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Determination of lansoprazole and two of its metabolites by liquid–liquid extraction and automated column-switching high-performance liquid chromatography: application to measuring CYP2C19 activity

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Abstract

A simple and sensitive column-switching high-performance liquid chromatographic (HPLC) method for the simultaneous determination of lansoprazole, a proton pump inhibitor and its major metabolites: 5-hydroxylansoprazole and lansoprazole sulfone in human plasma. The test compounds were extracted from 1 mL of plasma using diethyl ether–dichloromethane (7:3, v/v) mixture and the extract was injected into a column I (TSK-PW precolumn, 10 μm, 3.5 mm × 4.6 mm i.d.) for clean-up and column I (C_{18} STR ODS-II analytical column, 5 μm, 150 mm × 4.6 mm i.d.) for separation. The peak was detected by a ultraviolet detector set at a wavelength of 285 nm, and the total time for a chromatographic separation was ∼25 min. The method was validated for the concentration range from 3 to 5000 ng/mL. Mean recoveries were 74.0% for lansoprazole, 68.3% for 5-hydroxylansoprazole, and 79.4% for lansoprazole sulfone. Intra- and inter-day relative standard derivatives were less than 6.1 and 5.1% for lansoprazole, 5.8 and 5.8% for 5-hydroxylansoprazole, 4.4 and 5.9% for lansoprazole sulfone, respectively, at the different concentration ranges. This method is suitable for use in therapeutic drug monitoring and pharmacokinetic studies, and provides use tool for measuring CYP2C19 activity.

Keywords: Lansoprazole; Metabolite; HPLC; CYP2C19

1. Introduction

Lansoprazole [2-{(3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl)methyl}sulfinylbenzimidazole], structurally related to omeprazole, is a substituted benzimidazole and is a proton pump inhibitor that suppresses gastric acid secretion through an interaction with (H^+/K^+)-ATPase in gastric parietal cells [1]. Lansoprazole is effective in the treatment of various peptic diseases, including gastric and duodenal ulcer and reflux esophagitis, Zollinger–Ellison syndrome [2]. A combination of clarithromycin and amoxycillin with lansoprazole has proven to be highly effective in the eradication of *H. pylori* [3].

Lansoprazole is extensively metabolized in the liver; the major metabolites present in plasma are 5-hydroxylansoprazole and lansoprazole sulfone [4,5]. Formation of the 5-hydroxy metabolite is mainly by cytochrome P4502C19 (CYP2C19), whereas CYP3A4 is involved in the formation of the sulfone [4,5]. The area under the plasma concentration–time curve (AUC) values of poor metabolizers (PMs) of CYP2C19 were more than four times greater than those of extensive metabolizers (EMs) [6]. Several clinical trials have suggested that the eradication rate of *H. pylori* is influenced by CYP2C19 activity [7], because of higher plasma concentration of lansoprazole and hence higher intragastric pH in PMs [8]. Therefore, it is clinically important to measure CYP2C19 activity using the hydroxylation and sulfoxidation indexes of lansoprazole, which reflects phenotype, and genotype of CYP2C19 [9]. Omeprazole hydroxylation

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index has widely been used as a marker of CYP2C19 activity [10,11]. However, it is now difficult to obtain pure chemicals of metabolites of omeprazole from pharmaceutical company. Therefore, alternative biomarker of CYP2C19 activity other than omeprazole hydroxylation index is required.

Several high-performance liquid chromatographic (HPLC) methods for the determination of lansoprazole and lansoprazole metabolites concentrations have been previously published [12,13]. However, sample preparations in those methods have complicated, and compounds are determined at different wavelengths. Although Karol et al. [14] have reported a simple extraction procedure and fixed wavelength, relatively high quantification limit (10 ng/mL) is not enough to obtain concise pharmacokinetic parameters of lansoprazole metabolites. In the present study, we describe a simple and sensitive column-switching HPLC method for determination of lansoprazole and lansoprazole metabolites in plasma using liquid–liquid extraction. The assay is suitable for use in therapeutic drug monitoring with reference to the CYP2C19 genotypic status.

