 30 min. Add ca 5 g Celite (*Note: some grades may retain piperazine*) as slurry to Büchner containing 9.0 cm Whatman No. 3 paper and pull down under full vacuum. Wash pad with small portion feed extract and discard washing. Rapidly filter remaining feed extract and reserve filtrate for color development. *Do not delay; turbidity may form.*

Pipet 25.0 mL filtrate into 250 mL volumetric flask and dilute to volume with H₂O. Pipet 5 mL aliquot into test tube and immediately proceed with color development as in **D**. Include H₂O blank with each determination.

Prepare color blank for each feed as follows: Pipet 5 mL aliquot diluted extract into test tube and 5 mL H₂O into another test tube as reference. To each, add 5 mL solution containing 2.5 mL CH₃COOH diluted to 100 mL with alcohol. Mix by inverting and omit heating. Read *A* at same wavelength and subtract from test reading. Include 1 or 2 standards with each determination to detect shift in standard curve; adjust accordingly. Obtain g piperazine from standard curve.

$$\text{Piperazine, \%} = \frac{\text{g piperazine } 10^4}{\text{g test portion in aliquot}}$$

$$\text{Piperazine, mg/kg} = \frac{\text{g piperazine } 400}{\text{g test portion}}$$

Reference: *JAOAC* **50**, 268(1967).

CAS-110-85-0 (piperazine)