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Short communication

Chiral assay of omeprazole and metabolites and its application to a pharmacokinetics related to CYP2C19 genotypes

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ABSTRACT

Studies investigating the relationship between CYP2C19 genotype and the stereoselective metabolism of omeprazole have not been reported. In the present study, we developed a simple and sensitive analytical method based on column switching reversed phase high-performance liquid chromatography (HPLC) with UV detection to determine the concentrations of (*R*)- and (*S*)-omeprazole and of its principal metabolites, (*R*)- and (*S*)-5-hydroxyomeprazole, and the non-chiral, omeprazole sulfone, in human plasma. Sample preparation involved liquid–liquid extraction with diethyl ether:dichloromethane (60:40, v/v) followed by clean-up on a TSK BSA-ODS/S column (5 μm, 10 mm × 4.6 mm i.d.) using phosphate buffer:acetonitrile (97:3, v/v, pH 6.4). After column switching, separation was performed on a Shiseido CD-ph chiral column (5 μm, 150 mm × 4.6 mm i.d.) using phosphate buffer:methanol (45:55, v/v, pH 5.0) as mobile phase. The limit of quantitation (LOQ) was 5 ng/mL for all analytes with intra- and inter-day precisions (as coefficient of variation) of <9.5% and <9.6%, respectively for all analytes. The present method was successfully applied to a chiral pharmacokinetic study of omeprazole in human volunteers with different CYP2C19 genotypes. The results show that the formation of (*R*)-5-hydroxyomeprazole gives the best correlation with CYP2C19 genotype.

1. Introduction

Omeprazole is administered as a racemic mixture and is primarily metabolised to 5-hydroxyomeprazole and omeprazole sulfone by CYP2C19 and CYP3A4, respectively [1,2]. *In vitro* studies have shown that the metabolism by CYP2C19 is stereoselective [3] and that, in human liver microsomes, (*R*)-omeprazole produces mainly (*R*)-5-hydroxyomeprazole, whereas (*S*)-omeprazole produces mainly 5-*O*-desmethylomeprazole with virtually no formation of (*S*)-5-hydroxyomepraxole [4]. It is well known that CYP2C19 is polymorphic giving rise to 'extensive metaboliser' (EM) and 'poor metaboliser' (PM) phenotypes [5] and that this has clinical consequences. For example, the higher plasma concentration of omeprazole in PMs makes anti-*Helicobacter pylori* therapy more effective by creating a higher gastric pH and increasing the stability of antimicrobials in clinical situations [6,7]. However, the question of how CYP2C19 genotype affects the metabolism of omeprazole enantiomers has not been resolved. Several chiral assays for omeprazole have been reported [8–13] but few simultaneously analyse the enantiomers of omeprazole and its metabolites [14–16]. Although Kanazawa et al. [14] reported good separation of the enantiomers of omeprazole and 5-hydroxyomeprazole by reversed phase HPLC with mass spectrometric detection, the method was not applied to a full pharmacokinetic study. Similarly, Olsson et al. [15] achieved enantiomeric separation of omeprazole and 5-hydroxyomeprazole using non-aqueous capillary electrophoresis but did not apply their assay to human samples and pharmacokinetic study. Martens-Lobenhoffer et al. [16] validated a chiral assay for omeprazole, 5-hydroxyomeprazole and omeprazole sulfone in human serum using chiral HPLC/MS–MS and applied it to a pharmacokinetic study in two subjects with different CYP2C19 genotypes. Although satisfactory in many respects, the method employed normal phase chromatography and required isotope-labelled internal standards. Recently we developed a non-chiral assay for omeprazole and metabolites [17] and applied it to a study of the relationship between the metabolism of omeprazole and CYP2C19 genotype [18]. In this study we have extended this work and now report a simple and sensitive chiral assay for the simultaneous determination of (*R*)- and (*S*)-omeprazole, (*R*)- and (*S*)-5-hydroxyomeprazole and omeprazole sulfone in human plasma and its application to a chiral pharmacokinetic study of omeprazole in relation to CYP2C19 genotype.