2. Experimental

2.1. Chemicals

Lansoprazole and its metabolite 5-hydroxylansoprazole, and lansoprazole sulfone (Fig. 1) were kindly provided by Takeda Chemical Industries (Osaka, Japan). Omeprazole, the internal standard (Fig. 1) was kindly donated by Dr. Kobayashi, Chiba University, Japan. The purity of these materials was more than 99.5%. Potassium phosphate monobasic, acetonitrile, methanol, diethyl ether, and dichloromethane were purchased from Wako Pure Chemical Industries (Osaka, Japan). Water was deionized and purified using a Milli-Q system (MP-650, IWAKI Millipore, Tokyo, Japan).

2.2. Drug solutions

Stock solutions of lansoprazole, metabolite 5-hydroxylansoprazole, lansoprazole sulfone, and I.S. for generating standard curves were prepared by dissolving an appropriate amount of each compound in methanol to yield concentrations of 1.0 mg/mL. A very high working standard solution of lansoprazole (100 μg/mL) was obtained by 10 times diluting each stock solution with methanol. High working standard solutions of lansoprazole, metabolite 5-hydroxylansoprazole, lansoprazole sulfone, and I.S. (10 μg/mL) were obtained by 100 times diluting each stock solution with methanol. Middle (1.0 μg/mL) and low (0.1 μg/mL) working standard solutions of lansoprazole, 5-hydroxylansoprazole, and lansoprazole sulfone were obtained by further 10 and 100 times diluting each the working standard solution with methanol, respectively. Stock solutions were stable at 4 °C for at least three months. Drug-free plasma from healthy donors was used for validation studies. Calibration curves were prepared by spiking 10–50 μL of working solutions in 1 mL of blank plasma (final volume) to yield the final concentrations 1, 3, 5, 10, 50, 100, 500, 2000, and 5000 ng/mL for each analysis. Standard curves were prepared daily and constructed by linear regression analysis of the compounds/internal standard peak–height ratio versus the respective concentration of lansoprazole, 5-hydroxylansoprazole, and lansoprazole sulfone. Stock solution of lansoprazole, 5-hydroxylansoprazole, and lansoprazole sulfone were separately prepared for quality

