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Simultaneous determination of lansoprazole enantiomers and their metabolites in plasma by liquid chromatography with solid-phase extraction

Masatomo Miura, Hitoshi Tada, Toshio Suzuki*

Department of Pharmacy, Akita University Hospital, 1-1-1 Hondo, Akita 010-8543, Japan

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Abstract

A simple and highly sensitive high-performance liquid chromatography (HPLC) method for the simultaneous quantitative determination of lansoprazole enantiomers and their metabolites, 5-hydroxylansoprazole enantiomers and lansoprazole sulfone, in human plasma have been developed. Chromatographic separation was achieved with a Chiral CD-Ph column using a mobile phase of 0.5 M $NaClO_4$–acetonitrile–methanol (6:3:1 (v/v/v)). The analysis required only 100 μl of plasma and involved a solid-phase extraction with Oasis HLB cartridge, with a high extraction recovery (>94.1%) and good selectivity. The lower limit of quantification (LOQ) of this assay was 10 ng/ml for each enantiomer of both lansoprazole and 5-hydroxylansoprazole, and 5 ng/ml for lansoprazole sulfone. The coefficient of variation of inter- and intra-day assay was <8.0% and accuracy was within 8.4% for all analytes (concentration range 10–1000 ng/ml). The linearity of this assay was set between 10 and 1000 ng/ml ($r^2 > 0.999$ of the regression line) for each of the five analytes. This method is applicable for accurate and simultaneous monitoring of the plasma levels of lansoprazole enantiomers and their metabolites in the renal transplant recipients.

Keywords: Enantiomer separation; Lansoprazole; 5-Hydroxylansoprazole; Lansoprazole sulfone

1. Introduction

Lansoprazole (**1**), 2-[(3-methyl-4-(2,2,2-trifluoethoxy)-2-pyridyl)methyl]sulfinyl-benzimidazole, is one of the proton pump inhibitors (PPIs) that inhibits gastric acid secretion through an interaction with (H^+/K^+)-ATPase in gastric parietal cells [1]. This drug, which has an asymmetric sulfur in its chemical structure, is clinically administered as a racemic mixture of *R*(+)- and *S*(−)-enantiomers (**1a** and **1b**, respectively). Lansoprazole is extensively metabolized in the human liver to 5-hydroxylansoprazole (**2a** and **2b**) and lansoprazole sulfone (**3**), which are mainly catalyzed by CYP2C19 and CYP3A4, respectively [2]. The intrinsic clearance (V_{max}/K_m), which is useful for the estimation of in vivo clearance rate [3], for (*R*)-5-hydroxylansoprazole (**2a**) formation from (*R*)-lansoprazole (**1a**) by human liver microsomes was much greater than that for **3** formation from **1a** (0.028 ± 0.002 versus 0.006 ± 0.000 ml/(min mg protein)) [4], indicating that sulfoxidation by CYP3A4 is a relatively minor pathway in **1a**. On the other hand, the V_{max}/K_m values for the hydroxylation and sulfoxidation of (*S*)-lansoprazole (**1b**) were almost the same (0.023 ± 0.002 versus 0.023 ± 0.001 ml/(min mg protein)) [4]. Consequently, the V_{max}/K_m value for the sulfoxidation of **1b** was 3.8-fold higher than that for **1a**, showing that enantioselective metabolism occurs in human liver microsomes. Further, in vivo studies in humans have shown that the mean area under the plasma concentration–time curve (AUC) values of **1a** were approximately 5.7- and 8.5-fold greater in CYP2C19 poor and extensive metabolizers, respectively, than those of **1b** after oral administration of 30 mg recemic **1** [5]. These in vitro and in vivo studies indicate that the metabolic rate of **1b** in human is much greater than that of **1a**. Thus, the stereoselective differences in the biotransformation of **1a** and **1b** in humans have been previously studied using chiral HPLC columns (Chiralcel OD and Chiralpak AS) [5,6]. However, this previous method cannot assay **1a**, **1b**, **2**, and **3** simultaneously and requires two separate HPLC systems for the enantiomers of **1** and its metabolites, respectively. Although only one published analytical method for the simultaneous determination of **1a**, **1b**, and their metabolites

* Corresponding author. Fax: +81-18-836-2628.
E-mail address: suzuki@hos.akita-u.ac.jp (T. Suzuki).