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2. Experimental

2.1. Chemicals and reagents

Omeprazole (purity 99.0%) and its metabolites, 5-hydroxyomeprazole (purity 99.8%) and omeprazole sulfone (purity 99.5%), were kindly provided by Astra Zeneca R & D (Molndal, Sweden), and lansoprazole sulfone (purity 99.0%), which was used as an internal standard (I.S.), was kindly provided by Takeda Chemical Industries (Osaka, Japan). The esomeprazole magnesium (purity 98%) was purchased from Nakarai tesque (Kyoto, Japan). All of the other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan). All solvents were HPLC grade.

2.2. Preparation of the stock and working solutions and their levels of stability

The stock solutions for all analytes were prepared by dissolving an appropriate amount of each compound in methanol to yield a concentration of 1.0 mg/mL and then the working standard solutions of all analytes (100, 10 and 1 μg/mL) were prepared using a serial dilution method with methanol. The working standard solution of the I.S. (50 μg/mL) was obtained by diluting the stock solution (1.0 mg/mL) by 20-fold with methanol. The stock solutions were stable at −30 °C for at least 6 months.

2.3. Extraction procedure

The details of the present extraction procedure were described in our previous study [17]. After 10 min of vortex mixing (the extraction solvent and the plasma), the mixture was centrifuged at 3500 × *g* for 10 min at 4 °C (Himac CF16RX, Hitachi, Tokyo, Japan), and the organic phase was evaporated *in vacuo* at 50 °C until dry (EYELA MG-2200, Tokyo Rikakikai, Tokyo, Japan). The residue was dissolved with 30 μL of methanol and 100 μL of 50 mM disodium hydrogen phosphate buffer (pH 9.3), and an aliquot of 30 μL was injected onto the column.

2.4. Instruction and chromatographic condition

The column-switching HPLC system consisted of two Shimadzu (Kyoto, Japan) LC-10ADVP high-pressure pumps for eluents A and B (a Shimadzu SPD-10AV and a Shimadzu SIL-10ADVP (500 μL injection volume)), a Shimadzu CTO-10AVP column oven, and a Shimadzu Workstation LC solution chromatography integrator. A TSK BSA-ODS/S precolumn (for sample clean-up, column I: 10 mm × 4.6 mm i.d., particle size 5 μm; Tosho, Tokyo, Japan) and a Chiral CD-pH column (column II: 150 mm × 4.6 mm i.d., particle size 5 μm; Shiseido Co. Limit., Tokyo, Japan) were also used.

The column-switching chromatographic conditions were based on our previous report [17]. Briefly, from 0 to 7.5 min after the sample injection, the assay agents were separated from the interfering substances on column I with a mobile phase (eluent A) of a phosphate buffer (pH 6.4, 0.01 M) and acetonitrile (97:3, v/v). Between 7.5 and 8.2 min after the injection, all analytes that were retained on column I were eluted using a mobile phase (eluent B) of phosphate buffer (pH 5.0, 0.05 M) and methanol (45:55, v/v), and the effluent from column I was switched to column II. All analytes were separated on column II using eluent B (between 8.2 and 50 min). The flow rates of eluents A and B were 1.2 and 0.4 mL/min, respectively.

2.5. Assay validation

The calibration curve was obtained by spiking blank plasma samples with (*R*)-omeprazole, (*S*)-omeprazole and omeprazole sulfone (5–1000 ng/mL), and with (*R*)- and (*S*)-5-hydroxyomeprazole (5–500 ng/mL). The blank plasma samples were treated as described above. Calibration curves were constructed using the HPLC chromatograms' peak-area ratios for each analyte relative to the I.S.

The intra-day precision and accuracy were determined from the analyses of the control samples that were performed on six different days, whereas the intra-day precision and accuracy were determined by evaluating the spiked controls that were analysed in a random order six times over the course of one day. The precision level that was determined at each concentration level did not exceed 15% of the coefficient of variation (CV) that was expected for the lower limits of quantification (LOQ), where it should not exceed 20% of the CV [19]. The accuracy was calculated as the percent error (relative error (measured concentration − spiked concentration)/spiked concentration) × 100%)), whereas the precision values were quantified by calculating the intra- and inter-CV values. During all analytical runs, sample of blank plasma was analysed to evaluate the selectivity of the method. Quality control samples were run daily to ensure day-to-day repeatability. Sample stability was determined after stored at −30 °C for 6 month and following storage at room temperature for 72 h on the autosampler.

