Journal of Chromatography, 616 (1993) 144–150
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6845

Short Communication

Sensitive high-performance liquid chromatographic determination of EM523, a new erythromycin derivative, in human plasma and urine with ultraviolet detection using column switching

Kenji Yamashita*, Michio Motohashi and Takatsuka Yashiki

Takeda Analytical Research Laboratories, Ltd., Juso-Honmachi, Yodogawa-ku, Osaka 532 (Japan)

(First received January 5th, 1993; revised manuscript received March 11th, 1993)

ABSTRACT

A sensitive high-performance liquid chromatographic (HPLC) method using column switching is described for the determination of EM523 (I), a new erythromycin derivative, in human plasma and urine. The analyte was extracted from alkalinized plasma or urine with a mixture of *n*-hexane and acetone. After the evaporation of the organic layer, the reconstituted residue was injected into the HPLC system and separated on the first column. After column switching, the heart-cut fraction containing the analyte was further separated on the second column and monitored by ultraviolet absorbance at 210 nm. The detection limits were 1 ng/ml in plasma and 10 ng/ml in urine. The method was applied to the clinical trials of I.

INTRODUCTION

EM523 (I, Fig. 1), de(N-methyl)-N-ethyl-8,9-anhydroerythromycin A 6,9-hemiacetal, a new erythromycin derivative, has strong gastrointestinal motor-stimulating activity without antibiotic activity, with a mechanism similar to that of motilin [1–5]. This compound is expected to be useful in the treatment of gastrointestinal disorders and is now undergoing clinical trials.

High sensitivity for the determination of I in human plasma and urine is required because of its predicted low doses in the clinical trials. Several high-performance liquid chromatographic (HPLC) methods have been reported for the determination of erythromycin or its derivatives

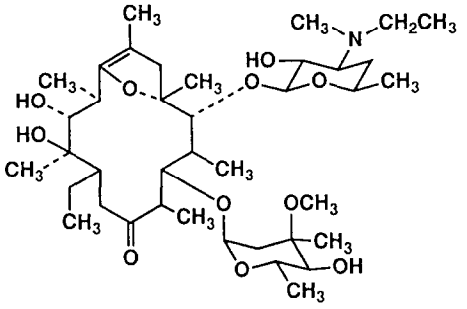

Fig. 1. Structure of I.

* Corresponding author.

0378-4347/93/$06.00

with ultraviolet (UV), fluorimetric or electrochemical detection [6–13]. However, these methods were not considered to be applicable to the clinical trials of I owing to their insufficient sensitivities.

Compound I shows UV absorption only in the short-wavelength region (molar absorptivity at 210 nm = 8000). A preliminary HPLC study using a standard solution of I showed that UV detection at 210 nm offered sufficient sensitivity provided that there were no interferences present at the retention time of I. The elution of I, a tertiary amine, was presumed to be delayed by ion-pair formation. Therefore, high sensitivity and selectivity was considered to be obtainable by using a column-switching technique combined with ion-pair chromatography, which we reported previously [14–17].

This paper describes a sensitive method for the determination of I in human plasma and urine using this technique.

EXPERIMENTAL

Reagents and materials

Compound I was synthesized by Takeda Chemical Industries (Osaka, Japan). Acetonitrile, *n*-hexane and acetone were of HPLC grade (Wako, Osaka, Japan). Sodium dodecyl sulphate (SDS) was of ion-pair reagent grade (Tokyo Kasei, Tokyo, Japan). All other reagents were of analytical reagent grade and were used without further purification.

Extraction from plasma and urine

To 1.0 ml of human plasma were added 50 μl of 1 *M* sodium hydroxide solution. The mixture was extracted twice with 4 ml of a mixture of *n*-hexane and acetone (6:1, v/v). The organic layer was evaporated to dryness under a nitrogen gas stream at 40°C. The residue was dissolved in 200 μl of a mixture of 20 m*M* potassium dihydrogenphosphate–acetonitrile (1:1, v/v). An aliquot of 150 μl was injected into the HPLC system.