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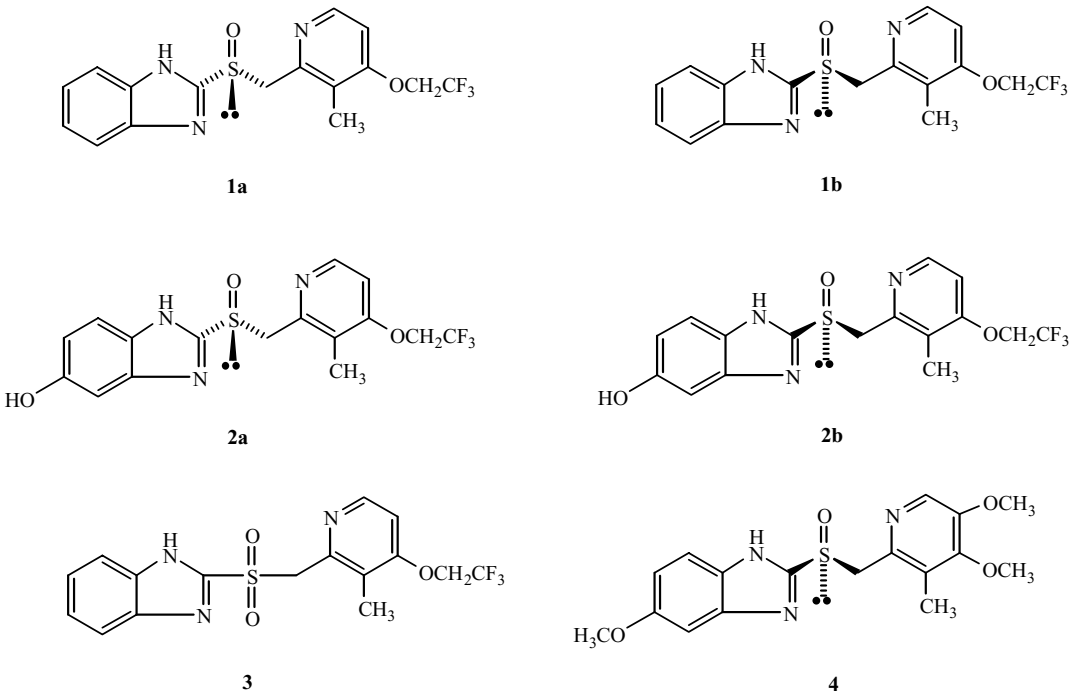

Fig. 1. Chemical structures of (*R*)-lansoprazole (**1a**), (*S*)-lansoprazole (**1b**), (*R*)-5-hydroxylansoprazole (**2a**), (*S*)-5-hydroxylansoprazole (**2b**), lansoprazole sulfone (**3**), and (*S*)-omeprazole (**4**).

is reported, but this was suitable for the analysis of microsomal samples, and cannot be applied for the plasma samples [7]. Up to now, no HPLC method for the simultaneous determination of **1a**, **1b**, and their metabolites in human plasma has been published. Therefore, a HPLC method for the simultaneous determination and the chiral separation of **1a**, **1b**, **2a**, **2b**, and **3** in human plasma was developed (Fig. 1).

On the other hand, the described methods for the chiral assay of **1** required a high amount of plasma (1 ml) and provided relatively low extraction recovery (70–78% for all analytes) by means of liquid–liquid extraction [5,6].

The method presented here is a rapid and simple solid-phase extraction method and selective HPLC method for the simultaneous determination of **1a**, **1b**, **2a**, **2b**, and **3** in human plasma. The proposed extraction procedure developed for the plasma sample pretreatment ensures high extraction recoveries and requires small amounts of plasma (100 μl) for a complete analysis. PPIs such as lansoprazole (**1**) are generally coadministered with tacrolimus in renal transplant recipients suffering from gastric ulcer disease [8]. The method was applied to investigate the pharmacokinetics of **1a**, **1b**, **2a**, **2b**, and **3** in renal transplant recipients receiving tacrolimus.

2. Experimental

2.1. Reagents and chemicals

Lansoprazole enantiomers and their metabolites (5-hydroxylansoprazole and lansoprazole sulfone) were purchased from Takeda Pharmaceutical (Osaka, Japan). (*S*)-Omeprazole used as the internal standard was kindly donated by the AstraZeneca (Mölndal, Sweden). The optical purity of (*S*)-omeprazole was 99.8–100% (data was provided by AstraZeneca). The Oasis HLB extraction cartridge was purchased from Waters (Milford, MA, USA). All solvents used were of HPLC grade (Wako Pure Chemical Industries, Osaka, Japan). All other reagents and chemicals were purchased from Wako Chemical Industries or Nacalai Tesque (Kyoto, Japan).