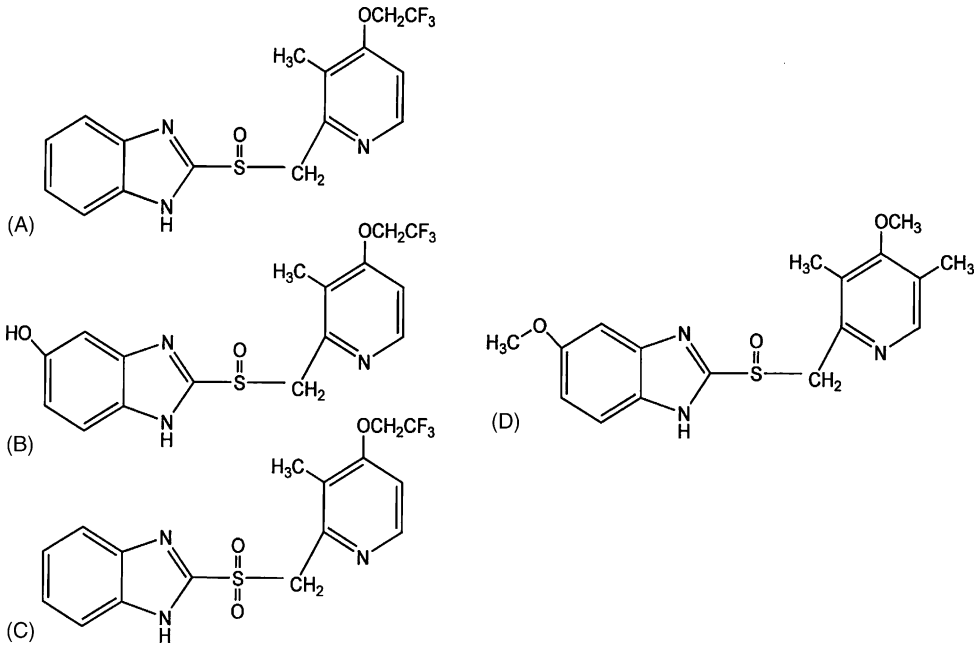

Fig. 1. Chemical structures of lansoprazole (A), 5-hydroxylansoprazole (B), lansoprazole sulfone (C), and omeprazole (D) as an internal standard.

controls in the same manner for standard curves. Quality control samples were obtained by spiking 10–50 μL of working plasma solutions in 1 mL of blank plasma (final volume) to yield the final concentrations range of 3, 5, 100, and 5000 ng/mL for lansoprazole, 5, 10, and 100 ng/mL for 5-hydroxylansoprazole, 3, 10, and 100 ng/mL for lansoprazole sulfone, and stored at −20 °C until analysis. All standard curves were checked using these quality control samples.

2.3. Subjects and CYP2C19 genotyping

Eighteen unrelated healthy subjects (7 women and 11 men) were enrolled in the current panel study. They ranged in age from 21 to 41 years and in weight from 40 to 78 kg. None had taken any drugs for at least one week before and during the study. Each subject was physically normal and had no antecedent history of significant medical illness or hypersensitivity to any drugs. Their health status was judged to be normal on the basis of physical examination with screening of blood chemistries, a complete blood count and urinalysis, and an electrocardiogram before the study. The study protocol was approved by Ethical Committee of Hirosaki University Hospital and a written informed consent was given from each subject.

The genotype of CYP2C19 was identified using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis as previously described [15]. Subjects were genotypically classified into the following three groups on the basis of PCR-RFLP analysis for CYP2C19 [15]: Homozygous (CYP2C19*1/*1) EM group (hmEMs, $n = 6$), heterozygous (CYP2C19*1/*2, CYP2C19*1/*3) EM group (htEMs, $n = 6$), and PM (CYP2C19*2/*2, CYP2C19*2/*3,) group (PMs, $n = 6$).

2.4. Sample collections

Two capsules containing 60 mg of lansoprazole as enteric for-coated granules (lansoprazole capsule, 30 mg) was orally administered to each of 15 healthy volunteers. Blood samples were obtained before and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after the dosing. Blood samples were collected in heparinized tubes and centrifuged immediately at 2500 × *g* for 10 min. The plasma was stored at −20 °C until analysis.

2.5. Apparatus

The column-switching HPLC system consisted of two Shimadzu LC-10AT high-pressure pumps (for eluent A and B), and a Shimadzu CTO-10AVP column oven and a Shimadzu Work station CLASS-VP chromatography integrator (Kyoto, Japan) and a Shimadzu SPD-10AVP (Kyoto, Japan) and a Shimadzu SIL-10ADVP (500-μL injection volume) (Tokyo, Japan). A TSK gel PW precolumn (a hydrophilic metaacrylate polymer column) for sample clean-up (column I: 35 mm × 4.6 mm i.d., particle size 10 μm; Tosoh, Tokyo, Japan) and a C_{18} STR ODS-II column as an analytical column (column II: 150 mm × 4.6 mm i.d., particle size 5 μm; Shinwa Chemical Industry, Kyoto, Japan) were used.

2.6. Extraction procedure

I.S. (omeprazolel) 100 μL of 2.5 μg/mL and 0.5 mL NaOH (0.001 M) were added to 1 mL of plasma. The tubes were vortex-mixed for 10 s and 5 mL of diethyl ether–dichloromethane (70:30, v/v) was added as extraction solvent. After 10 min of shaking, the mixture was centrifuged at 1700 × *g* for 10 min at 4 °C, and the organic phase was evaporated in vacuo at 60 °C to dryness (TAITEC VC-960, Shimadzu, Kyoto, Japan). The residue was dissolved with 50 μL of eluent A and 50 μL of methanol used as an extract.

