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Simultaneous determination of omeprazole and its metabolites in plasma and urine by reversed-phase high-performance liquid chromatography with an alkaline-resistant polymer-coated C_{18} column

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ABSTRACT

Omeprazole (OPZ) is a proton pump inhibitor in gastric parietal cells. A reversed-phase high-performance liquid chromatographic method was developed that enables concentrations of OPZ and its major metabolites, omeprazole sulphone (OPZ-SFN) and hydroxy-omeprazole (H-OPZ), to be determined simultaneously in plasma and that of H-OPZ in urine. To prevent decomposition of OPZ, all the processes (extraction, injection and elution) were carried out under alkaline conditions. Recoveries of the analytes and internal standard were >93.1%. The intra- and inter-assay coefficients of variation were <9.1 and 6.4% for plasma samples and <2.9 and

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3.9% for urine samples, respectively. The minimum determinable concentration (relative standard deviation 10–15%) was 10 ng/ml for all analytes in plasma and H-OPZ in urine samples. The clinical applicability of this assay method was evaluated by determining plasma concentration– and urinary excretion–time courses of the respective analyte(s) in four healthy volunteers after an oral dose of 20 mg of OPZ. The present assay is considered to be simple, precise and accurate and suitable for the study of the kinetic disposition and metabolism of OPZ, which is an extensively metabolized drug in the human liver.

INTRODUCTION

Omeprazole (OPZ), a substituted benzimidazole, suppresses acid secretion by the selective inhibition of H^+/K^+-ATPase in gastric parietal cells [1–3]. The drug is superior to histamine H_2-receptor antagonists in terms of the shorter healing rates of gastric and duodenal ulcers [1–3]. It is also effective for healing ulcers which failed to respond to H_2-receptor antagonists and is extremely valuable for the treatment of Zollinger–Ellison syndrome [1–3]. OPZ is extensively metabolized by the liver to several metabolites, omeprazole sulphone (OPZ-SFN), hydroxyomeprazole (H-OPZ) and omeprazole sulphide (OPZ-SFD) [3–5]. OPZ-SFN and H-OPZ are the major metabolites found in plasma and H-OPZ is the predominant one in urine [3–5]. The concentration of OPZ-SFD is usually too low to be determined in plasma [6], and that of OPZ-SFD or OPZ is also negligible in urine [5,7].

Several high-performance liquid chromatographic (HPLC) methods have been available for the determination of OPZ and its metabolites in biological fluids [6,8–10]. However, only one of the reversed-phase HPLC methods reported previously allows a simultaneous determination of OPZ and its major metabolites, OPZ-SFN and H-OPZ, in plasma [10]. Although two reversed-phase HPLC methods have enabled OPZ, OPZ-SFN and OPZ-SFD to be determined in plasma [8] and in both plasma and urine [9], these methods do not determine H-OPZ in human biological fluids [8,9]. Further, the standard type of octadecylsilica (ODS) column employed in these studies does not ensure prolonged use with an alkaline mobile phase [11–13], which is preferable for stabilizing OPZ that is easily decomposed under acidic conditions [14,15]. Although an HPLC method has been used to determine OPZ, OPZ-SFN and H-OPZ in plasma and H-OPZ in urine [6], it involves two separate HPLC systems: normal-phase for OPZ and OPZ-SFN in plasma and reversed-phase for H-OPZ in plasma and urine.

We report here a method for the determination of OPZ, OPZ-SFN and H-OPZ in plasma simultaneously and H-OPZ in urine by using a conventional HPLC system with an alkaline-resistant column packed with polymer-coated C_{18} packing material [11–13]. The method was applied to a preliminary pharmacokinetic study of OPZ and its metabolites in healthy volunteers.

EXPERIMENTAL

Materials

OPZ, OPZ-SFN and H-OPZ were generous gifts from Fujisawa-Astra (Osaka, Japan), Fujisawa Pharmaceutical (Osaka, Japan) and Astra Hässle (Mölndal, Sweden), respectively. Phenacetin, acetonitrile and methanol of HPLC grade were purchased from Wako (Osaka, Japan). All reagents and buffer solution were prepared with analytical-reagent grade chemicals.