2.2. Apparatus

The apparatus used for HPLC was a Model 510 chromatography pump (Waters) equipped with a Waters 486 ultraviolet detector. The wavelength was set at 285 nm. Test samples were introduced using a Waters 712 WISP auto sampler with an effective volume of 50 μl. The HPLC column used was a Chiral CD-Ph (250 mm × 4.6 mm i.d., Shiseido, Tokyo, Japan). The mobile phase consisted of 0.5 M $NaClO_4$–acetonitrile–methanol (6:3:1 (v/v/v)), which was degassed in an ultrasonic bath prior to use. A flow-rate of 0.5 ml/min was used at ambient temperature.

2.3. Standard solutions and stability

Stock solutions of **1**, **2**, **3**, and internal standard (each 1 mg/ml) were prepared in methanol and kept in glass tubes at −20 °C. Stock solutions were used for the preparation of calibration standards and quality control samples to yield concentrations of 1.0–1000 ng/ml of **1**, **2**, and **3**. Quality

control samples were used to determine inter- and intra-day accuracy and precision. The stability for the lansoprazole in plasma was reported in previous papers [9,10]. Short-term stability showed that lansoprazole is stable in plasma at least 16 h at room temperature, while long-term stability studies showed that lansoprazole is stable in plasma for at least 64 days when stored at −20 °C.

2.4. Extraction method

After (*S*)-omeprazole (20 ng) in methanol (10 μl) was added to plasma samples (100 μl) as an internal standard, the plasma samples were diluted with 1.0 ml of water, and the solution was vortexed for 30 s. This mixture was applied to an Oasis HLB extraction cartridge that had previously been activated with 1.0 ml of methanol followed by 1.0 ml of water. The cartridge was then washed with 1.0 ml of water and 1.0 ml of 40% methanol in water. The cartridge was then eluted with 1.0 ml of 80% methanol in water. The eluate was evaporated to dryness in vacuum at 60 °C by a rotary evaporator (Iwaki, Tokyo, Japan). The residue was dissolved in 50 μl of methanol with vortex-mixing for 30 s and then 50 μl of mobile phase with vortex-mixing for 30 s. A 50 μl aliquot of the solution was injected into the HPLC apparatus.

2.5. Calibration graphs

Calibration curves were obtained for spiked blank plasma samples in the concentration range of 10–1000 ng/ml for **1a**, **1b**, **2a**, **2b**, and **3**. The blank plasma samples were treated according to the procedure described above. The calibration graphs were constructed of the peak-height ratios of **1a**, **1b**, **2a**, **2b**, and **3** to (*S*)-omeprazole as an internal standard of the HPLC chromatograms, and plotted against the nominal concentration of **1a**, **1b**, **2a**, **2b**, and **3**.

2.6. Recovery

The recovery following the extraction procedure was determined by comparing the peak areas of blank plasma sample extracted according to the above procedure with those of non-extracted control samples. The control samples were prepared by mixing solutions containing the same amount of the compounds spiked in blank plasma and were not extracted, but were directly evaporated to dryness and the residues were reconstituted in methanol.

2.7. Assay validation

Inter-day precision and accuracy was evaluated from the analysis of each control once on each of 6 different days, while intra-day precision and accuracy was evaluated by analyzing the spiked controls six times over 1 day in random order. The precision of the method at each concentration was evaluated by the coefficient of variation (CV) by calculating the standard deviation (S.D.) as a percentage of the mean calculated concentration, while the accuracy was estimated for each spiked control by comparing the nominal concentration with assayed concentration. The limit of quantification (LOQ) was determined at the lowest non-zero concentration measured with an intra-day CV of <20% and an accuracy of <±20% [11], while the limit of detection (LOD) was determined as the concentration with a signal to noise ratio of 3.

2.8. Application to pharmacokinetics studies

The method was used to quantitate the plasma **1a**, **1b**, **2a**, **2b**, and **3** concentrations in renal transplant recipients. This study was approved by the Ethics Committee of Akita University Hospital, and all patients gave written, informed consent. The 30 mg Takepron® brand of lansoprazole (Takeda) at 8 a.m. (30 min after the breakfast) was taken in renal transplant recipients receiving combination immunosuppressive therapy consisting of tacrolimus and mycophenolate mofetil, given in equally divided doses every 12 h in designated time (9 a.m. and 9 p.m.). Meals were served at 7:30 a.m., 12:30 p.m., and 6 p.m. daily. Meal content (Japanese food) varied each day and for each patient, but energy, fat, protein, and water content was standardized (energy: 1700–2400 kcal, protein: 70–90 g, fat: 40–50 g, water: 1600–2000 ml) depending on body weight. On day 28 after renal transplantation, whole blood samples (2 ml) were collected by vein puncture at 1, 2, 3, 4, 5, 7, 10, 13, and 25 h after oral lansoprazole administration. The plasma was separated by centrifugation at 1900 × *g* for 15 min and stored at −30 °C until analysis, which was usually carried out within 1 week. Patient plasma (100 μl) was extracted as previously described and injected into the HPLC system. A pharmacokinetic analysis of **1a**, **1b**, **2a**, and **3** were carried out by a standard noncompartmental method using WinNonlin (Pharsight Co., CA, version 3.1). The total area under the observed plasma concentration–time curve (AUC) was calculated by using the linear trapezoidal rule. The maximum plasma level (C_{max}) and time to reach the peak (t_{max}) were obtained directly from the profile.