2.6. Application to pharmacokinetics studies

All of the subjects in the present study also participated in our previous studies [18]. The Ethics Committee of Hirosaki University School of Medicine approved the present study's protocol, and written informed consent was obtained from each participant before the examinations. After fasting overnight, the subjects were administered 40 mg of omeprazole (two tablets of Omepral, AstraZeneca Co., Osaka, Japan) with 240 mL of tap water. These alleles were divided into three groups: hmEMs (*1/*1, $n = 3$), htEMs (*1/*2 and *1/*3, $n = 7$) and PMs (*2/*2 and *2/*3, $n = 5$). The area under the plasma concentration–time curve from time zero to the last sampling time (AUC_{0-8}) was calculated using the linear trapezoidal rule. The hydroxylation index was calculated as (the AUC_{0-8} of omeprazole)/(the AUC_{0-8} of 5-hydroxyomeprazole). The values for the maximum plasma level (C_{max}) and the time to reach the peak value (T_{max}) were obtained directly from the profile. The terminal elimination rate constant (*ke*) was obtained using a linear regression analysis with at least three sampling points from the terminal log-linear declining phase to the last measurable concentration. The apparent elimination half-life ($t_{1/2}$) was calculated as 0.693 divided by *ke*.

2.7. Statistical analysis

The pharmacokinetic parameters from the three genotype groups were compared using a one-way ANOVA followed by Scheffe's test. A paired *t*-test was used for the comparison of the pharmacokinetic parameters for the (*R*)- and (*S*)-enantiomers. All of the data were analysed using the statistical program StatView 5.0 (Abacus Concept, Berkeley, Chicago, USA). A value of $P < 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1. Chromatography

In developing a chiral assay for omeprazole and its metabolites for clinical use, we chose reversed phase HPLC to avoid the use of organic solvents. Like Olssen et al. who used a β-cyclodextrin column [15], we selected a chiral β-cyclodextrin-phenylcarbamate column which provided good separation of enantiomers. A one-step liquid–liquid extraction followed by a non-chiral solid phase

