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Note

Analysis of trimethoprim and sulphamethoxazole in human plasma by high-pressure liquid chromatography

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Co-trimoxazole, a broad-spectrum antimicrobial combination of trimethoprim and the sulphonamide sulphamethoxazole, is used in the treatment of many common infections such as urinary and respiratory tract infections [1].

Several analytical procedures for the two drugs in plasma and other biological fluids have been reported. For sulphamethoxazole, spectrophotometric methods [2, 3] are available and are adequate in many instances. For trimethoprim, a microbiological assay [4], spectrofluorometric [5—7] and differential pulse polarographic [8] procedures have been used but most of these are laborious and require considerable attention to detail in order to obtain enough sensitivity for measurements in plasma. A sensitive and specific assay of trimethoprim in plasma or urine has recently been achieved using gas chromatography [9].

The use of high-pressure liquid chromatography (HPLC) on C-8 alkyl reversed-phase columns and variable-wavelength ultraviolet detection for analyses of trimethoprim and sulphamethoxazole in pharmaceuticals has been reported [10] and, more recently, has been applied to measurements in body fluids [11]. Using simpler HPLC apparatus and an octadecylsilane reversed-phase column in the present study, plasma concentrations of trimethoprim and sulphamethoxazole are determined separately. Sample preparation is identical for both drugs and merely involves precipitation of plasma protein and injection of supernatant directly into the chromatograph.

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MATERIALS AND METHODS

Plasma samples (1 ml) were pipetted into tubes containing 50 μl of 4 *M* trichloroacetic acid. After mixing for 20 sec on a vortex mixer, these were centrifuged for 15 min at 0° to separate the precipitated protein. Aliquots of 100 μl supernatant were injected into the chromatograph.

Analyses for both trimethoprim and sulphamethoxazole were performed on a reversed-phase column (Spherisorb, 10 μm ODS; 250 × 4.6 mm; Phase Separations, Queensferry, Great Britain) at ambient temperature. A single-piston high-pressure pump (Altex Model 110), which delivered solvent at constant flow-rates, a sample injection valve containing a 100-μl loop (Chromatronix) and a fixed-wavelength ultraviolet detector with a 20-μl flow cell (Altex Model 150) formed the basis of the chromatograph.

For trimethoprim, the eluting solvent was acetonitrile—aqueous 0.1 *M* KH_2PO_4 (pH 2.5) (30:70) containing 1% acetic acid and 1% ethyl acetate. The flow-rate was 1 ml/min. The mobile phase for sulphamethoxazole analysis was methanol—water—acetic acid (40:60:1) (pH 3.2) at a flow-rate of 2 ml/min.

Absorbance of the effluent from the column at 254 nm was monitored at a sensitivity of 0.02 a.u.f.s. for trimethoprim and at 0.32 a.u.f.s. for sulphamethoxazole. Peak heights were used for quantitation of both assays. Quality control was achieved by external standardization .

To prevent contamination of the reversed-phase column by plasma constituents remaining after the protein removal step, a pre-column (50 mm × 4.6 mm) containing 10 μm reversed-phase packing was incorporated into the system. Significant increases in perfusion pressure necessitating a change of pre-column (at 3000 p.s.i.) did not occur until several hundred samples had been assayed.

All chemicals used were analytical grade and water was double distilled. Sulphamethoxazole was B.P. grade. Trimethoprim lactate was donated by Wellcome, Australasia. All concentrations of drugs are in reference to the base.

Calibration curves were derived from pooled blood bank plasma.

RESULTS

Trimethoprim

Typical chromatograms of plasma samples (Fig. 1) show that control samples are free from contaminating peaks. Trimethoprim was eluted in 6 min. Sulphamethoxazole was eluted immediately after trimethoprim. However, under conditions optimized for measurement of trimethoprim, sulphamethoxazole was incompletely resolved from a major metabolite (vide infra). Calibration curves for trimethoprim passed through the origin and were linear to the maximum concentration used (10 μg/ml). The detection limit was 0.1—0.2 μg/ml. Recovery of trimethoprim added to plasma was 95%. The coefficient of variation for the analysis was determined to be 3.2% (n = 10) at a concentration of 2 μg/ml.

