

5.1.30

AOAC Official Method 956.11 Nicarbazin in Feeds

Spectrophotometric Method First Action 1956

Final Action

(Presence of furazolidone, nitrofurazone, or nihydrazone may cause high results. Confirm presence of nicarbazin by *Identification Test, F.*)

A. Reagents

- (a) *Dimethylformamide (DMF)*.—Reagent grade.
- (b) *Alumina*.—See **961.24B(b)** (see 5.1.08).
- (c) *Alcohol*.—Formulas SDA Nos. 2B, 3A, or 30 may be used.
- (d) *Alcoholic sodium hydroxide solution*.—Dilute 2.0 mL clear 50% NaOH solution, **936.16B(b)** (see A.1.12), to 100 mL with alcohol. Centrifuge in stoppered tube. Prepare fresh daily.
- (e) *Nicarbazin standard solutions*.—(1) *Stock solution*.—Weigh 25.0 mg Nicarbazin Reference Standard (available from Elanco Animal Health, PO Box 708, Greenfield, IN 46140, USA; www.elancous.com; or Phibro Animal Health, 65 Challenger Rd, Third Floor, Ridgefield, NJ 07660, USA; www.philbroah.com) into 500 mL volumetric flask, and dissolve in ca 150 mL DMF with aid of gentle heat. Cool, dilute to volume with DMF, and mix well. Store protected from light. (2) *Working solution*.—12.5 g/mL. Transfer 25.0 mL stock solution to 100 mL volumetric flask and dilute to volume with DMF. Mix well.

B. Preparation of Column

Use glass tube 22 mm id, ca 50 cm long, constricted at lower end. Place plug of glass wool in constricted end and add 30 g alumina in 3 portions. Tamp each portion with glass rod while applying gentle suction. Wash column with 25 mL DMF, draining to point 1–2 cm above bed level before adding test extract to column. Prepare column for each test portion and standard.

Never let column run dry; keep head of liquid at all times.

C. Preparation of Test Portion

Weigh 10.0 g test portion into 250 mL Erlenmeyer and add 100.0 mL DMF. Heat just to boiling on hot plate in hood with

intermittent stirring. Cool to room temperature by immersing in water bath. Decant supernate into centrifuge tubes and centrifuge 3 min.

D. Determination

Pipet 25.0 mL clear extract onto column and let pass through column with aid of gentle suction. Wash column with three 10 mL portions DMF and reject washings. Elute with nine 5 mL portions alcohol, discarding first 15 mL eluate and collecting next 25 mL eluate in 25 × 200 mm tube. Quantitatively transfer eluate into 50 mL volumetric flask and dilute to volume with alcohol. Mix well.

Pipet 25.0 mL working standard solution onto another column and proceed as for test portion.

Pipet two 15.0 mL diluted eluate solutions into separate 25 mL volumetric flasks. To one add 5.0 mL alcoholic NaOH solution and adjust volume of both solutions to 25 mL with alcohol. Read *A* of yellow solution formed in first flask within 5 min in spectrophotometer or colorimeter at 430 nm against second solution as blank. Calculate weight nicarbazin from standard curve.

E. Preparation of Standard Curve

Pipet 10, 15, and 20 mL aliquots of chromatographed working standard solution into separate 25 mL volumetric flasks, add 5 mL alcoholic NaOH, and dilute to volume with alcohol. Mix well. Measure *A* within 5 min at 430 nm against alcohol.

Prepare standard curve by plotting *A* against mg nicarbazin.

F. Identification Test

Place alcohol in 1 cm quartz cell and clear chromatographed test solution in matched cell. Determine *A* at 2 nm intervals from 340 to 349 nm with Beckman Model DU spectrophotometer, or equivalent, at minimum slit width, or scan wavelength 340 to 350 nm. Absorption maximum at 344 ± 4 nm confirms presence of nicarbazin.

References: *JAOAC* **39**, 321(1956); **40**, 469(1957); **41**, 326(1958).

CAS-330-95-0 (nicarbazin)