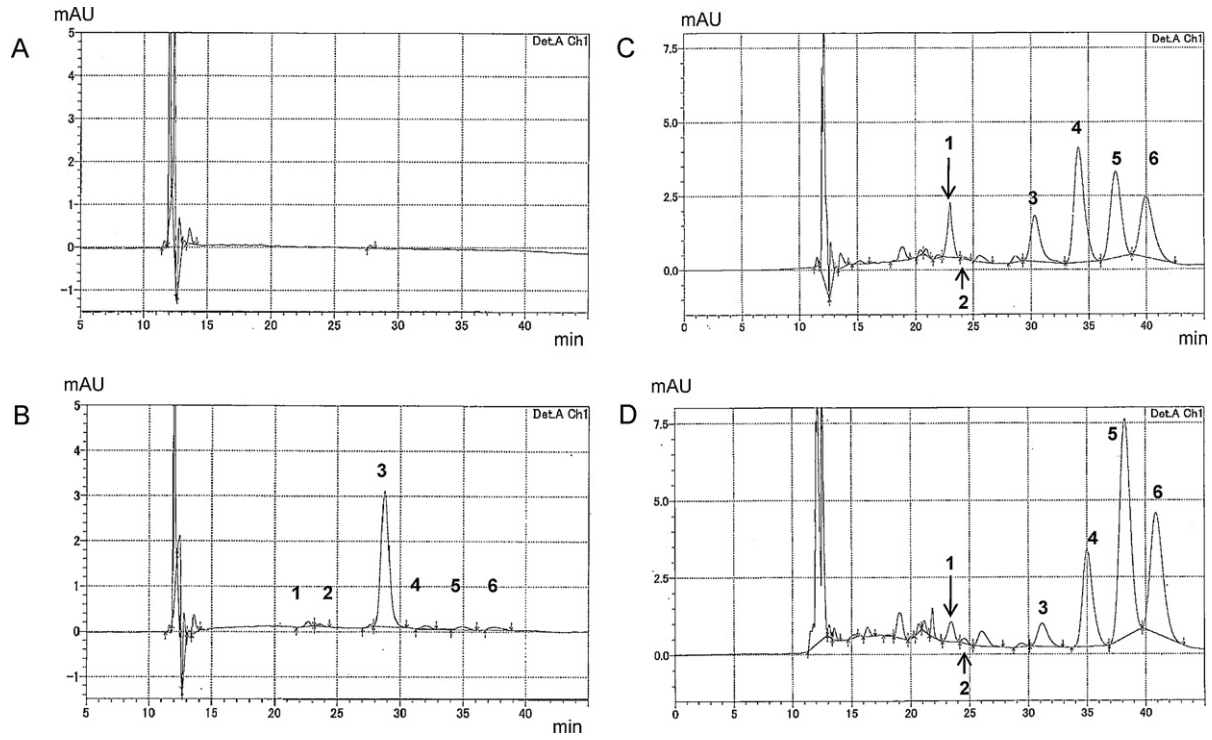

Fig. 1. Typical chromatograms of (A) a plasma blank; (B) the LOQ for a 500 ng/mL I.S. and 5 ng/mL of each analyte; 1: (*R*)-5-hydroxyomeprazole; 2: (*S*)-5-hydroxyomeprazole; 3: lansoprazole sulfone (I.S.); 4: omeprazole sulfone; 5: (*R*)-omeprazole; and 6: (*S*)-omeprazole, typical chromatograms of the plasma samples in the hmEMs (C) and PMs (D) from healthy subjects at 4 h after the oral administration of omeprazole (40 mg).

extraction was then necessary to remove interference from endogenous substances in human plasma prior to chiral separation. Chromatograms of blank and spiked plasma samples and of a real sample from a human volunteer after ingestion of a single oral dose of omeprazole are shown in Fig. 1. In terms of sensitivity, the LOQ of 5 ng/mL achieved for all analytes compares favourably with values determined in previous studies. Thus Kanazawa et al. achieved an LOQ of 2.7 ng/mL for omeprazole enantiomers [14] but their method required a large volume (2 mL) of plasma and a long HPLC run time (80 min). The method developed here uses less plasma (1 mL), has a shorter run time (<45 min) and provides excellent resolution of the 5 analytes.

3.2. Assay validation

3.2.1. Linearity

The calibration curves were linear for the concentration ranges of 5–1000 ng/mL for (*R*)-omeprazole ($r^2 = 0.9997$ and $F = 41{,}837.7$, $P < 0.001$, $n = 6$), of 5–1000 ng/mL for (*S*)-omeprazole ($r^2 = 0.9995$ and $F = 18{,}216.2$, $P < 0.001$, $n = 6$), of 5–500 ng/mL for (*R*)-5-hydroxomeprazole ($r^2 = 0.9994$ and $F = 4641.6$, $P < 0.001$, $n = 6$), of 5–500 ng/mL for (*S*)-5-hydroxomeprazole ($r^2 = 0.9996$ and $F = 7521.2$, $P < 0.001$, $n = 6$), and of 5–1000 ng/mL for omeprazole sulfone ($r^2 = 0.9995$ and $F = 8329.1$, $P < 0.001$, $n = 6$) (Table 1).

The lowest standard on the calibration curve was defined as the limit of quantification by which the analyte peaks for the six compounds were identifiable, discrete and reproducible, with a precision of 20% and an accuracy of 80–120%. The limits of quantification were 5 ng/mL for all of the analytes. Additionally, the CVs and the LOQ of 5 ng/mL for all of the analytes were less than 11.1%, and the accuracy of them was varied from 99.7 to 108.3%. For each analyte, the limit of detection corresponded to the analyte responses that were at least five times greater than the blank response (signal-noise ratio = 5), which was 3 ng/mL.

3.2.2. Specificity and sensitivity

A typical chromatogram is shown in Fig. 1B. The retention times of (*R*)-5-hydroxyomeprazole, (*S*)-5-hydroxyomeprazole, the I.S., omeprazole sulfone, (*R*)-omeprazole, and (*S*)-omeprazole were 22.8, 23.5, 29.3, 32.3, 35.2, and 37.9 min, respectively.