2.7. Chromatographic condition

Column-switching chromatographic condition was set based on our previous report [16]. An 80 μL portion of the extract was automatically injected into the HPLC system. The column-switching system and flow-rates were operated according to the time program depicted in Table 1. From 0 to 7.0 min after the sample injection were separated from the interfering substances present in the extract on column I with a mobile phase (eluent A) of phosphate buffer (0.02 M, pH 4.6), acetonitrile (90:10, v/v). Between 7.0 and 12 min after the injection, lansoprazole, 5-hydroxylansoprazole, lansoprazole sulfone, and I.S. retained on column I were eluted with a mobile phase (eluent B) of phosphate buffer (0.02 M, pH 4.6) and acetonitrile, and methanol (55:40:5, v/v), and effluent from column I was switched to column II. Then lansoprazole, 5-hydroxylansoprazole, lansoprazole sulfone, and omeprazole were separated on column II by eluting with eluent B (between 15 and 25 min). The flow-rates were 0.7 mL/min for 0–16 min and 1.2 mL/min for 16–25 min for eluent B, and 1.2 mL/min for eluent B, respectively. The temperatures of columns I and II were 40 °C, respectively. The peak was detected by a ultraviolet detector set at a wavelength of 285 nm. The peak area was used for the quantification of lansoprazole, 5-hydroxylansoprazole, and lansoprazole sulfone.

2.8. Statistical analyses

Percentages of control in pharmacokinetic parameters between three genotype groups were compared using one-way ANOVA followed by Bonferroni's correction. A *P* value of

Table 1
Time program for the column switching HPLC

Time after injection (min)	Column I		Column II	
	Mobile phase	Flow rate (mL/min)	Mobile phase	Flow rate (mL/min)
0.0–7.0	A	1.2	B	0.7
7.0–12.0	B	0.7	B	0.7
12.0–16.0	A	1.2	B	0.7
12.0–25.0	A	1.2	B	1.2

0.05 or less was regarded as significant. SPSS 8.0.1 for Windows (SPSS Japan Inc., Tokyo) was used for these statistical analyses.

3. Results and discussion

3.1. Chromatography

The chromatogram of an extracted blank plasma sample is shown in Fig. 2B, while the chromatogram of an extracted sample spiked with 5 ng/mL of lansoprazole, 5-hydroxylansoprazole, lansoprazole sulfone, and I.S. is shown in Fig. 2C. Several compounds were well separated from each other and from the front of the solvent peaks even though it is a little time consuming. The chromatograms of extracted plasma samples obtained from one volunteer receiving 60 mg lansoprazole did not show interference peaks (Fig. 2D). However, there was an interfering peak, probably another metabolite, with 5-hydroxylansoprazole peak without column-switching system (Fig. 2E).

3.2. Recovery and linearity

Recovery from plasma was calculated by comparing the peak areas of pure standards prepared in purified water, and injected directly into the analytical column with those of extracted plasma samples containing the same amount of the test compound ($n = 6$ each). Mean recoveries were 74.0% for lansoprazole, 68.3% for 5-hydroxylansoprazole, and 79.4% for lansoprazole sulfone. Their CV values were determined at three different concentrations ranging from 3 to 5000 ng/mL for lansoprazole, from 5 to 100 ng/mL for 5-hydroxylansoprazole, from 3 to 100 ng/mL for lansoprazole sulfone. Their CV values were less than 0.7% for lansoprazole, 0.8% for 5-hydroxylansoprazole, and 0.5% for lansoprazole sulfone, respectively. Calibration curves were linear over the concentrations range from 3 to 5000 ng/mL ($r = 0.9999$ for lansoprazole and $r = 0.9997$ for lansoprazole sulfone), from 5 to 5000 ng/mL ($r = 0.9997$ for 5-hydroxylansoprazole) (Table 2).

