CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Sulfamethazine in Serum

Purpose
Determination of sulfamethazine in serum.

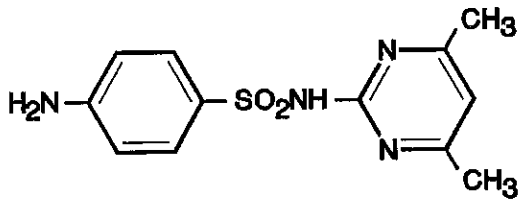

Figure 1. Structure of sulfamethazine.

Sulfamethazine (F1, 4-amino-N-(4,6,-dimethyl-2-pyrimidinyl)benzenesulfonamide) is a sulfonamide antibiotic used as an antibacterial agent in humans. Therapeutic levels in blood range from 50-150 µg/mL. The drug is rapidly converted to an inactive form by metabolic acetylation, but the rate of acetylation is a genetically controlled trait that varies among individuals. Measurement of sulfamethazine levels in serum can determine the rate of acetylation and is therefore an adjunct to dosage determinations. Sulfamethazine also prevents disease and promotes growth of farm animals, and it is widely used as a supplement to livestock feed. Screening tests for drug residues in meat and dairy products are thus needed.

Existing Methods
Bratton-Marshall colorimetric assay, TLC and LCUV. These may be time-consuming, non-specific and insensitive.

Conditions
EC Detector: BAS LC-44 Amperometric Detector
Electrode: BAS Glassy Carbon
Potential: 1.1 V vs Ag/AgCl
UV Detector: BAS UV-108 variable wavelength (266 nm)
Column: 3 µm, C 18 reverse-phase, 100 x 3.2 mm (PN MF-6213)
Mobile Phase: 90% (v:v) 0.01 M ammonium acetate pH 4.0, 10% acetonitrile. Flow rate was 1 mL/min.
Detection Limit: EC: 25 pg injected standard, 25 ng/mL serum (25 µL serum sample). UV: 100 pg injected standard, 100 ng/mL serum. (All at S/N = 3.)
Linear Range: 0.5-100 ng injected standards, 50-150 µg/mL serum.

Sample Preparation
1. Prepare Bond-Elut® C_{18} solid-phase extraction columns by washing with 1 mL methanol followed by 1 mL water.

2. Load the following onto each column: 25 µL serum, standards as appropriate, and 0.05 M NaH_2PO_4 (pH 4.0) for a final volume of 1 mL.

3. Wash the column with an additional 1 mL of phosphate buffer followed by two 1-mL washes of water.

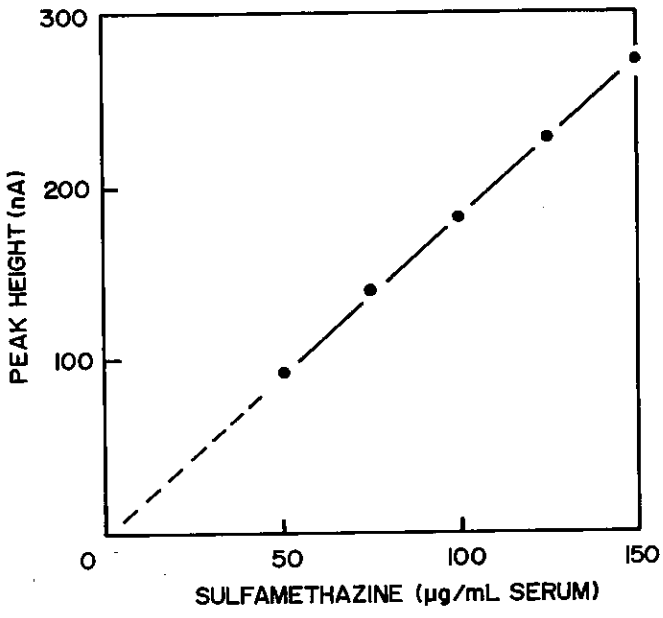

Figure 2. Calibration curve for spiked serum samples.

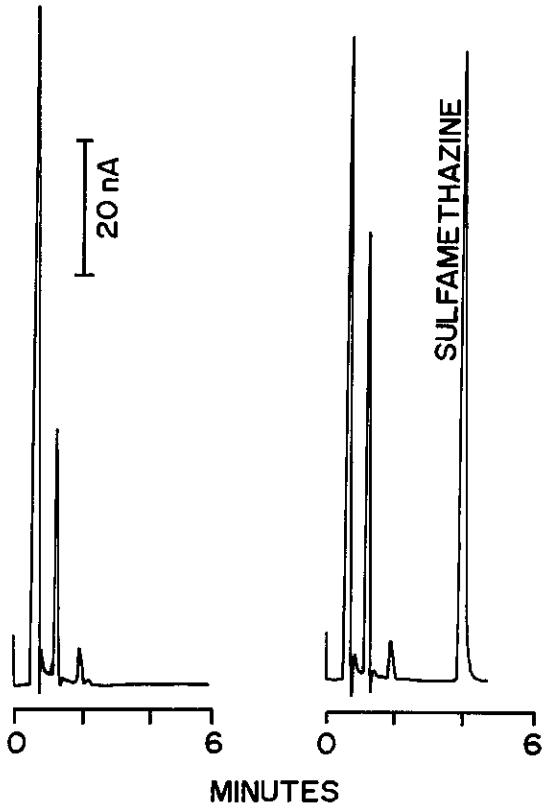

Figure 3. Sample chromatograms of blank (left) and spiked (50 µg sulfamethazine/mL) serum samples.

Figure 4. Chromatogram of 50 pg standard sulfamethazine.

4. Elute the samples with 1 mL methanol. Dry each in a stream of nitrogen or in a vacuum evaporator. Redissolve in 0.500 mL mobile phase and inject in 20 µL aliquots.

Notes

An EC calibration curve for spiked serum samples is presented in F2, and sample chromatograms are shown in F3 and F4.

Recovery of sulfamethazine from spiked serum samples was 93%.

Sulfadiazine has been used as an internal standard in the determination of sulfamethazine [1].

Detection limits were about 5 times lower for EC than for UV detection. Either type of detection was sufficiently sensitive for routine therapeutic monitoring, however.

The determination of sulfamethazine also can be performed on the BAS 200 Problem Solver.

References

1. Whelpton, R., G. Watkins and S.H. Curry, Clin. Chem. 27 (1981) 1911-1914.

2. Aerts, M.M.L. and W.M.J. Beek, J. Chromatogr. 435 (1988) 97-112.