3.2.3. Precision and accuracy

The CVs for the intra- and inter-day assays were determined at concentrations of 8–800 ng/mL for (*R*)-omeprazole, (*S*)-omeprazole and omeprazole sulfone and from 8 to 200 ng/mL for (*R*)- and (*S*)-5-hydroxyomeprazole. The CVs for the intra- and inter-day assays were as follows: less than 9.4% for (*R*)-omeprazole, less than 9.0% for (*S*)-omeprazole, less than 9.6% for (*R*)-5-hydroxyomeprazole, less than 8.9% for (*S*)-5-hydroxyomeprazole, and less than 9.5% for omeprazole sulfone. The accuracies for the intra- and inter-day assays were within 8.2%, 7.6%, 9.3%, 7.6% and 7.4% for (*R*)-omeprazole, (*S*)-omeprazole, (*R*)-5-hydroxyomeprazole, (*S*)-5-hydroxyomeprazole, and omeprazole sulfone, respectively (Table 2).

3.2.4. Recovery (extraction efficiency) from the biologic matrix

The recovery from the plasma was calculated by comparing the peak areas of the pure standards that were prepared in the working solutions and were directly injected into the analytical column with the peak areas of the extracted plasma samples that contained the same amount of the test compounds ($n = 6$). The mean absolute recoveries were 71.4–81.4% for omeprazole enantiomers, and 71.3–78.4% for omeprazole sulfone at 8, 400 and 800 ng/mL. In addition, the mean absolute recoveries were 64.5–79.2% for 5-hydroxyomeprazole enantiomers at 8, 200 and 400 ng/mL.

3.2.5. Stability

The stock solutions of omeprazole, 5-hydroxyomeprazole, omeprazole sulfone and the I.S. were stable at −30 °C for at

Table 1
Individual and mean values for slope, intercepts, correlation coefficients and *F*-values of calibration curves for (*R*)-, (*S*)-omeprazole, (*R*)-5-hydroxyomeprazole (*S*)-5-hydroxyomeprazole and omeprazole sulfone.