3.3. Sensitivity

The limit of detection was defined, as analyte responses are at least three times the response compared to blank response. The lowest standard on the calibration curve was defined as the limit of quantification as analyte peaks for both compounds were identifiable, discrete, and reproducible with a precision of 20% and accuracy of 80–125%. The limits of detections were 3 ng/mL for 5-hydroxylansoprazole, 1 ng/mL for lansoprazole and lansoprazole sulfone. The limits of quantification were 5 ng/mL for 5-hydroxylansoprazole, 3 ng/mL for lansoprazole and lansoprazole sulfone.

3.4. Precision and accuracy

Intra- and inter-day precision and accuracy were evaluated by assaying quality controls with different concentrations of lansoprazole, 5-hydroxylansoprazole, and lansoprazole sulfone. Intra- and inter-day precisions were assessed by analyzing six quality control samples at each concentration on the same day and mean values of two quality controls for six days, respectively. These extracts underwent the same column-switching procedure. Intra- and inter-day relative standard deviations were less than 2.8 and 5.3% for lansoprazole, 2.8

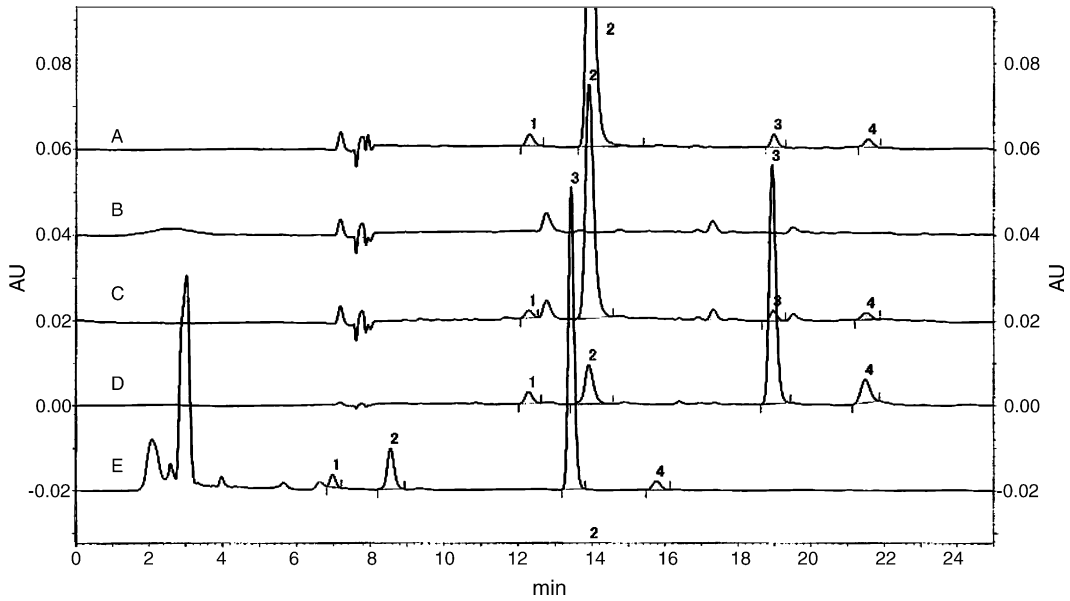

Fig. 2. The chromatogram of working solution (A), an extracted blank plasma sample (B), an extracted sample spiked with 5 ng/mL of lansoprazole, 5-hydroxylansoprazole, lansoprazole sulfone, and I.S. (C), extracted plasma samples obtained from one volunteer receiving 60 mg lansoprazole (D) and the same sample as D without column-switching system (E). Peaks: (1) 5-hydroxylansoprazole, (2) I.S., (3) lansoprazole, and (4) lansoprazole sulfone.

