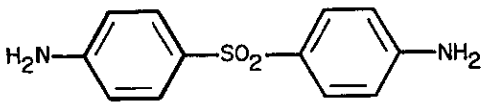
CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Determination of Dapsone and Pyrimethamine

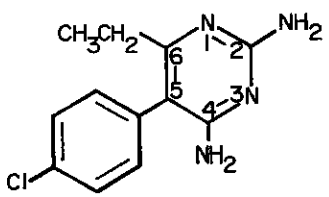
Purpose

Determination of dapsone and pyrimethamine in serum.



DAPSONE

Figure 1. Structure of dapsone.



PYRIMETHAMINE

Figure 2. Structure of pyrimethamine.

Dapsone (F1, 4,4'-sulfonylbisbenzamine) 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 folate metabolism in *Plasmodium falciparum*, the protozoan that causes the most severe form of malaria [1]. In addition, dapsone alone is used for treatment of leprosy [2]. Maloprim® is a trademarked prophylactic antimalarial that contains 100 mg dapsone and 12.5 mg pyrimethamine.

Existing Methods

TLC, which is time consuming and insensitive, and LC. The procedure below is rapid, sensitive, and

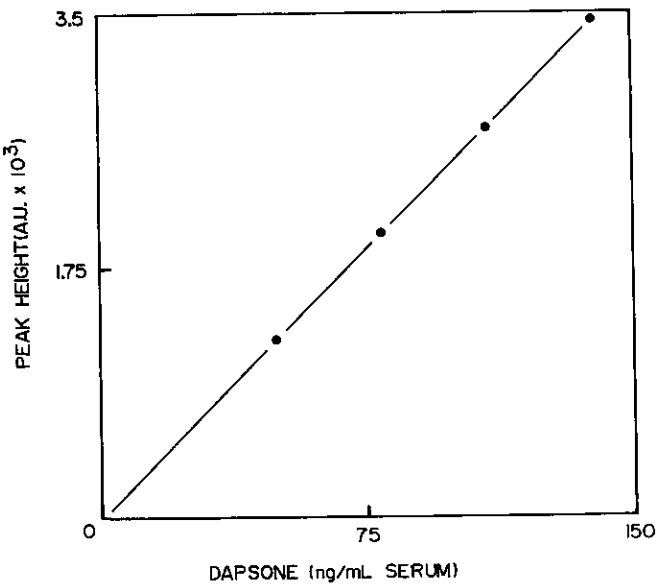


Figure 3. *Calibration curve for dapsone in serum.*

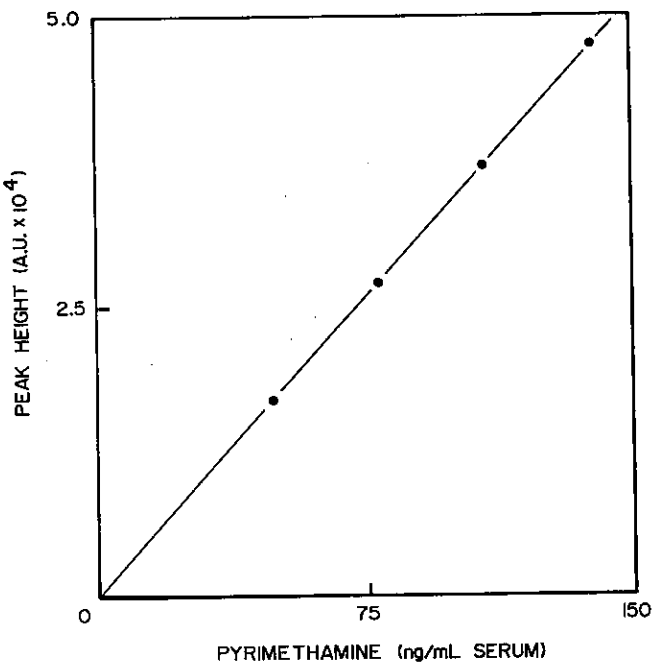


Figure 4. Calibration curve for pyrimethamine in serum.

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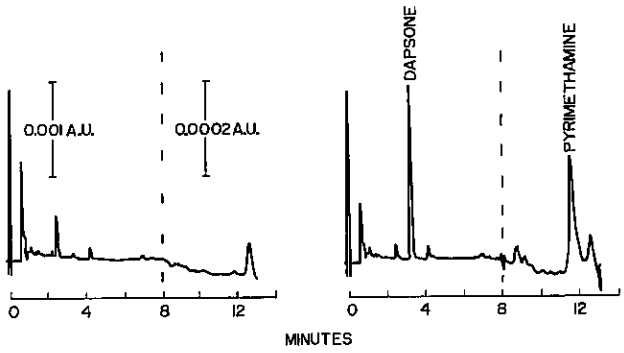

Figure 5. Sample chromatogram of unspiked serum (left) and serum spiked with 80 ng dapsone and 80 ng pyrimethamine per mL.

selective, and quantitates both compounds simultaneously.

Conditions

System: BAS 400 Liquid Chromatograph
Detector: BAS UV-116 dual channel, variable wavelength
Wavelengths: 295 nm (dapsone) and 230 nm (pyrimethamine)
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: dapsone: 25 pg injected standard, 2 ng/mL serum; pyrimethamine: 200 pg injected standard, 5 ng/mL serum. (All at S/N = 3.)
Linear Range: dapsone: 0.1 - 100 ng injected standards, 50 - 140 ng/mL serum; pyrimethamine: 0.2 - 100 ng injected standards, 50 - 140 ng/mL serum.

Sample Preparation

1. Combine in a 10 mL screw-capped tube: 0.5 mL serum, 0.150 mL 2 M NaOH, appropriate amounts of standards, and distilled water to make a final volume of 0.850 mL.

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

3. Centrifuge for 5 minutes at 1000 x g.

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

5. 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

The detector was operated at wavelengths for which the two compounds had high absorbances (295 nm for dapsone and 230 nm for pyrimethamine). Alternatively, both compounds can be detected at 254 nm (but with reduced sensitivity).

Recovery was 82% for dapsone and 86% for pyrimethamine.

Quinine [1] and *m*-aminophenyl sulphone [2] have been used as internal standards for the determination of dapsone.

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

References

1. Edstein, M., J. Chromatogr. 307 (1984): 426-431.

2. Horai, Y. and T. Ishizaki, J. Chromatogr. 345 (1985): 447-452.