To 200 μl of human urine was added 1 ml of 200 m*M* disodium hydrogenphosphate (pH 11 with 1 *M* sodium hydroxide solution). The mixture was extracted in the same way as that for plasma. The residue was dissolved in 300 μl of a mixture of 20 m*M* potassium dihydrogenphosphate–acetonitrile (1:1, v/v). An aliquot of 100 μl was injected into the HPLC system.

Instrument and conditions

The HPLC system consisted of two LC-6A pumps, two SPD-6A UV detectors, a CTO-6A column oven, an FCV-2AH six-port switching valve and an SIL-6A autoinjector, all of which were controlled automatically by an SCL-6A controller (all from Shimadzu, Kyoto, Japan). A U-228 dual-pen recorder (Nippon Denshi Kagaku, Kyoto, Japan) was used for the measurement of the peak height of the analyte. Both columns used (C1 and C2) were Nucleosil $5C_{18}$ (5 μm, 150 mm × 4.6 mm I.D.; Macherey & Nagel, Düren, Germany). The mobile phase for C1 (MP1) was 20 m*M* potassium dihydrogenphosphate–acetonitrile (1:1, v/v) containing 3.5 m*M* SDS, adjusted to pH 4 with 10% phosphoric acid. The mobile phase for C2 (MP2) was the same as MP1 but without SDS. The temperature and the flow-rate for both columns were 40°C and 1.0 ml/min, respectively. UV detection was carried out at 210 nm.

Analytical system and procedure

A schematic diagram of the HPLC system is shown in Fig. 2. The analytical system and the procedure were similar to those reported previously [14–17]. Before start-up, the retention time of I was checked daily by the detector A to determine the time programme for the column switching. The injected sample was first analysed by C1 with MP1 (valve position A). The eluate fraction containing I was transferred from C1 to C2 by switching the valve position from A to B. After the elution of I from C1 to C2, the valve position was switched back to A and the heart-cut fraction was further analysed by C2 with MP2 and monitored by detector B with UV absorbance at 210 nm. The valve operations were carried out automatically by the SCL-6A controller according to the predetermined time programme.

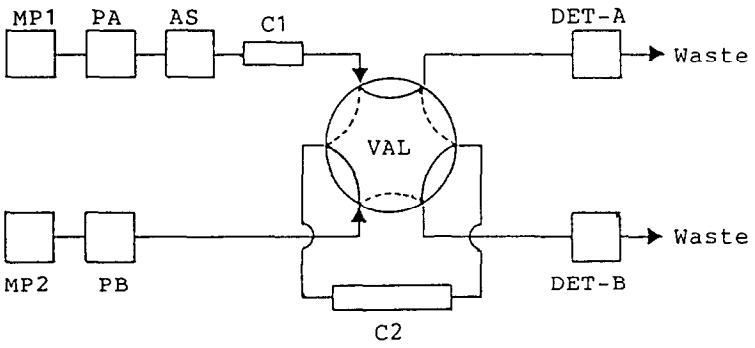

Fig. 2. Schematic diagram of the HPLC system. PA and PB = pumps A and B; AS = autosampler; VAL = six-port valve; C1 and C2 = columns 1 and 2; DET-A and DET-B = UV detectors A and B; MP1 and MP2 = mobile phases 1 and 2. The solid and dotted lines in the six-port valve indicate valve positions A and B, respectively.

RESULTS AND DISCUSSION

The preliminary investigations showed that the fluorimetric or electrochemical detection could not offer the required high sensitivity. Although UV detection at 210 nm offered sufficient sensitivity, it was predicted to be difficult to separate the analyte from interferences using an ordinary HPLC technique with such a short-wavelength UV detection. The elution of I, a tertiary amine, was predicted to be delayed by ion-pair formation. Therefore, the column-switching technique, which combines ion-pair and non-ion-pair chromatography [14–17], was considered to offer high sensitivity and selectivity even with this poorly selective detection. The investigations concerning various ion-pair reagents showed that the HPLC conditions shown in the experimental section using SDS as an ion-pair reagent offered the required high sensitivity and selectivity.