High-performance liquid chromatography

Analyses were performed with the following HPLC system: a Model L-6000 pump, a Model L-4000 ultraviolet absorbance detector, a Model AS-2000 autosampler, a Model D-2500 integrator (Hitachi, Tokyo, Japan) and a 25 cm × 4.6 mm I.D. Capcell Pak C_{18} SG 120 column, 5 μm particle size (Shiseido, Tokyo, Japan). The mobile phase was acetonitrile–0.05 *M* phosphate buffer (pH 8.5) (25:75, v/v) at a flow-rate of 0.8 ml/min. The eluate was monitored at UV wavelength of 302 nm.

Extraction procedure

Plasma. Frozen plasma was allowed to thaw at room temperature. Internal standard (phenacetin, 100 μl of a 0.1 mg/ml stock solution in methanol), dichloromethane (5 ml), sodium chloride (0.25 g) and 0.5 *M* phosphate buffer (pH 8.0) (500 μl) were added to 1.0 ml of plasma in a screw-capped tube. After shaking with a vortex mixer for 10 min and centrifugation at 1610 *g* for 30 min, the upper aqueous layer was aspirated and discarded. The remaining organic phase was transferred into a new glass tube and evaporated with a vacuum evaporator at 40°C. The residue was reconstituted with 300 μl of the mobile phase and passed through a 0.45-μm filter (Gelman Sciences, Ann Arbor, MI, USA). A 30-μl volume of the filtrate was injected into the HPLC apparatus.

Urine. Frozen urine was allowed to thaw at room temperature. Internal standard (phenacetin, 100 μl of a 0.2 mg/ml stock solution in methanol), dichloromethane (5 ml), sodium chloride (0.25 g) and 0.5 *M* phosphate buffer (pH 8.0) (500 μl) were added to 1.0 ml of urine in a screw-capped tube. After shaking with a vortex mixer for 10 min and centrifugation at 1610 *g* for 15 min, the upper aqueous layer was aspirated and discarded. The remaining organic phase was transferred into a new glass tube and evaporated with a vacuum evaporator at 40°C. The residue was reconstituted with 200 μl of the mobile phase and 10 or 30 μl were injected into the HPLC apparatus.

Quantification

To examine the linearity of the assay, we prepared calibration graphs for OPZ, OPZ-SFN and H-OPZ at concentrations ranging from 25 to 400 ng/ml in plasma and for H-OPZ at concentrations ranging from 0.25 to 2 μg/ml in urine. Standard samples were prepared by adding the analyte(s) to drug-free plasma or urine and were extracted and analysed as described above. Peak-height ratios of each analyte to the internal standard were measured and the calibration graph was obtained from the least-squares linear regression. The regression line was used to calculate the concentrations of the respective analytes in the unknown samples.

To assess the absolute recoveries of the analytes extracted from plasma or urine, we compared the peak heights of an extracted plasma or urine sample containing a known amount of each of the analytes with those obtained from a stock standard solution of each of the respective analytes. The final concentrations of OPZ, OPZ-SFN and H-OPZ in plasma were 50 and 400 ng/ml and those of H-OPZ in urine were 0.25 and 2 μg/ml.

The precision and accuracy of the assay were assessed by the intra- and inter-assay coefficients of variation (C.V.s) and relative errors by determining each of the three analytes at 50 and 400 ng/ml in plasma and 0.25 and 2 μg/ml H-OPZ in urine.

Preliminary pharmacokinetic study

To evaluate the applicability of the present assay, we measured plasma concentrations of OPZ, OPZ-SFN and H-OPZ and the cumulative urinary excretion of H-OPZ in four healthy male volunteers (aged 21–41 years). Informed consent was obtained from each volunteer. A 20-mg dose of OPZ (Losec; Yuhan, Seoul, Korea) was given orally with 100 ml of water after an overnight fast. Blood samples (3–5 ml each) were collected at 0 (before), 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 and 12 h after the drug administration. Plasma was separated after each blood sampling and stored at −80°C until assayed. Urine samples were also collected at 0 (before), 0–2, 2–4, 4–8, 8–12 and 12–24 h after the drug administration. Immediately after each collection of urine, the pH was adjusted to 7–8 with 1 *M* Na_2CO_3 and the sample was stored at −80°C until analysed.

