

5.1.47

AOAC Official Method 986.39 Roxarsone in Feeds

Atomic Absorption Spectrophotometric Method

First Action 1986

Final Action 1989

(Method determines total As and is not specific for roxarsone. Applicable range is 0–50 mg/kg 4-hydroxy-3-nitrobenzene arsonic acid.)

A. Principle

Test sample is extracted with aqueous ammonium carbonate solution and analyzed by furnace AAS for total As content, which is converted by factor to roxarsone concentration in finished feed.

B. Reagents

(a) *Water*.—Super Quality from Millipore Super Q system, or equivalent.

(b) *Nitric acid*.—Mallinckrodt Speciality Chemical Co., ACS grade, or equivalent.

(c) *Argon*.—Linde purified.

(d) *Nickel nitrate*.—Ni(NO₃)₂·6H₂O (Mallinckrodt Speciality Chemical Co., or equivalent).

(e) *Nickel nitrate solution*.—Approximately 2000 g/mL Ni. Dissolve 10.0 g Ni(NO₃)₂·6H₂O in H₂O and dilute to 1 L with H₂O.

(f) *Ammonium carbonate*.—Powder, purified (EM Science No. AX1260, or equivalent).

(g) *Methanol*.—Anhydrous, ACS (EM Science No. MX0485).

(h) *Diluting solution*.—Add 5 mL concentrated HNO₃ and 150 mL anhydrous methanol to 1 L volumetric flask, dilute to volume with H₂O, and mix.

(i) *Tantalum pentoxide*.—99.99% Ta₂O₅ (Aldrich Chemical Co.; No. 20,453-6, or equivalent).

(j) *Tantalum pentoxide solution*.—Suspend 2.0 g in 10 mL H₂O.

(k) *Roxarsone standard solution*.—1250 g/mL roxarsone (356 g/mL As). Accurately weigh 625.0 mg roxarsone reference standard (Alpharma, Inc.) into 500 mL volumetric flask. Dissolve and dilute to volume with 2% ammonium carbonate solution (w/v). (*Caution*: Wear protective clothing and avoid breathing dust.)

(l) *Dilute roxarsone standard solution*.—12.5 g/mL roxarsone (3.56 g/mL As). Dilute 10.0 mL roxarsone standard solution to 1 L with H₂O.

(m) *Control feed extract*.—Using typical nonmedicated poultry or swine ration, prepare feed extract as described under *Test Sample Preparation, D*. Test suitability of control feed extract by diluting 1 mL aliquot with diluting solution used in test sample preparation. Set up AAS system and furnace conditions as described in procedure. It is not necessary to perform calibration for this test; absorbance reading is satisfactory. Zero spectrophotometer on 20 L injection of diluting solution and measure A on 20 L control feed extract. Absorbance reading 0.010 indicates suitability.

(n) *Working standard solution*.—Transfer 1.0 mL dilute roxarsone standard solution to 10 mL volumetric flask. Dilute to volume with control feed extract. Transfer 1.0 mL aliquot of this solution to 25 mL Erlenmeyer and add 9.0 mL diluting solution. Twenty L working standard solution = 50 mg/kg roxarsone in feed for weights and volumes used in procedure.

C. Apparatus

(a) *Atomic absorption spectrophotometer*.—Perkin-Elmer Model 5000, or equivalent, with heated graphite atomizer furnace, autosampler, and printer sequencer.

(b) *Mechanical shaker*.—Wrist-action.

(c) *Pipets*.—Eppendorf: 10, 20, 50, and 1000 L.

(d) *Dispensing pipet*.—Repipet (Labindustries, 620 Hearst Ave, Berkeley, CA 94710, USA), or equivalent, 10 mL capacity set to deliver 9.0 mL diluting solution.

D. Extraction

Weigh 5.0 g ground test sample into 250 mL volumetric flask or 300 mL Erlenmeyer. Add 2.0 g ammonium carbonate powder and 200.0 mL H₂O, place on mechanical shaker, and shake vigorously 5 min at room temperature. Remove flask from shaker and let suspended feed particles settle 15–30 min. Transfer 1.0 mL aliquot of feed extract to 25 mL Erlenmeyer and add 9.0 mL diluting solution. Mix thoroughly. Repeat this step on reagent blank and on standard-fortified control feed extract equivalent to 50 mg/kg roxarsone in feed. Test extracts and standard are now ready for furnace AAS analysis.