Analyte	Curve	Slope	Intercepts	r^2	*F*-test for linearity		Concentration added (ng/mL)	Found (mean ± S.D.)	Accuracy (%)	CV (%)	*n*
					F	*P*-value					
(*R*)-Omeprazole	1	0.0051	0.0103	0.9998	16,430.7	<0.001	5	5.1 ± 0.5	101.5	9.5	6
	2	0.0051	0.0160	0.9992	4949.2	<0.001	25	24.8 ± 2.6	99.3	11.6	6
	3	0.0059	−0.0156	1.0000	219,147.3	<0.001	100	106.3 ± 7.0	106.3	6.6	6
	4	0.0061	−0.0129	0.9996	8890.6	<0.001	250	266.5 ± 17.3	106.6	6.4	6
	5	0.0055	−0.0471	0.9998	1264.6	<0.001	500	515.0 ± 46.2	103.0	9.0	6
	6	0.0059	−0.0636	0.9998	337.6	<0.001	1000	1103.3 ± 82.5	110.3	7.5	6
	Mean	0.0056	−0.0188	0.9997	41,837.7						
	S.D.	0.0004	0.0313	0.0003	87,062.0						
	S.E.	0.0002	0.0128	0.0001	35,542.9						
(*S*)-Omeprazole	1	0.0051	−0.0080	1.0000	97,815.8	<0.001	5	5.4 ± 0.6	108.3	11.1	6
	2	0.0051	0.0152	0.9995	4668.7	<0.001	25	23.4 ± 2.2	93.5	9.4	6
	3	0.0058	−0.0611	0.9990	305.4	<0.001	100	100.4 ± 5.9	100.9	5.9	6
	4	0.0051	0.0085	0.9999	4873.9	<0.001	250	243.9 ± 9.2	97.6	3.8	6
	5	0.0055	−0.0119	0.9997	1286.5	<0.001	500	482.6 ± 41.7	96.5	8.6	6
	6	0.0054	0.0129	0.9991	347.0	<0.001	1000	1049.8 ± 58.7	105.0	5.6	6
	Mean	0.0053	−0.0074	0.9995	18,216.2						
	S.D.	0.0003	0.0286	0.0004	39,049.6						
	S.E.	0.0001	0.0117	0.0002	15,941.9						
(*R*)-5-hydroxyomeprazole	1	0.0020	−0.0046	0.9991	1390.1	<0.001	5	4.9 ± 0.5	98.9	9.6	6
	2	0.0018	−0.0012	0.9993	2379.9	<0.001	12.5	13.8 ± 1.3	110.4	9.6	6
	3	0.0017	−0.0039	0.9999	13,580.7	<0.001	50	53.9 ± 4.3	107.9	7.9	6
	4	0.0016	0.0003	0.9997	5569.7	<0.001	100	100.6 ± 9.3	100.6	9.3	6
	5	0.0017	−0.0033	0.9994	3269.3	<0.001	250	258.1 ± 22.8	103.2	8.8	6
	6	0.0017	−0.0044	0.9991	1660.1	<0.001	500	496.4 ± 39.5	99.3	8.0	6
	Mean	0.0017	0.0028	0.9994	4641.6						
	S.D.	0.0001	0.0003	0.0003	4631.0						
	S.E.	0.00005	0.0001	0.0001	1890.6						
(*S*)-5-hydroxyomeprazole	1	0.0066	0.0051	0.9998	7937.2	<0.001	5	5.0 ± 0.5	99.7	10.7	6
	2	0.0016	0.0193	1.0000	23,217.6	<0.001	12.5	12.4 ± 1.2	99.2	9.5	6
	3	0.0016	0.0121	0.9994	3352.7	<0.001	50	51.8 ± 3.3	103.6	6.4	6
	4	0.0015	−0.0002	0.9997	9181.5	<0.001	100	99.7 ± 9.4	99.7	9.4	6
	5	0.0024	0.0036	0.9993	590.8	<0.001	250	264.6 ± 23.4	105.8	8.8	6
	6	0.0018	0.0179	0.9993	847.5	<0.001	500	496.5 ± 40	99.3	8.2	6
	Mean	0.0026	0.0096	0.9996	7521.2						
	S.D.	0.0020	0.0080	0.0003	8476.5						
	S.E.	0.0008	0.0033	0.0001	3460.5						
Omeprazole sulfone	1	0.0071	0.0101	0.9994	1948.5	<0.001	5	5.4 ± 0.4	108.0	6.9	6
	2	0.0070	−0.0306	0.9996	17,424.4	<0.001	25	25.1 ± 2.1	96.6	8.5	6
	3	0.0071	−0.0511	0.9996	11,608.1	<0.001	100	106.3 ± 10.5	106.3	9.9	6
	4	0.0068	0.0264	0.9998	1858.0	<0.001	250	208.7 ± 14.7	104.3	7.0	6
	5	0.0060	0.0284	0.9993	1171.2	<0.001	500	518.7 ± 42.9	103.7	8.3	6
	6	0.0061	−0.0364	0.9991	15,964.3	<0.001	1000	999.2 ± 67.5	99.9	6.8	6
	Mean	0.0067	−0.0089	0.9995	8329.1						
	S.D.	0.0005	0.0347	0.0003	7557.7						
	S.E.	0.0002	0.0141	0.0001	3085.4						

Table 2
Precision and accuracy for determination of analytes in spiked plasma.