Figs. 3 and 4 show the chromatograms obtained with C1 alone. The sensitive determination of I in plasma and urine was clearly impossible because of the interferences at the retention time of I. The satisfactory clean-up efficiency of the present method was demonstrated by the chromatograms obtained after column switching, which were free from interferences (Figs. 5 and 6). Further, the peak height of I after column switching was 2.7 times that obtained with C1 alone. It should be noted that only a simple liquid–liquid extraction was required as a clean-up procedure prior to HPLC analysis by adopting the above-mentioned highly selective column-switching technique.

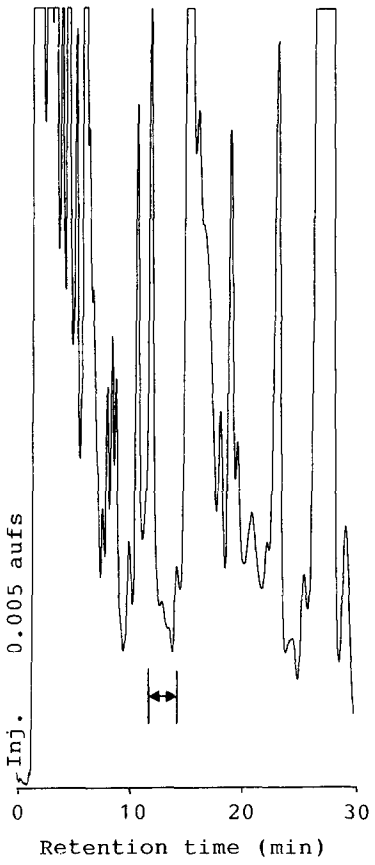

Fig. 3. Chromatogram of drug-free plasma obtained with C1 alone. The marked zone indicates the heart-cut fraction.

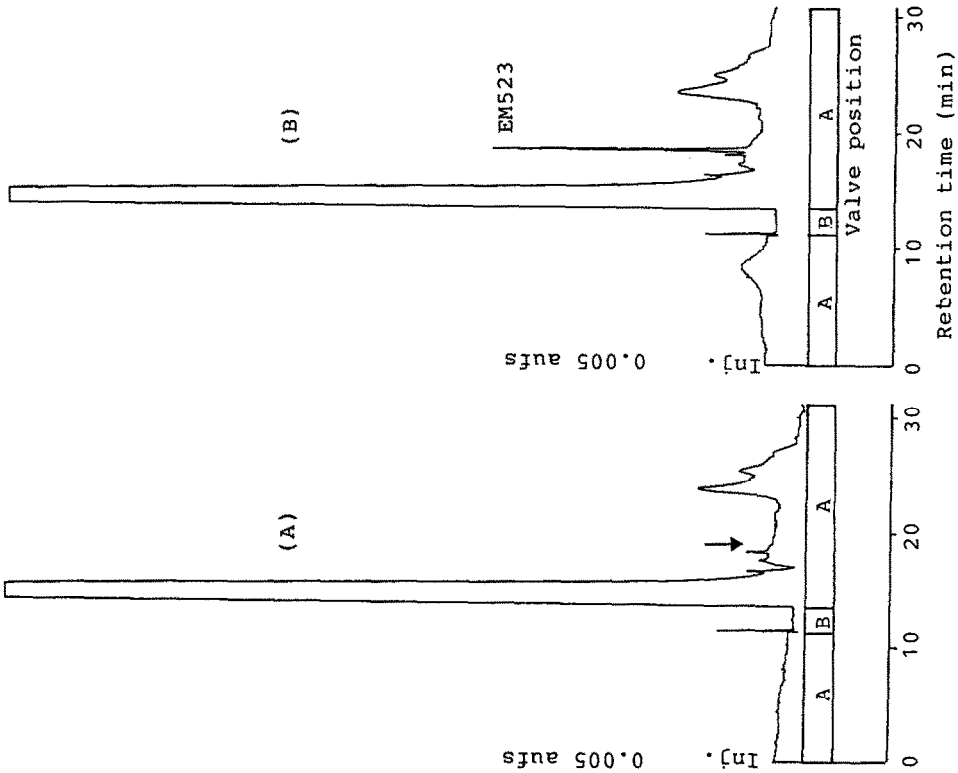

Fig. 5. Chromatograms of (A) drug-free plasma and (B) plasma spiked with I (40 ng/ml) obtained after column switching.