3. Results

3.1. Chromatograms

Fig. 2 shows typical chromatograms of a blank plasma (A), spiked plasma sample with racemic **1**, **2** (each 20 ng/ml), and **3** (10 ng/ml) (B) and with 200 ng/ml for each analyte (C), and with different concentrations of analytes (200 ng/ml for **2**, 5 ng/ml for **3**, and 400 ng/ml for **1**) (D). The separation of **1a**, **1b**, **2a**, **2b**, **3**, and (*S*)-omeprazole (**4**) was satisfactorily obtained from interfering peaks in biological matrix by our extraction method and chromatographic system. Retention times for **2a**, **2b**, **4**, **3**, **1a**, and **1b** were 17.0, 18.5, 27.5, 30.0, 31.6, and 36.6 min, respectively.

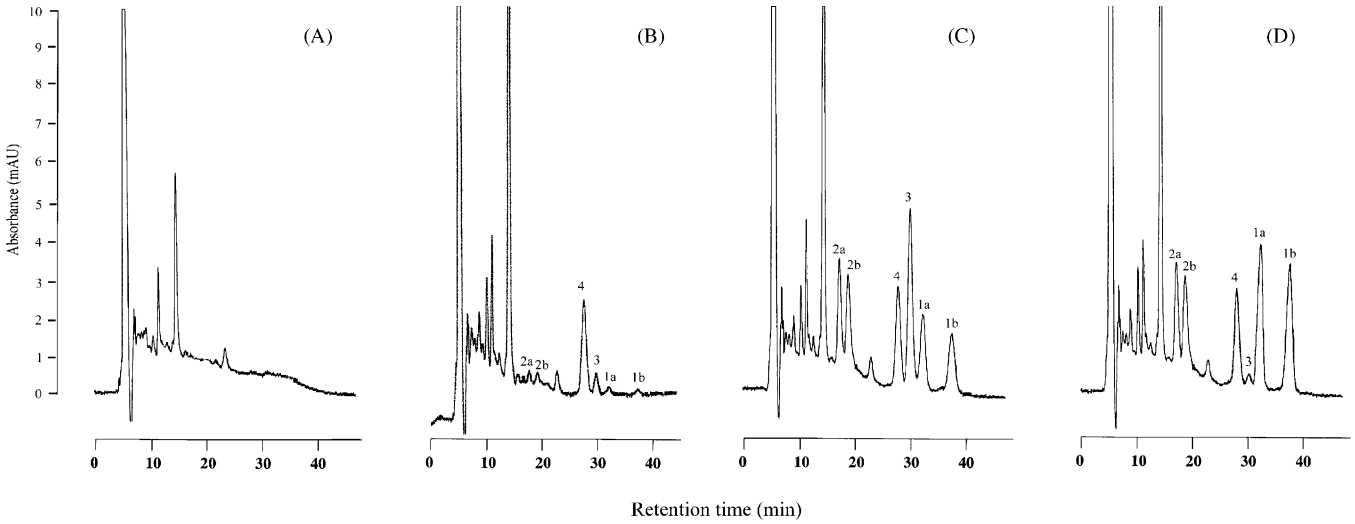

Fig. 2. Typical chromatograms of (A) plasma blank; (B) spiked with racemic **1** (2 ng), racemic **2** (2 ng), **3** (1 ng), and **4** (20 ng) in plasma (100 μl); (C) spiked with racemic **1** (20 ng), racemic **2** (20 ng), **3** (20 ng), and **4** (20 ng) in plasma (100 μl); and (D) spiked with racemic **1** (40 ng), racemic **2** (20 ng), **3** (0.5 ng), and **4** (20 ng) in plasma (100 μl).

