Journal of Pharmaceutical & Biomedical Analysis
Vol. 9, No. 6, pp. 507–510, 1991
Printed in Great Britain

0731–7085/91 $3.00 + 0.00

Short Communication

Rapid simultaneous determination of trimethoprim, sulphamethoxazole and acetylsulphamethoxazole in human plasma and urine by high-performance liquid chromatography

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Keywords: *Trimethoprim; sulphamethoxazole; acetylsulphamethoxazole; high-performance liquid chromatography; human plasma and urine.*

Introduction

Co-trimoxazole, a mixture of trimethoprim (TMP) and sulphamethoxazole (SMX) in an 1:5 ratio, is a widely used antimicrobial agent, particularly in urinary and lower respiratory tract infections. A major metabolite of SMX, like other sulphonamides, is N^4-acetylsulphamethoxazole (Ac-SMX) and a rapid simultaneous determination of TMP, SMX and Ac-SMX should be a valuable tool in clinical drug monitoring and pharmacokinetic studies. Several HPLC methods have been reported for the determination of both TMP and SMX in pharmaceutical preparations [1–3], and in plasma or other biological fluids [4–11], and for the determination of SMX and Ac-SMX [8–13]. The majority of these methods not only require tedious sample preparation and extraction techniques [7–9] but also do not allow the simultaneous measurement of both drugs. They either require two chromatographic columns [4] or determine the two drugs separately by employing two different eluting solvents [5, 6] and detection by dual-wavelength monitoring [10, 11]. In the present communication, a simple and rapid method is proposed, for the simultaneous analysis of TMP, SMX and Ac-SMX, in human plasma and urine. It involves minimal sample preparation and single wavelength monitoring. The detection limit of the method is 0.1 μg ml^{-1} for both TMP and SMX, and 1.0 μg ml^{-1} for Ac-SMX, and covers the entire concentration range normally encountered therapeutically.

Experimental

Materials

TMP and SMX were obtained from Geopharma (Milan, Italy) and Schweizerhall (Basel, Switzerland), respectively. Phenacetin obtained from Merck (Darmstadt, FRG) was used as internal standard (methanolic solution, 250 μg ml^{-1}). N^4-acetylsulphamethoxazole was prepared by acetylation of SMX as described by Sharma *et al.* [14]. Solvents for HPLC were purchased from Carlo Erba (Milan, Italy). All the reagents were of analytical grade and were used without further purification.

Instrumentation and chromatographic conditions

The HPLC system consisted of a Model G-802 liquid chromatograph (Gilson, Villiers-le Bel, France) equipped with a variable wavelength detector (set at 230 nm) and a Gilson NI chart recorder.

The separations were performed at room temperature on a stainless-steel column (250 × 4.5 mm i.d.) packed with 5-μm Spherisorb

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ODS (Perkin–Elmer, Norwalk, CT, USA). Analytical samples were introduced onto the column using a 100-μl loop valve (Rheodyne, Cotari, CA, USA). The mobile phase consisted of acetonitrile–0.1 M sodium acetate (30:70, v/v) adjusted to pH 6.8 by the dropwise addition of glacial acetic acid and was pumped through the column at 1 ml min^{-1} (inlet pressure, *ca* 1800 p.s.i. or 12.4 MPa). Absorbance of the effluent from the column, at 230 nm, was monitored at a sensitivity of 0.05 a.u.f.s.

Sample preparation

Heparinized plasma samples (1 ml), internal standard solution (100 μl) and 1 M trichloroacetic acid (100 μl) were vortex-mixed for 20 s. The samples were centrifuged for 10 min at 2105*g* (3000 rpm) and aliquots of 100 μl supernatant were injected into the chromatograph.

Urine samples (1 ml) were analysed after the addition of 4 M hydrochloric acid (100 μl). The acid treated samples were vortex-mixed, and then internal standard solution (100 μl) and methanol (1 ml) were added. After mixing, 100 μl of the resulting solution were injected into the column: generally no precipitate was formed.