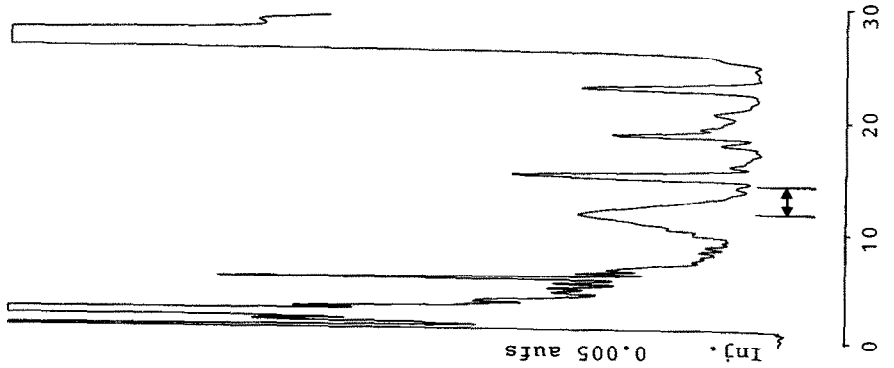

Fig. 4. Chromatogram of drug-free urine obtained with C1 alone. The marked zone indicates the heart-cut fraction.

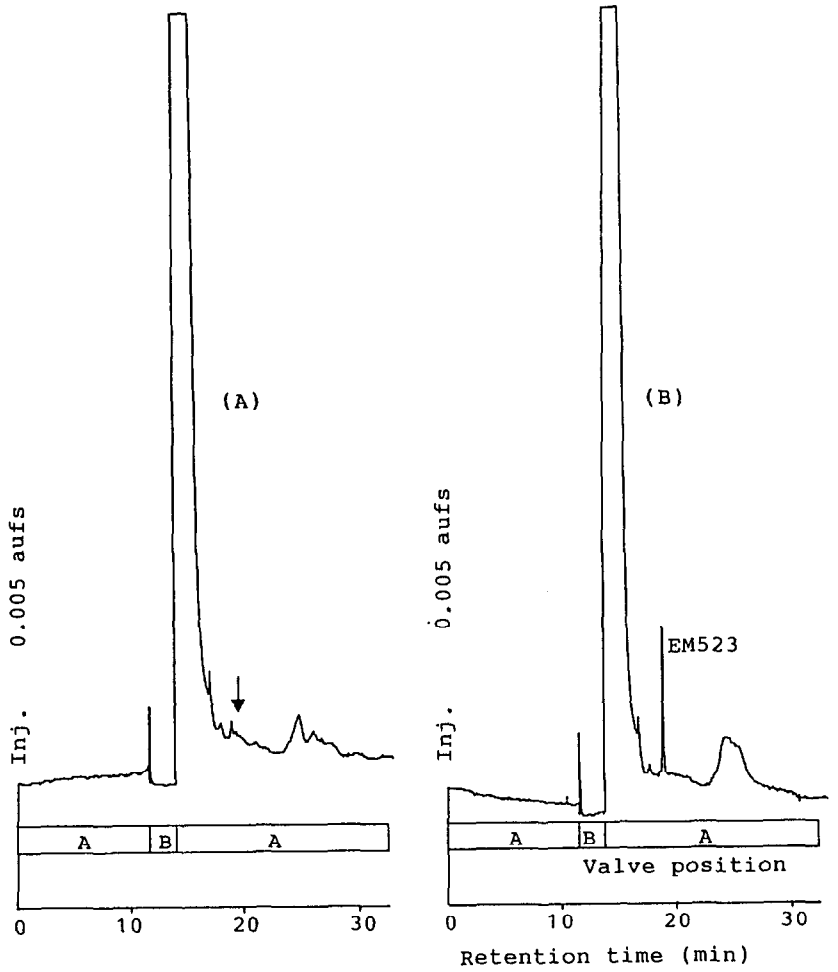

Fig. 6. Chromatograms of (A) drug-free urine and (B) urine spiked with I (200 ng/ml) obtained after column switching.