Calculations

Pharmacokinetic parameters were determined by a model-independent method. The slope (k) of the terminal log–linear portion of the plasma concentration *versus* time curve was determined by least-squares regression analysis. The apparent elimination half-life ($t_{1/2}$) of each of the analytes from plasma was calculated as $t_{1/2}$ =

0.693/k. Areas under the plasma concentration–time curve (AUCs) of OPZ, OPZ-SFN and H-OPZ were calculated by the trapezoidal rule with the time extrapolated to infinity. The apparent oral clearance (Cl/F) of OPZ was calculated as Cl/F = dose/AUC, where F is the oral bioavailability. The maximum plasma concentration (C_{max}) and the time to reach C_{max} (t_{max}) for all the analytes were read from the observed data. Cumulative urinary excretion of H-OPZ was plotted against the up to 24-h post-dose time and expressed as the percentage relative to the molar equivalent dose of OPZ. The data are expressed as mean ± S.D.

RESULTS AND DISCUSSION

The chromatograms of blank plasma, spiked plasma and plasma obtained from a volunteer who had received OPZ are shown in Fig. 1A, B and C, respectively. Those of blank, spiked, and volunteer's urine are shown in Fig. 1D, E and F, respectively. The retention times for H-OPZ, OPZ-SFN, phenacetin (internal standard) and OPZ were 6.6, 11.5, 13.6 and 16.8 min, respectively. The peaks of these compounds were sharp, symmetrical and well resolved. Although an unknown peak arising from plasma had a retention time close to that of H-OPZ (Fig. 1A), such a small overlapping did not interfere with the determination of H-OPZ in plasma (B and C). No other interference peaks were observed in the chromatograms of blank plasma and urine samples. Histamine H_2-receptor blocking drugs such as cimetidine, ranitidine and famotidine, which may be administered before starting omeprazole treatment [16,17], did not co-elute with the analytes or internal standard under the HPLC conditions described.

The mean recoveries of OPZ, OPZ-SFN, H-OPZ and phenacetin from plasma ranged from 93.1 to 115.5% and those of H-OPZ and phenacetin from urine ranged from 101.4 to 102.3% (Table I), indicating that the present method can achieve complete recoveries from plasma and urine for all the compounds studied. The C.V.s were less than 8.7 and 3.2% for the analytes in