Table 2
Individual and mean values for slope, intercepts, and correlation coefficients of five calibration curves for analytes

Analyte	Curve	Slope	Intercepts	*r*
Lansoprazole	1	0.0038	0.0068	0.9998
	2	0.0031	0.0046	1
	3	0.0030	0.0038	1
	4	0.0027	0.0029	0.9998
	5	0.0031	0.0013	0.9999
	Mean	0.0032	0.0041	
	S.D.	0.0004	0.0020	
	S.E.	0.0002	0.0009	
5-Hydroxylansoprazole	1	0.0016	0.0034	0.9998
	2	0.0012	0.0011	0.9999
	3	0.0015	−0.0030	1
	4	0.0009	0.0001	0.9998
	5	0.0014	−0.0036	0.9992
	Mean	0.0013	−0.0004	
	S.D.	0.0002	0.0026	
	S.E.	0.0001	0.0012	
Lansoprazole sulfone	1	0.0028	0.0100	0.9997
	2	0.0049	0.0005	0.9998
	3	0.0027	0.0005	0.9999
	4	0.0034	0.0049	0.9999
	5	0.0033	0.0013	0.9993
	Mean	0.0034	0.0031	
	S.D.	0.0009	0.0045	
	S.E.	0.0004	0.0020	

and 5.3% for 5-hydroxylansoprazole, and 2.4 and 4.4% for lansoprazole sulfone, respectively (Table 3). Accuracy was expected as percent error (relative error) [(measured concentration − spiked concentration)/spiked concentration] × 100 (%), while precisions was quantitated by calculating intra- and inter-CV values.

3.5. Specificity

Potential interference from co-administered drugs was investigated by determining their retention times in this system. No peaks were observed until 60 min after injections of extracts with clarithromycin and amoxycillin. Also, their metabolites were not investigated.

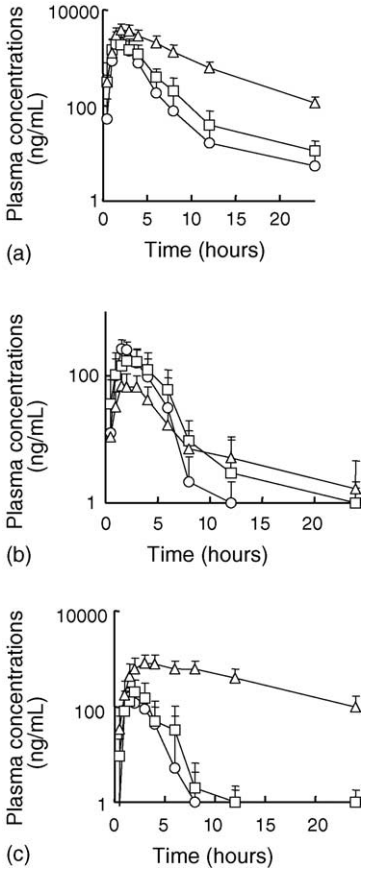

Fig. 3. Plasma concentration–time curves (mean ± S.D.) of lansoprazole (a), 5-hydroxylansoprazole (b), and lansoprazole sulfone (c) from 0 to 24 h to 18 healthy volunteers after a single-oral dose of lansoprazole 60 mg. Circles, squares, and triangles indicate values of homozygous EMs, heterozygous EMs and PMs, respectively.

3.6. Drug concentrations in human plasma

Fig. 3 shows concentration versus time curves obtained after an oral administration of lansoprazole (60 mg) to three different CYP2C19 genotype groups. The mean kinetic parameters of lansoprazole and its primary metabolites, 5-hydroxylansoprazole, and lansoprazole sulfone, in the three

Table 3
Precision (CV) and accuracy (relative error) for determination of analytes in spiked plasma ($n = 6$)