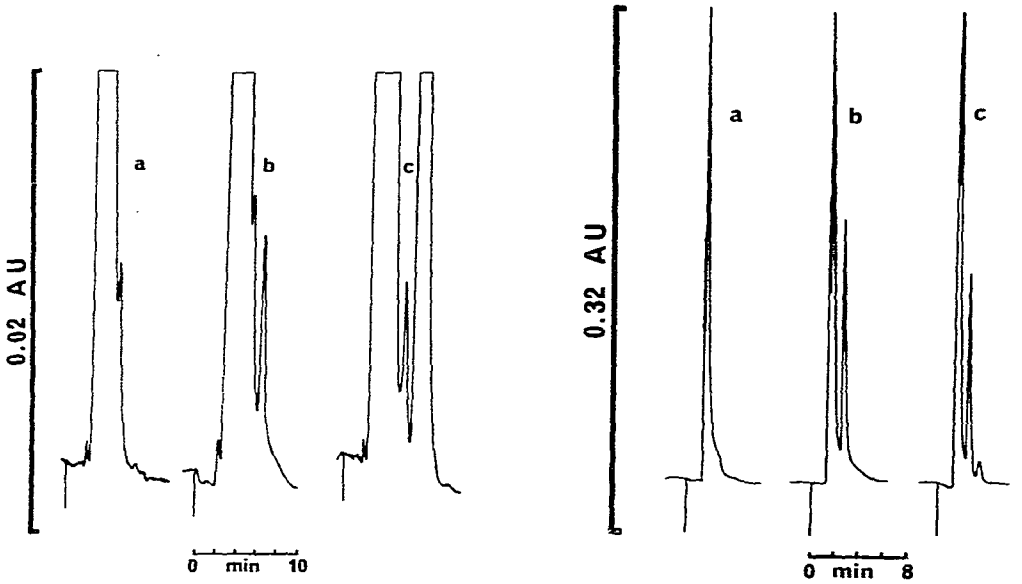

Fig. 1. Trimethoprim assay. Chromatograms of deproteinized plasma. (a) Blank plasma, (b) plasma containing 3.85 μg/ml trimethoprim, and (c) plasma from a patient receiving co-trimoxazole; the major component of the large peak following trimethoprim is sulphamethoxazole.

Fig. 2. Sulphamethoxazole assay. Chromatograms of deproteinised plasma: (a) blank plasma, (b) plasma containing 60 μg/ml sulphamethoxazole, and (c) plasma from a patient receiving co-trimoxazole; the small peak eluting after sulphamethoxazole is presumably due to the acetylated metabolite.

Sulphamethoxazole

Typical chromatograms of plasma samples (Fig. 2) show that control samples are free from interfering peaks. Sulphamethoxazole was eluted in 3 min and a peak, presumed to be its acetylated metabolite, appeared at 4 min (Fig. 2). The latter peak disappeared following hydrolysis by autoclaving acidified deproteinised plasma for 30 min, and there was a corresponding elevation of the sulphamethoxazole peak. Procurement of pure acetylated sulphamethoxazole standard should enable its quantitation in plasma. Trimethoprim could not be detected due to prolonged retention on the column. The calibration curves for sulphamethoxazole passed through the origin and were linear up to 200 μg/ml, the maximum concentration used. A sulphamethoxazole concentration of less than 1 μg/ml in plasma was easily detectable. Recovery of sulphamethoxazole added to plasma was 81%. At a concentration of 50 μg/ml, the coefficient of variation was 1.8% (n = 10).

To demonstrate the effectiveness of the assays in a clinical situation, blood samples from ten hospitalised patients dosed orally with co-trimoxazole (two tablets*, twice daily) were taken at least three days after starting treatment. In each patient, blood was taken 2 h before the evening dose on the day of sampling. Plasma concentrations of trimethoprim ranged from 0.39 to 3.16 μg/ml (mean 1.83 μg/ml). Sulphamethoxazole concentrations ranged from 28.2 to 128.1 μg/ml (mean 66.6 μg/ml).

*Each tablet contained 400 mg sulphamethoxazole and 80 mg trimethoprim.

No interference with the measurement of trimethoprim or sulphamethoxazole was observed despite concurrent medication with a wide range of drugs. These included amiloride, ampicillin, aspirin, chloral hydrate, chlorothiazide, chlorpromazine, cloxicillin, codeine, cortisone, dextropropoxyphene, digoxin, dioctyl sodium sulphosuccinate, frusemide, glyceryl trinitrate, hyoscine-N-butylbromide, indomethacin, mebendazole, metoclopramide, morphine, neomycin, nitrazepam, nystatin, oxycodone pectinate, paracetamol, pethidine, phenytoin, prednisolone, pindolol, quinine, salbutamol, sodium valproate and triamterene.

DISCUSSION

Assay of sulphonamide levels has been available for decades but it is only recently that such data have made a contribution to clinical deliberations. Most assays of trimethoprim have been rather laborious and unsuitable for routine use. The HPLC method described in this paper permits specific determination of the level of both drugs to be accomplished rapidly and therefore to influence decisions on dosing. Its value for routine use is enhanced by lack of interference from a large number of other drugs and their circulating metabolites. Furthermore, only relatively inexpensive HPLC equipment is required.

Co-trimoxazole is now one of the most commonly used antimicrobial agents and is given by both oral and parenteral routes. It is increasingly being employed in severely ill patients and monitoring of plasma levels is likely to make a substantial contribution to ensuring that maximum benefit is derived from this valuable treatment. This is emphasised by the eight fold variation in trimethoprim levels found among ten patients in this study.

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