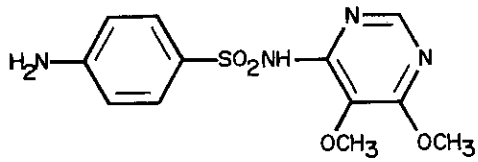
CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Determination of Sulfadoxine and Pyrimethamine

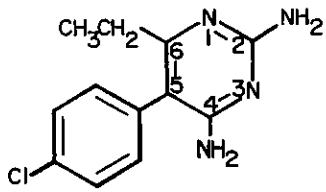
Purpose

Determination of sulfadoxine and pyrimethamine in serum.



SULFADOXINE

Figure 1. Structure of sulfadoxine.



PYRIMETHAMINE

Figure 2. Structure of pyrimethamine.

Sulfadoxine (F1, 4-amino-N-(5,6-dimethoxy-4- pyrimidinyl)benzenesulfonamide) and pyrimethamine (F2, 5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine) are antimicrobial compounds used in the treatment of chloroquine-resistant cases of malaria. The two drugs act synergistically to block enzymes responsible for pyrimidine synthesis in *Plasmodium falciparum*, the protozoan that causes the most severe form of malaria [1]. Fansidar® is a trademarked antimalarial that contains 500 mg sulfadoxine and 25 mg pyrimethamine. Therapeutic concentrations in blood may range from 0.5 - 90 µg/mL for sulfadoxine and 0.05 - 5 µg/mL for pyrimethamine [1].

Existing Methods

Spectrophotometry, which is non-specific; TLC and GC, which are time consuming; microbiological assay, which is both time consuming and non-specific, and LC. In contrast to most LC methods, the method reported below [1] extracts and detects both compounds simultaneously.

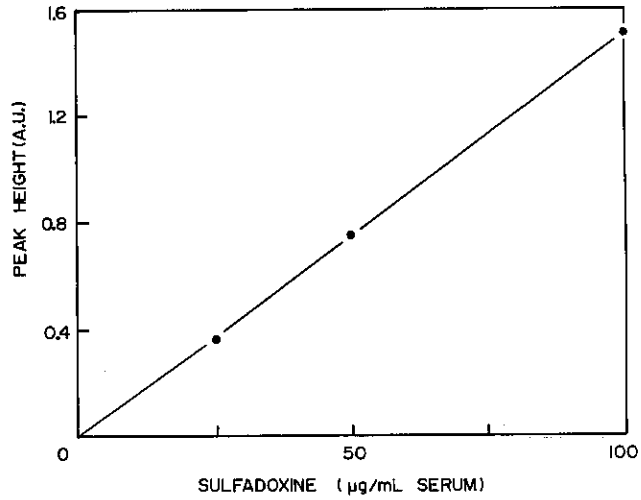


Figure 3. Calibration curve for sulfadoxine in serum.

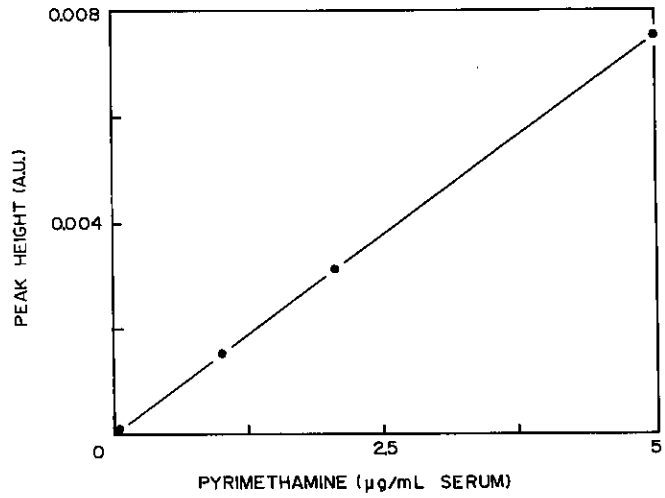


Figure 4. Calibration curve for pyrimethamine in serum.

