19.7.05

AOAC Official Method 974.41 Polythiazide in Drugs Spectrophotometric Method First Action 1974 Final Action 1976

(Applicable to formulations containing vanillin.)

A. Principle

Vanillin, which interferes in method, is condensed through aldehyde group with primary amine group of sulfanilic acid to form strongly polar and H₂O-soluble Schiff's base, which is retained in aqueous immobile phase of column. Less polar polythiazide is eluted with mobile phase, ether-isooctane, and determined by UV spectrophotometry.

B. Apparatus and Reagent

(Use H₂O-washed solvents throughout.)

(a) Chromatographic tube and tamping rod.—See **967.31A** (see 19.1.02).

(b) Diatomaceous earth.—See 960.53B (see 18.1.01).

(c) Dilute animonium hydroxide.—1M. Dilute 17 mL NH_4OH to 250 mL with H_2O .

(d) Ammonium sulfanilate solution.—6%. Dissolve 6.0 g sulfanilic acid in $1M NH_4 OH$ and dilute to 100 mL with $1M NH_4 OH$.

(e) Polythiazide standard solution.—10 μ g/mL. Accurately weigh ca 100 mgUSP polythiazide Reference Standard, transfer to 100 mL volumetric flask, and dilute to volume with methanol. Fur-

ther dilute 10 mL of this solution to 100 mL with methanol and 10 mL diluted solution to 100 mL with methanol.

C. Preparation of Test Portion

Accurately weigh test portion containing ca 1 mg polythiazide and transfer to 150 mL beaker. Add 0.25 mL dimethylsulfoxide (DMSO) and mix thoroughly to wet entire test sample. Let stand 3–4 min.

D. Preparation of Column

(a) *Lower layer.*—Mix 6 g diatomaceous earth, (b), and 5 mL ammonium sulfanilate solution in 150 mL beaker, transfer to tube, and tamp to uniform mass.

(b) Upper layer.—Add 4 mL ammonium sulfanilate solution to test sample solution, and mix. Add 4 g diatomaceous earth, mix, transfer to tube, and tamp to uniform mass. Dry-wash beaker with 1 g diatomaceous earth and few drops H_2O , transfer to tube, and tamp. Top with glass wool pad.

E. Determination

Pass 100 mL isooctane through column; discard eluate. Elute polythiazide with 100 mL isooctane-ether (1 + 1), receiving eluate in 250 mL beaker. Immediately evaporate eluate to dryness. Dissolve residue in small amount methanol and transfer quantitatively to 100 mL volumetric flask. Dilute to volume with methanol. Filter through glass wool, discarding first 20 mL. Determine *A* of test and standard solutions against methanol in 1 cm cell at maximum, ca 268 nm.

Reference: JAOAC 57, 716(1974).

CAS-346-18-9 (polythiazide)