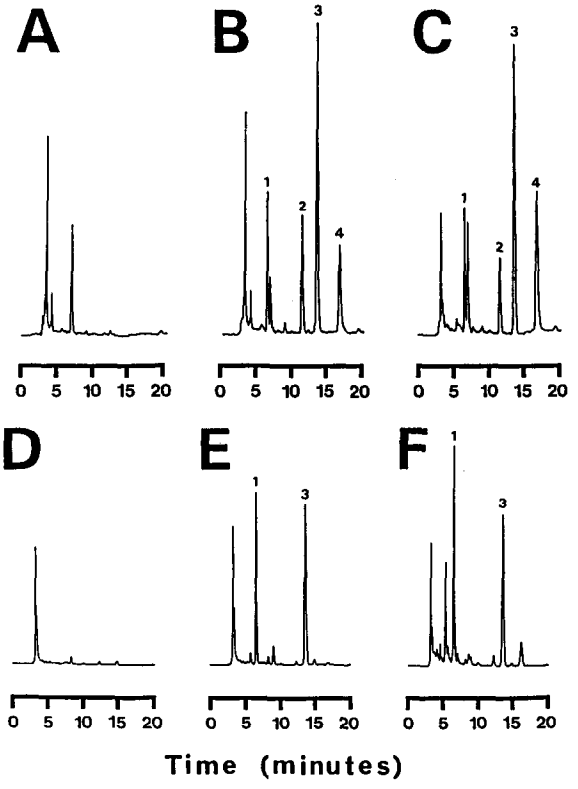

Fig. 1. Representative chromatograms obtained from (A) blank plasma, (B) blank plasma spiked with omeprazole, omeprazole sulphone and hydroxyomeprazole to give a final concentration of 200 ng/ml of each, (C) plasma obtained from a volunteer 1.5 h after an oral dose of 20 mg of omeprazole, (D) blank urine, (E) blank urine spiked with hydroxyomeprazole to give a final concentration of 2 μg/ml and (F) urine obtained from a volunteer between 4 and 8 h after an oral dose of 20 mg of omeprazole. Peaks: 1 = hydroxyomeprazole; 2 = omeprazole sulphone; 3 = phenacetin (internal standard); 4 = omeprazole.

plasma and urine, respectively (Table I). The calibration graphs for both plasma and urine samples were linear over the concentration range examined ($r > 0.999$ for all the analytes).

The data on the analytical precision and accuracy are given in Table II. The intra- and inter-assay C.V.s for the three analytes were less than 9.1 and 6.4% in plasma and those of H-OPZ were less than 2.9 and 3.9% in urine, respectively. Relative errors for plasma and urine samples ranged from −3.7 to +3.8% and from +0.6 to +2.0%, respectively. The minimum determinable concentration (or quantification limit), de-

TABLE I

RECOVERY OF OMEPRAZOLE, OMEPRAZOLE SULPHONE, HYDROXYOMEPRAZOLE AND PHENACETIN FROM PLASMA AND URINE

Compound	Concentration	Mean recovery ($n=6$) (%)	C.V. (%)
Plasma			
Omeprazole	50 ng/ml	93.6	8.7
	400 ng/ml	98.1	1.8
Omeprazole sulphone	50 ng/ml	115.5	3.0
	400 ng/ml	101.3	2.0
Hydroxyomeprazole	50 ng/ml	93.1	5.8
	400 ng/ml	93.5	2.4
Phenacetin	10 μg/ml	102.8	0.8
Urine			
Hydroxyomeprazole	0.25 μg/ml	101.4	3.2
	2 μg/ml	101.4	1.4
Phenacetin	20 μg/ml	102.3	1.1

fined as relative standard deviation between 10 and 15%, was 10 ng/ml for all analytes in plasma and H-OPZ in urine. These data for precision and limit of determination are comparable to or better than those reported previously [6,8–10] and appear to be applicable for clinical pharmacokinetic studies.

The mean (± S.D.) plasma concentration *versus* time data for OPZ and its metabolites after an oral dose of 20 mg of OPZ in four healthy volunteers are shown in Fig. 2A. Mean pharmacokinetic parameters calculated from the data were as follows: for OPZ, t_{max} = 1.4 ± 0.5 h, C_{max} = 384 ± 166 ng/ml, AUC = 720 ± 212 ng · h/ml, Cl/F = 518 ± 221 ml/h · kg and t_{12} = 1.2 ± 0.7 h; for OPZ-SFN, t_{max} = 1.6 ± 0.5 h, C_{max} = 90 ± 36 ng/ml, AUC = 553 ± 244 ng · h/ml and $t_{1/2}$ = 3.3 ± 1.0 h; for H-OPZ, t_{max} = 1.4 ± 0.5 h, C_{max} = 217 ± 50 ng/ml, AUC = 487 ± 43 ng · h/ml and $t_{1/2}$ = 1.2 ± 0.3 h. The cumulative urinary

TABLE II

PRECISION AND ACCURACY OF INTRA- AND INTER-ASSAY OF OMEPRAZOLE, OMEPRAZOLE SULPHONE AND HYDROXYOMEPRAZOLE IN PLASMA AND URINE