Table 1
Accuracy and precision of the determination of lansoprazole enantiomers and their metabolites in human plasma (mean ± S.D., $n = 6$)

Compounds	Added (ng/ml)	Inter-day			Intra-day			Recovery (%)
		Found (mean ± S.D.)	CV (%)	Accuracy (%)	Found (mean ± S.D.)	CV (%)	Accuracy (%)	
(*R*)-5-Hydroxylansoprazole (**2a**)	10	10.7 ± 0.6	5.7	7.0	10.6 ± 0.6	5.6	6.1	94.9
	50	51.6 ± 2.5	4.9	3.1	48.9 ± 2.1	4.2	−2.2	96.2
	100	106 ± 4.6	4.3	6.1	105 ± 4.2	4.0	5.2	97.4
	200	199 ± 9.4	4.7	−0.5	208 ± 7.7	3.7	4.1	97.8
	500	493 ± 14	2.9	−1.5	514 ± 12	2.4	2.8	98.1
	1000	1022 ± 25	2.4	2.2	997 ± 25	2.5	−0.3	97.4
(*S*)-5-Hydroxylansoprazole (**2b**)	10	10.8 ± 0.6	5.6	8.4	10.7 ± 0.6	5.3	7.2	94.1
	50	51.2 ± 2.6	5.1	2.4	48.4 ± 2.2	4.5	−3.1	95.9
	100	98.3 ± 3.9	4.0	−1.8	102 ± 4.1	4.0	2.1	97.3
	200	210 ± 11	5.4	5.1	212 ± 8.7	4.1	6.1	97.7
	500	508 ± 15	2.9	1.6	505 ± 13	2.5	1.1	98.5
	1000	1021 ± 27	2.6	2.1	997 ± 25	2.5	−0.4	97.1
Lansoprazole sulfone (**3**)	5	5.1 ± 0.4	8.0	2.2	5.1 ± 0.4	7.5	1.8	94.1
	10	9.9 ± 0.5	5.0	−1.2	9.9 ± 0.5	4.7	−0.8	95.2
	50	48.6 ± 2.1	4.4	−2.8	49.0 ± 1.6	3.2	−2.0	96.8
	100	107 ± 2.2	2.1	6.5	102 ± 2.1	2.1	1.5	97.3
	200	195 ± 5.3	2.7	−2.4	196 ± 4.7	2.4	−1.9	96.7
	500	485 ± 19	4.0	−3.1	496 ± 18	3.7	−0.8	98.3
	1000	983 ± 25	2.5	−1.7	1040 ± 22	2.1	3.9	98.0
(*R*)-Lansoprazole (**1a**)	10	9.5 ± 06	5.9	−4.9	9.7 ± 0.6	5.7	−3.3	94.8
	50	50.8 ± 3.0	5.8	1.8	51.8 ± 2.6	5.7	3.7	95.2
	100	98.8 ± 47	4.8	−1.2	99.9 ± 4.3	4.3	−0.0	96.1
	200	195 ± 6.1	3.1	−2.4	201 ± 4.8	2.4	0.5	97.4
	500	492 ± 11	2.3	−1.5	508 ± 12	2.3	1.7	96.9
	1000	988 ± 34	3.4	−1.1	1021 ± 30	2.9	2.1	97.2
(*S*)-Lansoprazole (**1b**)	10	9.7 ± 0.6	5.9	−3.2	9.8 ± 0.6	5.6	−2.1	95.2
	50	48.8 ± 2.8	5.7	−2.4	51.3 ± 2.9	5.7	2.6	95.2
	100	103 ± 5.6	5.4	3.0	104 ± 4.5	4.3	3.8	96.8
	200	201 ± 10	5.1	−0.6	204 ± 8.6	4.2	2.1	98.2
	500	505 ± 16	3.2	1.0	496 ± 17	3.4	−0.8	97.4
	1000	1024 ± 43	4.2	2.4	987 ± 39	4.0	−1.3	97.9

3.2. Calibration curves

The calibration curves for **1a**, **1b**, **2a**, **2b**, and **3** in plasma were linear in the concentration range of 10–1000 ng/ml. The mathematical expression (obtained by means of the least-squares method) of the curves for **2a**, **2b**, **3**, **1a**, and **1b** can be expressed by the following equations: $y = 0.0054(\pm0.0002)x + 0.0046(\pm0.0028)$ ($r^2 = 0.9999$), $y = 0.0041(\pm0.0002)x + 0.0023(\pm0.0026)$ ($r^2 = 0.9998$), $y = 0.0082(\pm0.0003)x + 0.006(\pm0.0034)$ ($r^2 = 0.9999$), $y = 0.0028(\pm0.0001)x + 0.0047(\pm0.0031)$ ($r^2 = 1.0000$), and $y = 0.0023(\pm0.0001)x + 0.0018(\pm0.0025)$ ($r^2 = 0.9999$), respectively, y being the peak height ratio and x being the concentration in ng/ml.