Quantification

The peak height ratio method was used to calculate the concentrations of TMP, SMX and Ac-SMX, by reference to the internal standard, from a series of previously prepared calibration graphs. The calibration graphs were constructed by treating, as described above, drug-free plasma and urine samples spiked with 100-μl aliquots of standard methanolic solutions of TMP, SMX and Ac-SMX in order to achieve a concentration range of 0.1–100.0 μg ml^{-1} for plasma and 0.1–300.0 μg ml^{-1} for urine.

Results and Discussion

Typical chromatograms of drug-free plasma and plasma from a volunteer following the oral ingestion of 160 mg TMP and 800 mg SMX are shown in Fig. 1(A). Corresponding chromatograms from the analysis of urine samples are shown in Fig. 1(B).

The retention times were 4.04, 4.46, 6.04

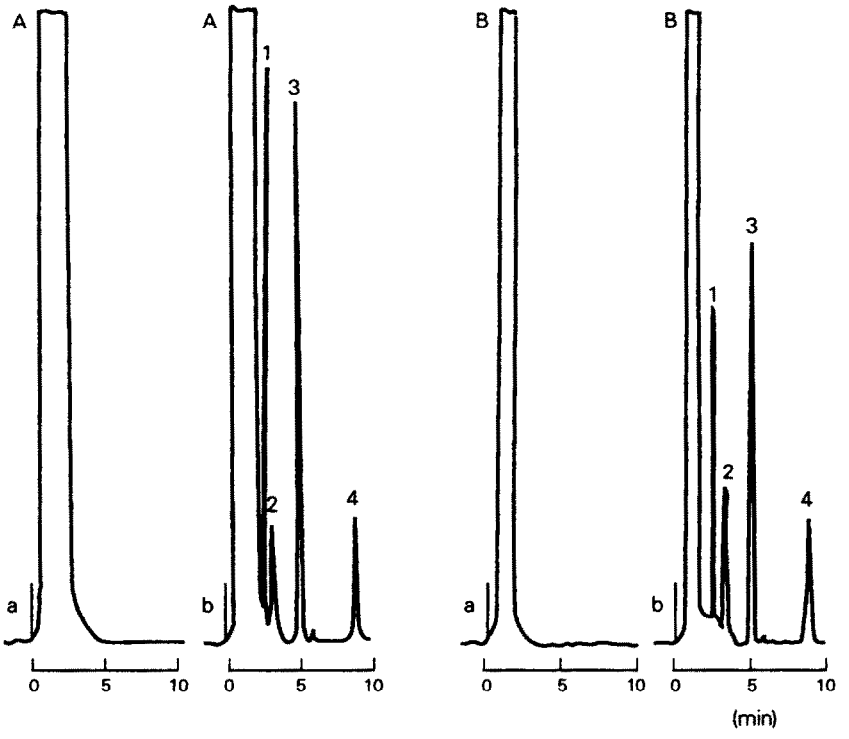

Figure 1
Chromatograms of (A) plasma and (B) urine samples. a, Blank; b, from a volunteer 2 h after oral administration of 160 mg TMP and 800 mg SMX. Peaks: (1) Ac-SMX, (2) TMP, (3) SMX and (4) internal standard (phenacetin). Liquid chromatography on a 250 × 4.5 mm i.d. Spherisorb-ODS column with acetonitrile–0.1 M sodium acetate (30:70, v/v) isocratic elution and UV detection at 230 nm.

and 9.49 min for Ac-SMX, TMP, SMX and Phenacetin (internal standard), respectively. All the peaks were consistently symmetrical and no interference was observed due to endogenous constituents. Ac-SMX elutes before TMP and SMX, while, in other HPLC methods reported [8–10, 12, 13], it elutes afterwards. The efficiency of the system employed for Ac-SMX may be attributed to the high proportion of the aqueous component (70% v/v 0.1 M sodium acetate) in the mobile phase and the relative solubility of Ac-SMX.

All the calibration graphs displayed good linearity over the range examined and almost passed through the origin. The linear regression equations, in plasma and urine respectively, were:

$X = 0.90\ (Y - 0.165)$ and $X = 1.90\ (Y - 0.015)$ for TMP,
$X = 16.39\ (Y - 0.114)$ and $X = 5.15\ (Y + 0.036)$ for SMX,
and $X = 1.81\ (Y - 0.331)$ and $X = 1.10\ (Y - 0.047)$ for Ac-SMX,

where X is the concentration and Y is the peak height ratio. The regression coefficients were 0.999 or better for at least eight points.