Compound	Concentration	Intra-assay (n = 6)		Inter-assay (n = 6)	
		C.V. (%)	Relative error (%)	C.V. (%)	Relative error (%)
Plasma					
Omeprazole	50 ng/ml	9.1	0.0	4.9	+2.0
	400 ng/ml	3.5	−3.7	1.0	−0.3
Omeprazole sulphone	50 ng/ml	3.0	0.0	4.2	−1.2
	400 ng/ml	3.2	−2.2	0.9	−0.8
Hydroxyomeprazole	50 ng/ml	5.0	−1.6	6.4	+3.8
	400 ng/ml	6.3	−3.0	2.4	−1.0
Urine					
Hydroxyomeprazole	0.25 μg/ml	2.9	+0.6	1.6	+1.1
	2 μg/ml	0.7	+2.0	3.9	+1.3

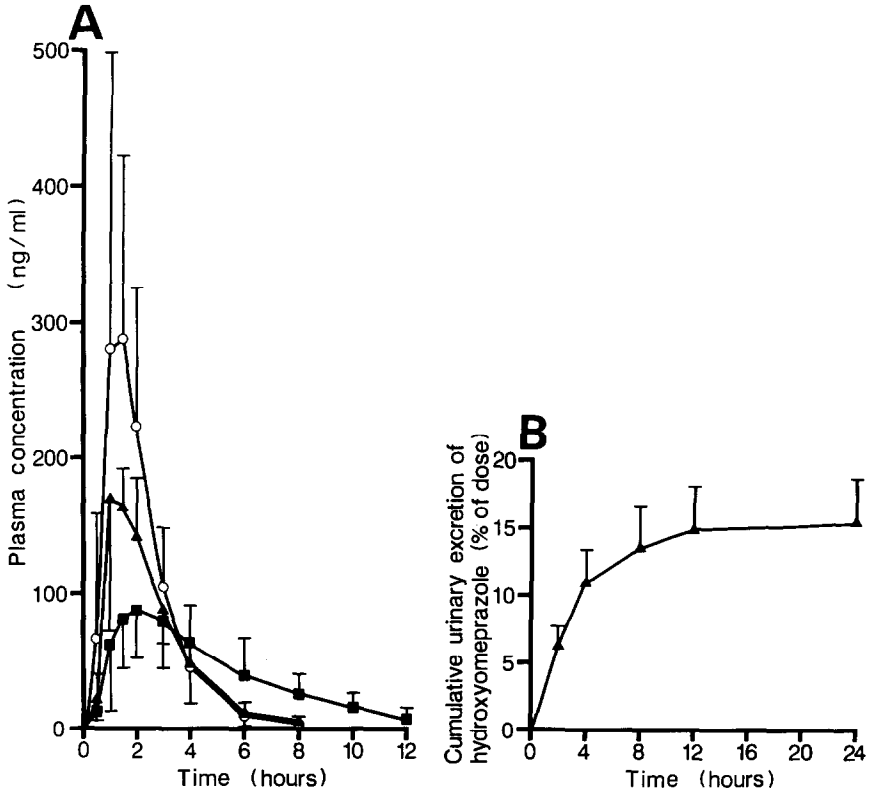

Fig. 2. (A) Plasma concentration–time data for omeprazole and its two metabolites and (B) cumulative urinary excretion–time data for hydroxyomeprazole after an oral dose of 20 mg of omeprazole administered to four healthy volunteers. The data given are mean ± S.D. ○ = Omeprazole; ■ = omeprazole sulphone and ▲ = hydroxyomeprazole.

excretion of H-OPZ for up to 24 h post-dose is also shown in Fig. 2B. The mean post-dose 24-h urinary recovery of H-OPZ was 14.2 ± 3.5% of the dose. Although these pharmacokinetic parameters were derived from a limited number of study subjects, the mean values appear to be comparable to those reported previously [5].