3.3. Recovery

The results of recovery studies are shown in Table 1. The recovery of **1a**, **1b**, **2a**, **2b**, and **3** were determined by adding six known concentrations (10, 50, 100, 200, 500, and 1000 ng/ml) to drug-free plasma. The mean extraction recovery values for **2a**, **2b**, **1a**, and **1b** were 94.9–98.1, 94.1–98.5, 94.8–97.4, and 95.2–98.2%, respectively, in the concentration ranges of 10–1000 ng/ml, while that for **3** was 94.1–98.3% in the 5–1000 ng/ml concentration range.

3.4. Precision and accuracy

The coefficients of variation and accuracy for the intra- and inter-day assay of this method were determined at concentration of 10–1000 ng/ml for **1a**, **1b**, **2a**, and **2b**, and 5–1000 ng/ml for **3**. The coefficients of variation for the intra- and inter-day assay for **1a**, **1b**, **2a**, **2b**, and **3** were all less than 8.0% (Table 1). The accuracy for the intra- and inter-day assay was within 4.9% for **1a** and 3.8% for **1b**. The values of % error of metabolites were within 7.0% for **2a**, 8.4% for **2b**, and 6.5% for **3**. The accuracy and precision of the proposed method were defined from these results.

3.5. Sensitivity

The lower limit of quantification for **1a**, **1b**, **2a**, and **2b** was 10 ng/ml, whereas that for **3** was 5.0 ng/ml. The limit of detection for **1a**, **1b**, **2a**, and **2b** were 5.0 ng/ml, and that for **3** was 1.0 ng/ml.

3.6. Application

The concentrations of **1a**, **1b**, **2a**, **2b**, and **3** in plasma samples obtained from renal transplant recipients were determined using our present HPLC method. Fig. 3 shows chromatograms of plasma sample collected just before (A) and at 3 h (B) and at 10 h (C) after 30 mg oral dose of racemic **1**. The peaks corresponding to **1a**, **1b**, and **3** were well separated and no interfering peaks were detected at the retention of each analyte when blank pre-dose plasma samples were analyzed. Another peak (at retention time 11.0 and 15.2 min) is also present in the chromatogram, which was not detectable in spiked blank plasma (Fig. 2A), and it is probably due to one of the coadministered drugs. Patients were subjected to simultaneous immunosuppressive therapy consisting of tacrolimus and mycophenolate mofetil.