Analyte	Concentration added (ng/mL)	Intra-day				Inter-day			
		Found (mean ± S.D.) (ng/mL)	Accuracy (%)	CV (%)	Relative error (%)	Found (mean ± S.D.) (ng/mL)	Accuracy (%)	CV (%)	Relative error (%)
(*R*)-Omeprazole	8	7.9 ± 0.6	98.9	7.1	−1.1	8.1 ± 0.5	98.8	4.9	−1.2
	40	39.8 ± 3.7	99.4	9.4	−0.6	43.3 ± 1.5	108.2	3.5	8.2
	450	442.4 ± 22.2	98.3	5.0	−1.7	444.8 ± 8.0	98.9	1.8	−1.2
	800	802.2 ± 25.4	100.3	3.2	0.3	845.3 ± 26.4	105.7	3.1	5.7
(*S*)-Omeprazole	8	8.4 ± 0.4	105.1	4.3	5.1	8.5 ± 0.4	106.5	4.3	6.3
	40	39.6 ± 3.6	98.9	9.0	−1.0	43.0 ± 3.3	107.6	7.6	7.6
	450	437.2 ± 21.9	97.2	5.0	−2.9	449.0 ± 8.2	99.8	1.8	−0.2
	800	792.6 ± 29.1	99.1	3.7	−0.9	807.0 ± 26.7	100.9	3.3	0.9
(*R*)-5-hydroxyomeprazole	8	7.4 ± 0.7	93.0	9.2	−7.0	7.8 ± 0.6	97.7	8.0	−3.0
	40	40.1 ± 3.6	100.2	6.5	0.2	40.6 ± 3.9	101.4	9.6	1.4
	125	132.7 ± 12.5	106.2	9.4	−6.2	136.7 ± 11.2	109.3	8.2	9.3
	200	210.9 ± 9.1	105.4	4.3	5.4	217.4 ± 14.3	108.7	6.6	8.7
(*S*)-5-hydroxyomeprazole	8	8.6 ± 0.9	107.6	9.9	7.6	8.3 ± 0.7	104.1	8.9	4.1
	40	42.7 ± 2.8	106.9	6.5	6.9	40.1 ± 2.9	100.2	7.3	0.2
	125	133.1 ± 7.9	106.5	5.9	6.5	130.5 ± 7.4	104.4	5.7	4.4
	200	210.1 ± 12.6	105.1	6.0	5.1	199.9 ± 13.5	99.9	6.7	−0.1
Omeprazole sulfone	8	7.7 ± 0.7	96.4	9.5	−3.7	7.8 ± 0.7	97.2	9.1	−2.8
	80	85.9 ± 4.6	107.4	5.3	7.4	76.2 ± 5.5	95.2	7.3	−4.8
	400	422.6 ± 37.4	105.7	8.9	5.7	407.8 ± 32.0	102.0	7.8	2.0
	800	791.6 ± 66.6	98.9	8.4	−1.1	778.4 ± 20.2	97.0	2.6	−2.7

Table 3
Pharmacokinetic parameters of (*R*)-, (*S*)-omeprazole and (*R*)-, (*S*)-5-hydroxyomeprazole.

	hmEMs		htEMs		PMs	
	n = 3		*n* = 7		*n* = 5	
	(*R*)-	(*S*)-	(*R*)-	(*S*)-	(*R*)-	(*S*)-
Omeprazole						
C_{max} (ng/ml)	217±63	299±168	554±293	639±322	968±143 *, # §	728±92
t_{max} (h)	2.5±1.3	2.5±1.3	2.6±1.2	2.6±1.2	2.6±1.3	2.5±1.0
AUC (0-8) (ng*h/ml)	489±102	623±263	1733±742	1852±802	5304±2214 *, # §	2618±923 *
$t_{1/2}$ (h)	0.6±0.1	0.5±0.3	0.7±1.2 §§§	0.3±0.2	1.2±0.4 *, # §	0.7±0.2 #
5-hydroxyomeprazole						
C_{max} (ng/ml)	832±555	14.4±8.3	826±293 §§§	40.2±17.5	105±37 *, # §§	28.3±30.8
AUC (0-8) (ng*h/ml)	1989±590 §§	15.7±3.6	3201±963 §§§	148±82	831±403 *, # §	166±243 *
$t_{1/2}$ (h)	0.4±0.2	0.8±0.5	0.6±0.2	0.6±0.4	2.7±1.6	0.9±0.8
Hydroxylation index	0.2±0.1 §	41.7±18.9	0.6±0.3 §	17.0±16.0	6.6±1.2 ***, ###	131.2±131.9

Data are shown as mean ± S.D. values. The hydroxylation index was calculated as $AUC_{(0-8)}$ of 5-hydroxyomeprazole/$AUC_{(0-8)}$ of omeprazole, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the homozygous EM group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ compared with the heterozygous EM group. $^{\S}P < 0.05$, $^{\S\S}P < 0.01$, $^{\S\S\S}P < 0.001$ between with the *R*-, *S*-enantiomers.