E. AAS Conditions

Set up graphite furnace and spectrophotometer according to following conditions and allow 30 min warm-up time. Operating conditions: lamp, As EDL operated at 8 watts, properly aligned; lamp current, 0 ma; wavelength, 193.7 nm; slit, 0.7 nm bandpass, low position; readtime, 5 s; mode, AA-BG; readout, concentration; signal, peak height on instrument display and A on recorder if used; standard 1, S1 = 50.0 ppm (g/g) roxarsone (use 3 digits), do not use S2 and S3.

Install furnace assemble in AAS system and align as in manufacturer's instructions.

F. Furnace Tube Coating Procedure

Prepare 20% Ta₂O₅ aqueous suspension. Shake suspension vigorously, introduce 50 L aliquot into pyrolytically coated graphite furnace tube, and perform following sequence of operations: H₂O flow, 1–2 L/min to cool furnace; Ar pressure, 35 psi; on/off switch, on; gas control, on.

Step 1 (drying).—Temperature, 100°C; ramp time, 10 s; hold time, 90 s.

Step 2 (charring).—Temperature, 1000 C; ramp time, 10 s; hold time, 30 s.

Step 3 (atomizing).—Temperature, 2700 C; ramp time, 5 s; hold time, 10 s; stop flow, on.

Repeat coating procedure twice (3 applications). Tube is now ready for furnace AAS use.

(With initial coating, some material may flake after approximately 35 firings. If this happens, pass small brush or Kimwipe through tube to remove loose tantalum and apply single recoating.)

G. Furnace Conditions for Assay

Step 1 (drying).—Temperature, 100°C; ramp time, 10 s; hold time 50 s.

Step 2 (charring).—Temperature, 1000 C; ramp time, 10 s; hold time, 30 s.

Step 3 (atomizing).—Temperature, 2300 C; ramp time, 0 s; hold time, 5 s; read, on; stop flow, on.

Step 4 (burnout).—Temperature, 2400 C; ramp time, 0 s; hold time, 5 s; read, off; flow, 300 mL/min (stop flow, off).

H. Autosampler Conditions

Install autosampler assembly in furnace and align as per manufacturer's instructions. Operating conditions: power switch, on, and let autosampler go through count down; program sequence, press standby key to bring programmer into operating mode; method number, enter 1 and press method # key; recalibrate, for full tray recalibrate at 9A, 18B, and 27C; last test portion, program number for last test portion volume key; alternate volume, enter 10 L and press alternate volume key [Ni(NO₃)₂·6H₂O solution, 2000 ppm Ni]; instrument program, enter 1 and press instrument program key; HGA program, enter 1 and press HGA program key.

Use test portion tray 1 with method 1 in autosampler sequence. This method uses external standard technique for instrument calibration. In this procedure, only position S1 is used because calibration is based on single point standard with calibration standard equivalent to 50.0 mg/kg roxarsone in finished feeds.

I. Test Sample Analysis

Load test portion tray 1 as follows: Place blank of reagents [diluting solution, (h)] in AZ location of tray; place 50 g/g roxarsone feed standard in S1 location and at position 1 (check

sample), then load test extracts in sequence around tray, starting at position 2. Place 2000 g/mL Ni solution in reagent container and place in appropriate location for alternate test extract in autosampler. Cover test portion tray with cover provided to minimize evaporation; these solutions contain methanol.

Instrument and test extracts are now ready for calibration and test extract analysis. Press start/stop key to start program in sampling cycle. AZ and S1 calibration should be done in duplicate. Observe *A* for duplicate S1 values. These values should be within reasonable agreement (±5%) before program is allowed to proceed with test extract. Instrument is recalibrated by autosampler in setup instructions which will monitor calibration for any changes and update calibration as time progresses.

J. Calculations

Instrument is programmed to calculate test sample g/g on basis of single point standard equivalent to 50 g/g roxarsone. For weights or volumes other than those specified, manual calculation is required.

Reference: *JAOAC* **69**, 838(1986).

CAS-121-19-7 (roxarsone)