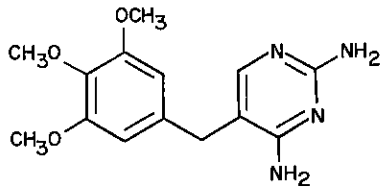
CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Trimethoprim in Serum

Purpose

Determination of trimethoprim in serum.



TRIMETHOPRIM

Figure 1. Structure of trimethoprim.

Trimethoprim [F1, 2,4,-diamino-5-(3,4,5- trimethoxybenzyl)pyrimidine] is an antibacterial agent used for the treatment of various infections, but particularly those of the urinary system and respiratory tract. Trimethoprim and sulfamethoxazole are frequently given in combination because of their complementary effect on one enzymatic pathway: the former inhibits dihydrofolate reductase while the latter inhibits the formation of folic acid. Therapeutic concentrations of trimethoprim in blood range from 2-30 µg/mL, while those for sulfamethoxazole may be 20 times greater.

Existing Methods

Microbiological assay, which lacks selectivity, and LC with fluorometric or UV detection. The procedure outlined below uses solid-phase extraction to provide a relatively clean sample with minimum preparation [1].

Conditions

System: BAS 400 Liquid Chromatograph
Detector: BAS UV-108 variable wavelength (230 nm)
Column: BAS 3 µm Phase II ODS reverse-phase (100 x 3.2 mm) (PN MF-6213)
Mobile Phase: 85% (v:v) 0.05 M sodium acetate, 15% acetonitrile. Flow rate was 1 mL/min.

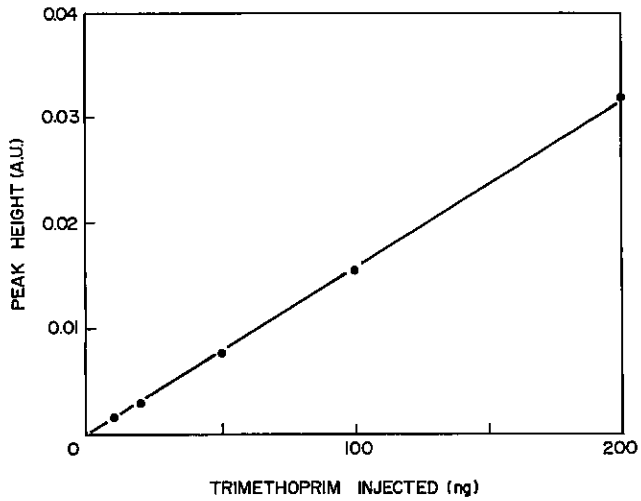


Figure 2. Calibration curve for trimethoprim standards. Each point represents the mean of two determinations.

Detection Limit: 150 pg injected standard (S/N = 3), 15 ng/mL serum (S/N = 3).
Linear Range: 0.5-200 ng injected standards, 2-50 µg/mL serum.

Sample Preparation

1. Prepare an extraction buffer (0.1 M sodium carbonate).

2. Combine the following in a plastic microcentrifuge tube: 0.2 mL extraction buffer, 0.2 mL serum, standards, and distilled water to make a total volume of 0.9 mL.

3. Vortex the tubes for 30 seconds.

4. Prepare Bond-Elut® C_{18} extraction columns by washing twice with 1 mL methanol and twice with 1 mL distilled water.

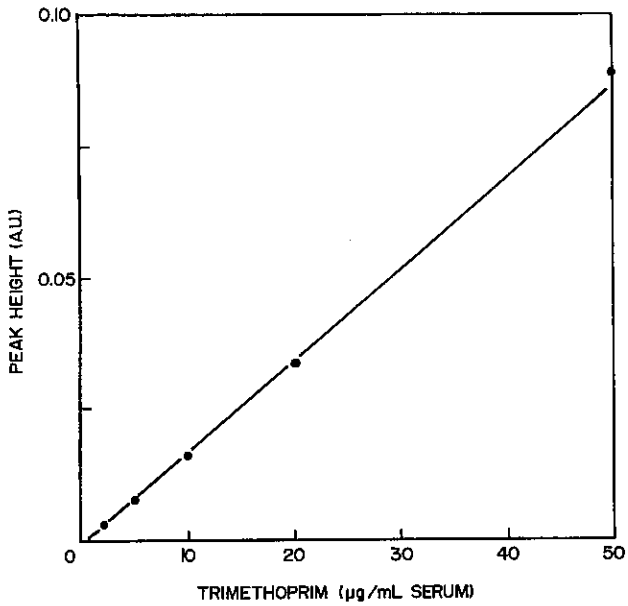


Figure 3. Calibration curve for spiked serum samples. Each point represents the mean of two determinations.

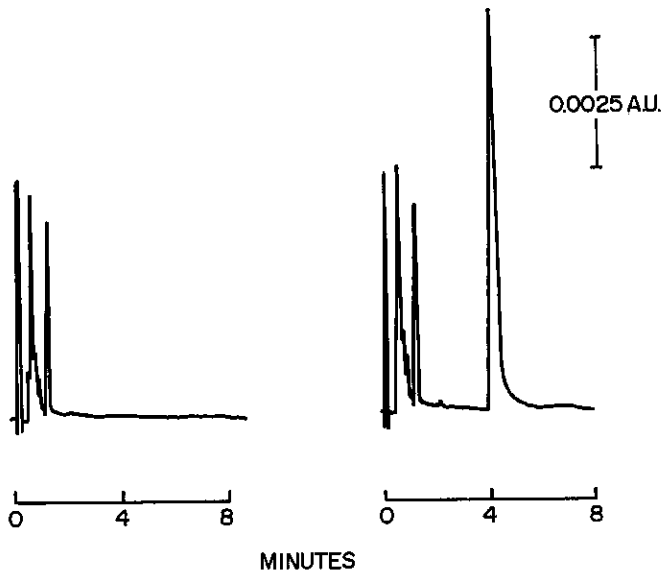


Figure 4. Sample chromatograms for unspiked (left) and spiked (5 µg/mL) serum.

5. Load the samples onto the columns and apply vacuum. Wash with an additional 1 mL distilled water. Add 0.1 mL methanol and allow 30 seconds for it to soak onto the column. Then apply vacuum and collect the eluent. Repeat the methanol extraction step and combine the eluents.

6. Dry the eluents, redissolve in 0.350 mL mobile phase, and inject. Injection volume was 20 µL.

Notes

A calibration curve for trimethoprim standards is presented in F2. A calibration curve for spiked serum samples, and a sample chromatogram, are presented in F3 and F4.

Recovery of trimethoprim from spiked serum was 99%.

2,4-Diamino-5-(3,5-dimethoxy-4-methylbenzyl)-pyrimidine [1] and timolol maleate [2] have been used as internal standards.

Most commonly-used drugs do not interfere with this determination of trimethoprim. A list of drugs that do and do not interfere is provided in [1].

The determination of trimethoprim presented above can be duplicated using the BAS 200 Problem Solver.

References

1. J.E. Svirbely and A.J. Pesce, Therap. Drug Monitoring 9 (1987): 216-220.

2. C.T. Hung and D.G. Perrier, J. Liq. Chrom. 8 (1985): 521-536.