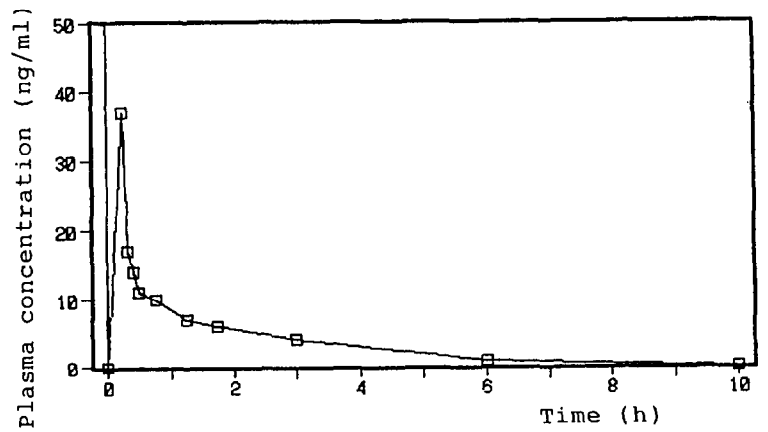

Fig. 7. Plasma levels of I after constant-rate intravenous infusion of 4 mg of I to a healthy human volunteer for 15 min.

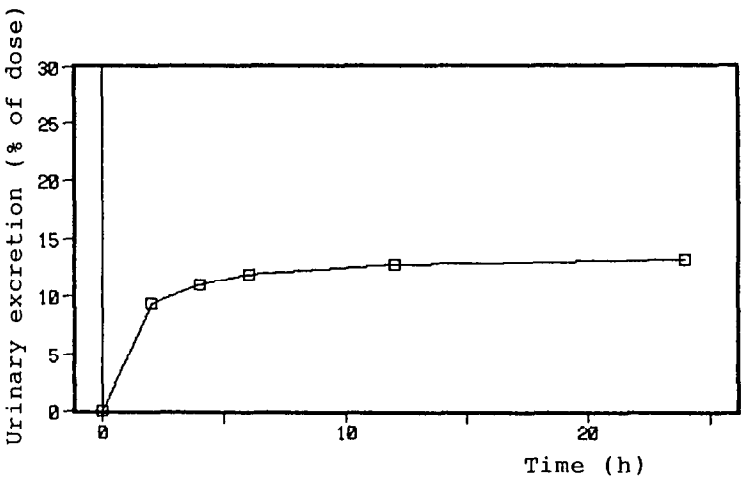

Fig. 8. Cumulative urinary excretion of I after constant-rate intravenous infusion of 4 mg of I to a healthy human volunteer for 15 min.

The calibration graphs were obtained by analysing spiked plasma and urine samples over the concentration ranges 1–50 and 10–5000 ng/ml, respectively. The least-squares regression fit showed good linearity, passing through the origin (correlation coefficient = 0.999) for both plasma and urine. The intra-assay recoveries of I from spiked plasma were 96.5% with a coefficient of variation (C.V.) of 4.0% (12 ng/ml, $n = 6$) and 93.9% with a C.V. of 4.7% (50 ng/ml, $n = 5$). The inter-assay recovery from plasma was 91.0% with a C.V. of 3.0% (50 ng/ml, $n = 3$). The intra- and inter-assay recoveries from urine were 95.1% with a C.V. of 3.7% (400 ng/ml, $n = 5$) and 92.9% with a C.V. of 2.1% (400 ng/ml, $n = 3$), respectively. The detection limits were 1 ng/ml in plasma and 10 ng/ml in urine, both at a signal-to-noise ratio of 3. Accuracy and precision data at the upper and lower limits of the concentration ranges are presented in Table I. The compound in plasma and urine was stable for at least thirty days at −20°C (data not shown).