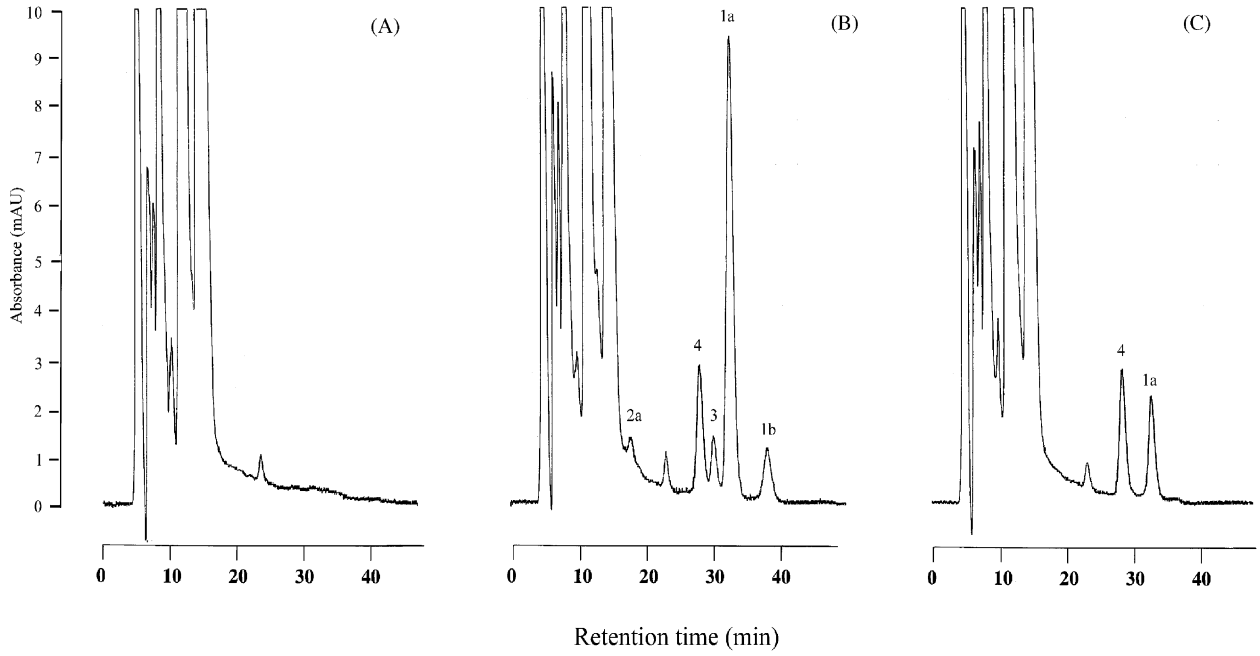

Fig. 3. Typical chromatograms of plasma sample of (A) pre-dosing; (B) at 3 h (the calculated concentrations of **2a**: 15 ng/ml, **3**: 40 ng/ml, **1a**: 615 ng/ml, and **1b**: 85 ng/ml, and **4**: 200 ng/ml); and (C) at 10 h (**1a**: 140 ng/ml and **4**: 200 ng/ml) after 30 mg oral dose of racemic lansoprazole to patient.

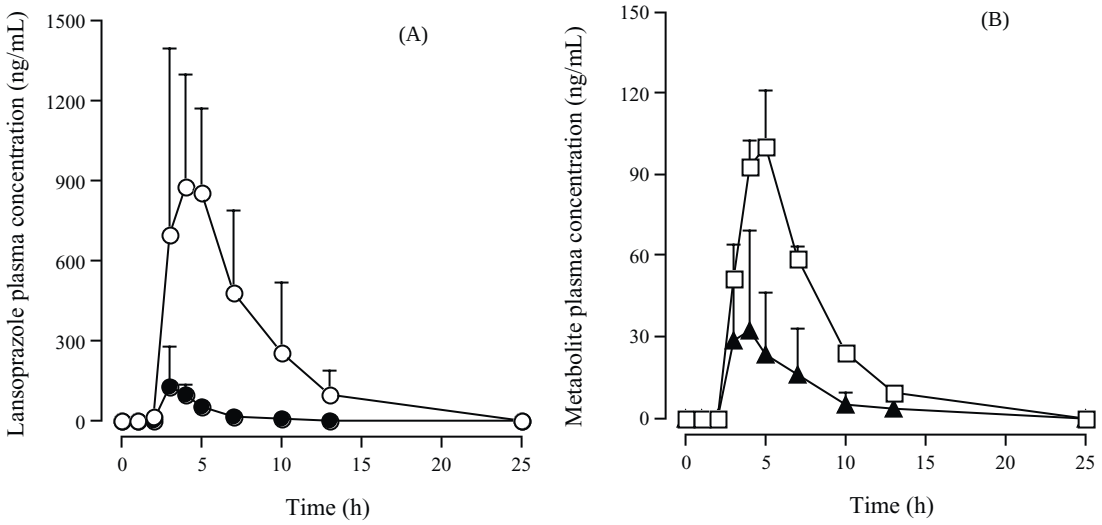

Fig. 4. Plasma concentration–time profiles of (A) (*R*)-lansoprazole (**1a**, open circles) and (*S*)-lansoprazole (**1b**, solid circles); (B) (*R*)-5-hydroxylansoprazole (**2a**, open squares) and lansoprazole sulfone (**3**, solid triangles) after 30 mg oral dose of racemic lansoprazole to three renal transplant recipients.

The time course of the mean concentrations of **1a**, **1b**, **2a**, and **3** in plasma samples from three renal transplant recipients taking 30 mg of daily lansoprazole are shown in Fig. 4. The pharmacokinetic parameters for each compound in individual patients are given in Table 2. The time required to reach the maximum concentration (t_{max}) of both **1a** and **1b** was around 3.8 h. The mean maximum concentration (C_{max}) for **1a** was 1075 ng/ml (range: 745–1718 ng/ml), whereas C_{max} of **1b** was 147 ng/ml (range: 58.5–299 ng/ml), respectively. The half-life ($t_{1/2}$) values for the **1a** and **1b** were around 2.2 and 1.5 h, respectively, whereas the mean $AUC_{(0-\infty)}$ values were 5162 (range: 1514–9712) and 357 ng h/ml (range: 196–632 ng h/ml), respectively. The differences between **1a** and **1b** were significant in AUC and C_{max}. **2a** in humans was only observed by the stereospecific oxidation of **1**. The mean C_{max} and $AUC_{(0-\infty)}$ values

Table 2
Pharmacokinetic parameters of lansoprazole enantiomers and their metabolites in three renal transplant recipients