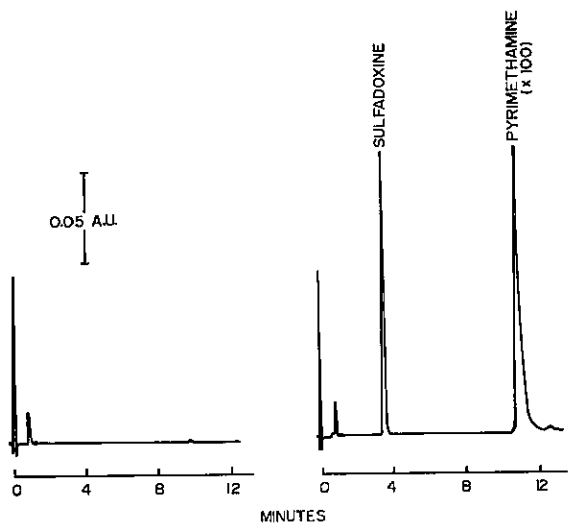


Figure 5. Sample chromatogram of unspiked serum (left) and serum spiked with 25 µg sulfadoxine and 0.5 µg pyrimethamine per mL.

Conditions

System: BAS 400 Liquid Chromatograph

Detector: BAS UV-108 variable wavelength (275 nm)

Column: BAS 3 µm Phase II Octyl reverse-phase (100 x 3.2 mm) (PN MF-6214)

Mobile Phase: 82.5% (v:v) 0.01 M KH_2PO_4, adjusted to pH 2.3 with phosphoric acid; 17.5% acetonitrile. Flow rate was 0.9 mL/min.

Detection Limit: sulfadoxine: 30 pg injected standard, 5 ng /mL serum; pyrimethamine: 300 pg injected standard, 10 ng/mL serum. (All at S/N = 3.)

Linear Range: sulfadoxine: 0.5 - 100 ng injected standards, 0.5 - 100 µg/mL serum; pyrimethamine: 0.5 - 100 ng injected standards, 0.05 - 5 µg/mL serum.

Sample Preparation

1. Prepare an extraction buffer: 0.1 M KH_2PO_4 adjusted to pH 2.3 with phosphoric acid.

2. Combine in a 10 mL screw-capped tube: 0.5 mL serum, 0.1 mL extraction buffer, appropriate amounts of standards, and distilled water to make a final volume of 0.850 mL.

3. Add 5 mL dichloroethane to each tube and agitate vigorously for 1 minute.

4. Centrifuge for 5 minutes at 1000 x g.

5. Remove the upper aqueous layer and discard. Dry the lower layer in a vacuum evaporator or under a stream of nitrogen.

6. Redissolve the samples in 0.250 mL mobile phase. Filter through 0.45 µm membranes (PN MF-5655) by centrifuging at 1600 x g in MF-1 microfiltration tubes (PN MF-5500). Inject 20 µL onto the chromatograph.

Notes

Pyrimethamine concentrations in blood are expected to be 1/10 those of sulfadoxine [1]. Moreover, pyrimethamine elutes later than sulfadoxine so its peak height will be further reduced. (Peak area, of course, is unaffected by elution time.) Detector gain was increased after the sulfadoxine peak to allow pyrimethamine detection at a more sensitive range. Alternatively, a dual-pen recorder can be connected with one pen set at an appropriate range to record the sulfadoxine peak and the other set at a range appropriate for pyrimethamine.

Recovery was 80% for sulfadoxine and 86% for pyrimethamine.

Quinine has been used as an internal standard for the determination of sulfadoxine and pyrimethamine [1].

The determination of sulfadoxine and pyrimethamine also can be performed on the BAS 200 Problem Solver. The BAS 200 is programmable, so gain changes can be automatically incorporated into each chromatographic run.

Reference

1. Edstein, M., J. Chromatogr. 305 (1984): 502-507.