Fig. 7 shows a plasma concentration profile after constant-rate intravenous infusion of 4 mg of I to a healthy human volunteer for 15 min. A cumulative urinary excretion profile is shown in Fig. 8. The present method was thus sensitive enough for pharmacokinetic studies of the compound in humans. The method has been successfully applied to many healthy human volunteers and patients.

TABLE I

ACCURACY AND PRECISION DATA FOR I IN PLASMA AND URINE

Values in parentheses are coefficients of variation (%).

Sample	Added concentration (ng/ml)	Mean found concentration (ng/ml)	
		Intra-assay ($n = 5$)	Inter-assay ($n = 3$)
Plasma	50	49.5 (5.1)	50.3 (1.8)
	1	0.99 (9.5)	1.03 (3.7)
Urine	5000	5020 (2.6)	5030 (2.0)
	10	10.0 (10.3)	10.6 (9.0)

In conclusion, the column-switching technique combined with ion-pair chromatography enabled the highly sensitive and selective determination of I in human plasma and urine with UV detection at 210 nm. The method was applied to the clinical trials of I.

REFERENCES

1 K. Tsuzuki, T. Sunazuka, S. Marui, H. Toyoda, S. Omura, N. Inatomi and Z. Itoh, *Chem. Pharm. Bull.*, 37 (1989) 2687.
2 T. Sunazuka, K. Tsuzuki, S. Marui, H. Toyoda, S. Omura, N. Inatomi and Z. Itoh, *Chem. Pharm. Bull.*, 37 (1989) 2701.

3 N. Inatomi, H. Satoh, Y. Maki, N. Hashimoto, Z. Itoh and S. Omura, *J. Pharmacol. Exp. Ther.*, 251 (1989) 707.
4 T. Satoh, N. Inatomi, H. Satoh, S. Marui, Z. Itoh and S. Omura, *J. Pharmacol. Exp. Ther.*, 254 (1990) 940.
5 I. Depoortere, T. L. Peeters and G. Vantrappen, *Peptides*, 11 (1990) 515.
6 C. Stubbs, J. Haigh and I. Kanfer, *J. Pharm. Sci.*, 74 (1985) 1126.
7 K. Tsuji, *J. Chromatogr.*, 158 (1978) 337.
8 M. L. Chen and W. L. Chiou, *J. Chromatogr.*, 278 (1983) 91.
9 G. S. Duthu, *J. Liq. Chromatogr.*, 7 (1984) 1023.
10 D. Croteau, F. Vallee, M. G. Bergeron and M. LeBel, *J. Chromatogr.*, 419 (1987) 205.
11 L. G. Nilsson, B. Walldorf and O. Paulsen, *J. Chromatogr.*, 423 (1987) 189.
12 M. Shirotsuka, T. Yamamoto, M. Hirayama, K. Saitoh and K. Okui, *Chemotherapy (Tokyo)*, 36 S-4 (1988) 138.
13 T. Ohtake, K. Ogura, C. Iwatate and T. Suwa, *Chemotherapy (Tokyo)*, 36 (1988) 916.
14 K. Yamashita, M. Motohashi and T. Yashiki, *J. Chromatogr.*, 487 (1989) 357.
15 T. Miyabayashi, K. Yamashita, I. Aoki, M. Motohashi, T. Yashiki and K. Yatani, *J. Chromatogr.*, 494 (1989) 209.
16 K. Yamashita, M. Motohashi and T. Yashiki, *J. Chromatogr.*, 527 (1990) 103.
17 K. Yamashita, M. Motohashi and T. Yashiki, *J. Chromatogr.*, 527 (1990) 196.