OPZ is relatively stable at neutral and alkaline pH but is decomposed rapidly under acidic conditions [14,15]. In addition, it has been reported that an alkaline mobile phase is required occasionally in order to obtain a better resolution of OPZ-SFN in plasma [10]. Hence the analysis of OPZ is preferably carried out under alkaline conditions. However, a commonly used packing material, ODS, dissolves in alkaline solution, and therefore it is difficult to use it under alkaline conditions [11–13]. Organic porous polymer packing material is resistant to alkaline conditions, but it has some disadvantages such as a lower peak resolution and weaker pressure resistance to and swelling in certain solvents [11–13]. In contrast, the silicone polymer-coated silica is a hybrid type of packing material possessing the advantages of both types of packing materials: a separation efficiency similar to that of typical silica-based packings and alkali resistance similar to that of porous polymer packings [11–13]. Hence, such a silicone polymer-coated silica column as used in the present assay method appears to be suitable for the determination of compounds such as OPZ which prefer alkaline conditions. In fact, the chromatograms obtained under the HPLC conditions described showed that all of the peaks are sharp and symmetrical with a baseline resolution (Fig. 1). In addition, the pre-

cision and accuracy of the present assay (Table II) are similar to or better than those of methods reported previously, which employed a standard type of ODS column [8–10]. We also confirmed that the sharpness and resolution of the peaks did not change after at least 200 h of running the system with an alkaline mobile phase (pH 8.5).

In conclusion, the results reported here, coupled with the emphasis that the current assay is the first simultaneous determination of OPZ and its major metabolites, OPZ-SFN and H-OPZ, in plasma and determination of H-OPZ in urine using a conventional HPLC system with an alkali-resistant polymer-coated C_{18} column, suggest that the present method will be useful for determining acid-labile compounds such as OPZ in human biological fluids and be suitable for the study of the kinetic disposition and metabolism of OPZ in humans.

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REFERENCES

1 S. Holt and. C. W. Howden, *Dig. Dis. Sci.*, 36 (1991) 385.
2 P. N. Maton, *N. Engl. J. Med.*, 324 (1991) 965.
3 C. W. Howden, *Clin. Pharmacokin.*, 20 (1991) 38.
4 L. Renberg, R. Simonsson and K.-J. Hoffman, *Drug Metab. Dispos.*, 17 (1989) 69.
5 C.-G. Regårdh, T. Andersson, P. O. Lagerström, P. Lundborg and I. Skanberg, *Ther. Drug Monit.*, 12 (1990) 163.
6 P.-O. Langerström and B.-A. Persson, *J. Chromatogr.*, 309 (1984) 347.
7 J. Naesdal, T. Andersson, G. Bodemar, R. Larsson, C.-G. Regårdh, I. Skanberg and A. Walan, *Clin. Pharmacol. Ther.*, 40 (1986) 344.
8 M. A. Amantea and P. K. Narang, *J. Chromatogr.*, 426 (1988) 216.
9 G. W. Mihaly, P. J. Prichard, R. A. Smallwood, N. D. Yeomans and W. J. Louis, *J. Chromatogr.*, 278 (1983) 311.
10 I. Grundevik, G. Jerndal, K. Balmér and B.-A. Persson, *J. Pharm. Biomed. Anal.*, 4 (1986) 389.
11 Y. Ohtsu, H. Fukui, T. Kanda, K. Nakamura, M. Nakano, O. Nakata and Y. Fujiyama, *Chromatographia*, 24 (1987) 380.
12 T. Kanda, J. Koyama, K. Nakamura, Y. Ohtsu and T. Ohta, *Nippon Kagaku Kaishi*, (1989) 702.
13 S. Sirota, Y. Ohtsu and O. Nakata, *J. Chromatogr. Sci.*, 28 (1990) 553.
14 A. Pibrant and C. Cederberg, *Scand. J. Gastroenterol.*, 20 (Suppl. 108) (1985) 113.
15 P. Erlandsson, R. Isaksson, P. Lorentzon and P. Lindberg, *J. Chromatogr.*, 532 (1990) 305.
16 W. Londong, V. Londong, C. Cederberg and H. Steffen, *Gastroenterology*, 85 (1983) 1373.
17 K. E. McArthur, M. J. Collen, P. N. Maton, J. A. Cherner, J. M. Howard, C. A. Ciarleglio, M. J. Cornelius, R. T. Jensen and J. D. Gardner, *Gastroenterology*, 88 (1985) 939.