Table 4
Pharmacokinetic parameters of omeprazole sulfone.

	hmEMs $n=3$	htEMs $n=7$	PMs $n=5$
C_{max} (ng/mL)	88 ± 32	227 ± 80	450 ± 133$^{***,\#}$
$AUC_{(0-8)}$ (ng/mL h)	608 ± 298	2136 ± 942	4713 ± 1880$^{**,\#}$
$t_{1/2}$ (h)	2.1 ± 0.4	2.1 ± 1.3	9.8 ± 12.8

Data are shown as mean ± S.D. values.
$^{*}P<0.05$ compared with the homozygous EM group.
** $P<0.01$ compared with the homozygous EM group.
*** $P<0.001$ compared with the homozygous EM group.
$^{\#}$ $P<0.05$ compared with the heterozygous EM group.
$^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ compared with the heterozygous EM group.

least 6 months. When these solutions were spiked in the plasma blank, these compounds were also stable at −30 °C for at least 6 months. Additionally, the omeprazole enantiomers, 5-hydroxyomeprazole enantiomers, omeprazole sulfone and I.S. in the extracts from the reconstituted plasma samples were stable at ambient temperature for 72 h in the autosampler.

3.3. Applications for pharmacokinetic studies

The PM peak concentrations of (*R*)-, and (*S*)-omeprazole were greater than the EM peak concentrations of these compounds. Similarly, the plasma concentration of omeprazole sulfone in PMs was also significantly higher than that found in hmEMs and htEMs. Additionally, although the plasma concentration of (*R*)-5-hydroxyomeprazole was determined in every sample point for every subject in the present study, (*S*)-5-hydroxyomeprazole was not always determined at each sample point. A previous report has shown that, *in vitro*, CYP2C19 is mainly catalysed by 5-hydroxylation to (*R*)-omeprazole, whereas 5-*O*-desmethylation is the primary catalysis reaction for (*S*)-omeprazole [17]. Thus, our *in vivo* results are in agreement with previous reports and significant differences were observed in the pharmacokinetic parameters between (*R*)- and (*S*)-5-hydroxyomeprazole for the three CYP2C19 genotypes (Tables 3 and 4). However, since we could not detect the metabolite 5-*O*-desmethylomeprazole from (*S*)-omeprazole, a further study in relation to stereoselective metabolism of omeprazole will be needed.

Omeprazole sulfone, an achiral metabolite of omeprazole, is predominantly metabolised by CYP3A4 [1,2]. The data from the present study suggest that the plasma concentration of omeprazole sulfone in PMs is significantly higher than that found in hmEMs and htEMs. The relative AUC ratios that were previously reported for omeprazole sulfone in the hmEMs, htEMs and PMs were 1:2.2:8.3 [18], whereas those for the present data were 1:3.5:7.6, respectively. These results are also in agreement with at least one previous study.

Of all previous reports, only one study pertains to an analytical method that was developed for the simultaneous determination of omeprazole, 5-hydroxyomeprazole enantiomers, and omeprazole sulfone [16], however, this previous method did not simultaneously assess the pharmacokinetic parameters for (*R*)- and (*S*)-omeprazole and their chiral metabolites. To our knowledge, the present study is the first report of the simultaneous determination of the pharmacokinetic parameters for (*R*)- and (*S*)-omeprazole, their chiral metabolites and omeprazole sulfone, a racemic metabolite.

4. Conclusions

The HPLC procedure described in the present study for the simultaneous determination of (*R*)-omeprazole, (*S*)-omeprazole, (*R*)-5-hydroxyomeprazole, (*S*)-5-hydroxyomeprazole and omeprazole sulfone is suitable for routine analyses. Satisfactory validation data were achieved for the linearity, precision and recovery of this method. The limit of quantification that was obtained may determine the *in vivo* pharmacokinetics of chiral omeprazole and, this compound's chiral and non-chiral metabolites.

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