

### 5.1.33

#### AOAC Official Method 961.25 Nihydrazone in Feeds

##### Colorimetric Method

First Action 1961

Final Action 1962

##### A. Reagents

(a) 95% Dimethylformamide (DMF).—Dilute 95 parts DMF (Eastman Kodak Co. No. 5870, or equivalent) with 5 parts H<sub>2</sub>O.

(b) Nihydrazone standard solutions.—(1) Stock solution.—Weigh 110 mg crystalline nihydrazone into 100 mL volumetric flask, dissolve in DMF, and dilute to volume with DMF. Protected from light, solution is stable several months. (2) Working solution.—For feeds containing 0.011% nihydrazone, pipet 1 mL aliquot stock solution into 100 mL volumetric flask, add 50 mL DMF, and dilute to volume with H<sub>2</sub>O.

##### B. Determination

Weigh 10 g test portion into 125 mL Erlenmeyer, add exactly 50 mL 95% DMF, stopper loosely, and place in boiling water bath 5 min (or until temperature of solvent reaches 90 C). Shake mechanically 10 min and filter through rapid paper. To 25 mL filtrate add 25 mL H<sub>2</sub>O and mix. Let stand, protected from light, 30 min. (Some solids may separate; standing for longer time is permissible.)

Prepare ca 20 mm diameter adsorption column containing adsorbent, 960.63A(e) (see 5.1.24), to height of 5 cm. (With highly colored feeds, use somewhat longer column.) Use plug of cotton or glass wool to support column, and similar plug or layer of washed sea sand on top. Pass test solution through column, collecting ca 15 mL eluate. Pipet 5 mL aliquots into each of 2 tubes. Protect one tube from light; to other add 3 drops freshly prepared 2% sodium hydrosulfite solution (w/v), mix, and let stand 5 min. Treat 5 mL aliquots diluted standard solution similarly.

Pipet 5 mL phenylhydrazine solution, 960.63A(a) (see 5.1.24), into all test tubes, mix, and heat 20 min in 40 C water bath. Cool by placing tubes in 15 C water bath 5 min. Add exactly 10 mL toluene to each tube, stopper, and shake vigorously 40 times. Separate and centrifuge toluene layer, and read *A* at 440 nm.

$$\text{Nihydrazone, \%} = \frac{(A_{\text{test portion}} - A_{\text{reduced test portion}}) \cdot 0.011}{A_{\text{standard}} - A_{\text{reduced standard}}}$$

Reference: *JAOAC* 44, 2(1961).

CAS-67-28-7 (nihydrazone)

Revised: March 2002