The reproducibility of the method was examined by the repeated analysis of 10 samples spiked with TMP, SMX and Ac-SMX at certain concentrations and the results for within assay determination are presented in Table 1. The between assay precision (RSD%) for plasma samples ranged from 8.5% (0.4 μg ml^{-1}) to 3.1% (1.2 μg ml^{-1}) for TMP, 2.2% (8.0 μg ml^{-1}) to 1.2% (12.0 μg ml^{-1}) for SMX and 2.6% (4.0 μg ml^{-1}) to 0.6% (8.0 μg ml^{-1}) for Ac-SMX. Between assay precision in urine was 3.4% (4.0 μg ml^{-1}) to 1.6% (8.0 μg ml^{-1}) for TMP, 0.9% (16.0 μg ml^{-1}) to 1.1% (20.0 μg ml^{-1}) for SMX and 0.4% for Ac-SMX (24.0–32.0 μg ml^{-1}). The reproducibility, the correlation coefficients for the regression equations ($r > 0.999$) and the detection limit of 0.1 μg ml^{-1} for both TMP and SMX and 1.0 μg ml^{-1} for Ac-SMX, render the method suitable for monitoring TMP, SMX and Ac-SMX concentrations commonly found in man after therapeutic administration of co-trimoxazole. Furthermore, the stability of the drugs in frozen aliquots of human plasma and urine at concentrations of 0.16 and 32.0 μg ml^{-1} were examined in six samples over a time period of 10 days to 3 months. There was no trend indicative of compound instability as a result of freezing and thawing over this period.

The applicability of the proposed method was tested out by analysing blood samples from a hospitalized patient. A single oral dose of 160 mg TMP and 800 mg SMX (two tablets, Septrin, Wellcome) was administered to the patient after an overnight fast. Blood and urine samples were collected at scheduled intervals. Blood samples were collected from a forearm vein and the plasma was separated by centrifugation and then frozen. Urine samples (5 ml) were frozen immediately after their collection. The plasma concentration and urinary excretion rate–time profiles of TMP, SMX and

Table 1
Reproducibility of TMP, SMX and Ac-SMX determination in human plasma and urine samples ($n = 10$)

Sample	Drug	Spiked concentration (μg ml^{-1})	Found concentration (μg ml^{-1})	RSD (%)
Plasma	TMP	0.40	0.40 ± 0.03	7.5
		0.60	0.60 ± 0.05	8.3
		1.20	1.20 ± 0.03	2.5
	SMX	8.00	7.99 ± 0.15	1.9
		10.00	10.01 ± 0.07	0.7
		12.00	11.99 ± 0.10	0.8
	Ac-SMX	4.00	4.01 ± 0.08	2.0
		6.00	6.01 ± 0.05	0.8
		8.00	8.01 ± 0.03	0.4
Urine	TMP	4.00	3.99 ± 0.12	3.0
		6.00	6.01 ± 0.15	2.5
		8.00	7.99 ± 0.10	1.3
	SMX	16.00	16.01 ± 0.09	0.6
		18.00	18.01 ± 0.12	0.7
		20.00	19.99 ± 0.19	1.0
	Ac-SMX	24.00	23.99 ± 0.06	0.3
		28.00	28.01 ± 0.10	0.4
		32.00	31.99 ± 0.05	0.2