Analyte	Concentration added (ng/mL)	Between-day		Within-day	
		CV (%)	Relative error (%)	CV (%)	Relative error (%)
Lansoprazole	3	5.14	−0.35	6.14	4.51
	10	4.05	−0.23	4.65	−0.31
	100	4.76	0.97	1.85	0.24
	5000	2.27	−0.162	2.72	−0.72
5-Hydroxylansoprazole	5	5.79	−2.45	5.77	4.09
	10	3.31	6.4	4.65	4.05
	100	2.92	−1.29	3.67	−3.93
Lansoprazole sulfone	3	5.86	0.35	4.36	3.05
	10	1.83	3.65	1.88	−1.65
	100	2.08	−1.27	3.11	0.43

Table 4
Pharmacokinetic parameters of lansoprazole and its two primary metabolites in the three different CYP2C19 genotyping groups receiving lansoprazole 60 mg dose

	Homozygous EMs	Heterozygous EMs	PMs
Lansoprazole			
C_{max} (ng/mL)	2685 ± 971	2690 ± 1327	4231 ± 1519
T_{max} (h)	1.8 ± 0.7	1.9 ± 0.9	2.3 ± 0.6
$T_{1/2}$ (h)	1.0 ± 0.3	1.7 ± 0.5	3.7 ± 1.4$^{***,\#\#}$
AUC_{0-24} (ng h/mL)	6373 ± 3446	8299 ± 3669	26666 ± 8058$^{***,\#\#\#}$
5-Hydroxylansoprazole			
C_{max} (ng/mL)	293 ± 91	197 ± 44*	82 ± 28$^{***,\#}$
T_{max} (h)	1.9 ± 0.6	1.9 ± 0.9	2.2 ± 0.6
AUC_{0-24} (ng h/mL)	752 ± 412	665 ± 363	359 ± 216
Hydroxylation index	0.12 ± 0.03	0.09 ± 0.05	0.02 ± 0.01$^{**,\#\#}$
Lansoprazole sulfone			
C_{max} (ng/mL)	219 ± 174	238 ± 152	883 ± 482$^{**,\#\#}$
T_{max} (h)	1.7 ± 0.7	2.2 ± 0.9	3.5 ± 0.5$^{**,\#}$
AUC_{0-24} (ng h/mL)	381 ± 374	557 ± 555	9536 ± 5473$^{***,\#\#}$
Sulfoxidation index	0.05 ± 0.03	0.07 ± 0.04	0.33 ± 0.13$^{***,\#\#\#}$

AUC, area under plasma concentration–time curve; C_{max}, peak concentration; T_{max}, time to C_{max}; $T_{1/2}$, elimination half-life; hydroxylation index, AUC (5-hydroxylansoprazole)/AUC (lansoprazole); sulfoxidation index, AUC (lansoprazole sulfone)/AUC (lansoprazole).
* $P < 0.05$ 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.
$^{\#\#}$ $P < 0.01$ compared with the heterozygous EM group.
$^{\#\#\#}$ $P < 0.001$ compared with the heterozygous EM group.

genotype groups are summarized in Table 4. There were significant differences ($P < 0.001$) between hmEMs and PMs, and between htEMs and PMs in AUC_{0-24} of lansoprazole. The relative AUC ratio values of lansoprazole in hmEMs, htEMs, and PMs were 1:1.3:4.2, respectively. The mean pharmacokinetic values for lansoprazole sulfone differed among the three groups. In contrast, the tendency of pharmacokinetic parameters for 5-hydroxylansoprazole was opposite to that observed for lansoprazole and lansoprazole sulfone. Significant differences in hydroxylation indexes and sulfoxidation indexes were found between different genotypes.

4. Conclusion

The HPLC procedure described for determination of lansoprazole, 5-hydroxylansoprazole, and lansoprazole sulfone is suitable for routine analysis even though it is a little time consuming. Satisfactory validation data were achieved for linearity, precision, and recovery. The limit of quantification obtained allows measurement of therapeutic concentration of lansoprazole and its metabolites and application of measuring CYP2C19 activity with reference to the CYP2C19 genotypic status.

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