

## 5.1.50

### AOAC Official Method 969.57 Sulfamethazine in Feeds Spectrophotometric Method First Action 1969

(Applicable to feeds containing procaine penicillin. Not applicable to feeds made from granule-stabilized Tylan-Sulfa premixes.)

#### A. Reagents

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

(a) 50% Methanol solution.—50% (v/v) aqueous solution of methanol.

(b) Sulfamethazine standard solutions.—(1) Stock solution.—Accurately weigh 0.100 g USP Sulfamethazine Reference Standard into 100 mL volumetric flask. Add 50 mL 50% methanol solution and shake until dissolved. Dilute to volume with 50% methanol solution. Solution is stable at least several weeks. (2) Intermediate solution.—Pipet 5 mL stock solution into 200 mL volumetric flask, dilute to volume with 50% methanol, and mix well. Solution is stable several weeks. (3) Working solution.—2.5 g/mL. Pipet 10 mL intermediate solution into 100 mL volumetric flask, add 1 mL HCl and 50 mL 50% methanol, dilute to volume with H<sub>2</sub>O, and mix well. Solution is stable ca 2 weeks.

#### B. Preparation of Test Solution

Weigh 5.00 g ground test portion into 250 mL glass-stoppered Erlenmeyer. Add 100.0 mL 50% methanol solution, shake well on mechanical shaker 1 h, and centrifuge. Pipet aliquot supernate containing ca 250 g sulfamethazine into 100 mL volumetric flask, add 50% methanol, if necessary, to volume of ca 60 mL, followed by 1.0 mL HCl, and 10 mL 1% (w/v) ZnSO<sub>4</sub> solution. Let stand 10 min, dilute to volume with H<sub>2</sub>O, and mix.

#### C. Determination

Filter portion of prepared solution through Whatman No. 42 paper, or equivalent, into 250 mL flask. Filtrate should be clear. Pipet two 10 mL aliquots filtrate and 10 mL working standard solution into separate 50 mL centrifuge tubes. To each tube add 1.0 mL 0.1% NaNO<sub>2</sub> solution; mix and let stand 3 min. Add 1.0 mL 0.5% ammonium sulfamate solution; mix and let stand 2 min. Add 1.0 mL 0.1% *N*-(1-naphthyl)ethylenediamine 2HCl solution to one of test solutions and to standard solution. To second test solution add 1.0 mL H<sub>2</sub>O (test blank). Mix all solutions well and let stand 10 min.

To test, test blank, and standard solution add ca 10 mL CHCl<sub>3</sub>, stopper, and shake vigorously 30 s (30 s is required to ensure complete removal of procaine dye). Add 0.8 mL 10M NaOH to test solution, test blank, and standard solution. Stopper and shake vigorously 1 min to ensure complete removal of procaine dye. Centrifuge solutions at 2000 rpm 5 min or until aqueous layer is completely clear. Remove 10.0 mL aqueous phase with pipet and transfer to 50 mL Erlenmeyer or 50 mL beaker. Add 1.0 mL HCl and remove fumes formed in flask with aspirator or air stream.

Read *A* of test solution, test blank, and standard solution (*A*) at 540 nm in spectrophotometer, against H<sub>2</sub>O blank. Correct *A* of test solution by subtracting that of test blank.

$$\text{mg/kg Sulfamethazine} = \left( \frac{A}{A} \right) \left( \frac{2.5 \text{ g/mL}}{100 \text{ mL/mL extract aliquot taken}} \right) \left( \frac{100 \text{ mL/5 g}}{100 \text{ mL/5 g}} \right)$$

References: *JAOAC* **51**, 1282(1968); **72**, 106(1989).

CAS-57-68-1 (sulfamethazine)