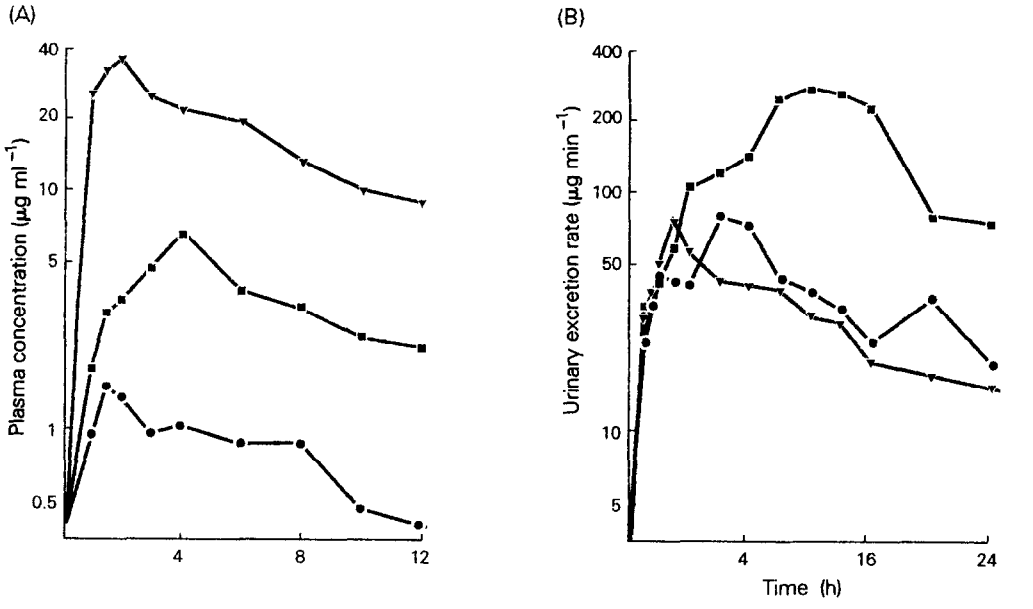

Figure 2
Plasma concentration (A) and urinary excretion rate (B) versus time for TMP (●), SMX (▼) and Ac-SMX (■) in a volunteer after a single oral administration of 160 mg TMP and 800 mg SMX.

Ac-SMX are shown in Fig. 2. The cumulative amounts excreted in the urine over 48 h were calculated and they were found to be 100 mg TMP, 92 mg SMX and 365 mg Ac-SMX.

The HPLC assay described here utilizes standard HPLC equipment, has good sensitivity and each analytical run is completed within 12 min, allowing good sample throughput. The method is currently being used in an investigation of some formulation factors on the pharmacokinetics and bioavailability of co-trimoxazol.

References

[1] P. Helboe and M. Thomsen, *Archs Pharm. Chem. Sci. Ed.* **5**, 25–28 (1977).
[2] U.S.P. (XXI), United States Pharmacopeial Convention, Rockville, MD, p. 995 (1985).
[3] R.O. Singletary Jr and F.D. Sancilio, *J. Pharm. Sci.* **69**, 144–146 (1980).
[4] A. Bye and M.E. Brown, *J. Chromatogr. Sci.* **15**, 365–371 (1977).
[5] T.B. Vree, Y.A. Hekster, A.M. Baars, J.E. Damsma and E. Vander Kleijn, *J. Chromatogr.* **146**, 103–112 (1978).
[6] R.W. Bury and M.L. Mashford, *J. Chromatogr.* **163**, 114–117 (1979).
[7] V. Ascalone, *J. High Resolut. Chromatogr., Chromatogr. Commun.* **3**, 261–264 (1980).
[8] R. Gochin, J. Kanfer and J.M. Haigh, *J. Chromatogr.* **223**, 139–145 (1981).
[9] O. Spreux-Varoquaux, J.P. Chapalain, P. Cordonnier, C. Advenier, M. Pays and L. Lamine, *J. Chromatogr.* **274**, 187–199 (1983).
[10] A. Weber, K. Opheim, G.R. Siber, J.F. Ericson and A.L. Smith, *J. Chromatogr.* **278**, 337–345 (1983).
[11] G.R. Erdmann, D.M. Canafax and G.S. Giebink, *J. Chromatogr.* **433**, 187–195 (1988).
[12] N.E. Basci, A. Bozkurt, S.O. Kayaalp and A. Isimer, *J. Chromatogr.* **527**, 174–181 (1990).
[13] S.C. Laizure, C.L. Holden and R.C. Stevens, *J. Chromatogr.* **528**, 235–242 (1990).
[14] J.P. Sharma, E.G. Perkins and R.F. Bevill, *J. Pharm. Sci.* **65**, 1606–1608 (1976).

[Received for review 12 September 1990; revised manuscript received 12 February 1991]