Parameters	Patients			Mean	S.D.
	1	2	3		
(*R*)-Lansoprazole (**1a**)					
t_{max} (h)	4.0	3.0	4.0	3.7	0.6
C_{max} (ng/ml)	745	1718	763	1075	556
$t_{1/2}$(h)	2.7	3.2	0.5	2.2	1.4
CL/F (ml/(h kg))	67.0	28.0	198	97.7	89.1
$AUC_{(0-\infty)}$ (ng h/ml)	4260	9712	1514	5162	4173
(*S*)-Lansoprazole (**1b**)					
t_{max} (h)	4.0	3.0	4.0	3.7	0.6
C_{max} (ng/ml)	84.4	299	58.5	147	132
$t_{1/2}$ (h)	1.2	1.9	1.4	1.5	0.3
CL/F (ml/(h kg))	1171	431	1532	1045	561
$AUC_{(0-\infty)}$ (ng h/ml)	244	632	196	357	239
(*R*)-5-Hydroxylansoprazole (**2a**)					
t_{max} (h)	5.0	4.0	n.c.	4.5	0.7
C_{max} (ng/ml)	115	99.5	n.c.	107	11.0
$t_{1/2}$ (h)	2.2	2.5	n.c.	2.3	0.2
$AUC_{(0-\infty)}$ (ng h/ml)	566	524	n.c.	545	29.7
Lansoprazole sulfone (**3**)					
t_{max} (h)	5.0	3.0	3.0	3.7	1.2
C_{max} (ng/ml)	20.9	74.3	4.6	33.3	36.5
$t_{1/2}$ (h)	4.0	2.2	0.8	2.4	1.6
$AUC_{(0-\infty)}$ (ng h/ml)	160	439	13.1	204	216

n.c., not calculated.

for **2a** were 107 ng/ml and 545 ng h/ml, respectively. On the other hand, the mean C_{max} and $AUC_{(0-\infty)}$ values for **3** were 33.3 ng/ml (range: 4.6–74.3 ng/ml) and 204 ng h/ml (range: 13.1–439 ng h/ml), respectively.

4. Discussion

In this report, we present a sensitive and specific HPLC method for simultaneous determination of **1a**, **1b**, **2a**, **2b**, and **3** concentrations in human plasma. All peaks of the analytes were clearly separated with chiral separation using a Chiral CD-Ph analytical column (Shiseido, Tokyo, Japan). Further, each enantiomer of both **1** and **2** were also separated. Although the total run time is relatively long (about 40 min) compared with other HPLC method [6,7], the method described in this paper is the first method suitable for enantioselective determination of **1** with **2** in plasma. Therefore, the difference between the pharmacokinetics of **2a** and **2b** in human can also be examined by using this method. Furthermore, it should be noted that the present method is also sufficiently sensitive for the determination of **1b** plasma concentrations that are usually very low in humans. The C_{max} value for **1b** in CYP2C19 extensive metabolizers was 84 ± 51 ng/ml after oral administration of 30 mg racemic **1** [5]. The LOQ value for both **1a** and **1b** of the previous method using a Chiralcel OD-R column was 0.25 μM (=92.3 ng/ml) [7], on the other hand, our data was 10 ng/ml for each enantiomer of both **1** and **2**, and 5 ng/ml for **3** using 100 μl of plasma.

For routine drug monitoring in patients, assays that require small sample volumes are very useful. The previous extractive procedures developed for the analysis of **1** in human plasma have been based on liquid–liquid extraction, which require a plasma volume of 1 ml [5,6]. The novel solid-phase extraction procedure described here needs a small amount of plasma sample (100 μl) for one complete analysis and provides a high extraction recovery (>94.1% for all compounds) and good selectivity. These results proved better than in previously described method [5–7]. Furthermore, the use of an Oasis HLB cartridge effectively eliminated the interfering material in plasma.

The sensitivity and the calibration range of the present method were also appropriate for the therapeutic drug monitoring (TDM) of lansoprazole in patients. Using this assay, **1a**, **1b**, and their metabolites in human plasma can be measured reliably over a wide range of plasma concentrations: **1a**, **1b**, **2a**, and **2b** in the range 10–1000 ng/ml and **3** in the range 5–1000 ng/ml. The correlation coefficients for each individual calibration curves for **1a**, **1b**, **2a**, **2b**, and **3** were greater than $r^2 = 0.999$, showing good proportionality between concentration and detector response. The accuracy of the assay was within 8.4% for different concentrations for different analytes. These results are acceptable. This method is currently used as a part of our routine TDM of lansoprazole for renal transplant recipients. They are also receiving tacrolimus and mycophenolate mofetil for immunosuppression. In present method, however, no analytical interference with these compounds was found (Fig. 3A).

Before application to clinical study, we evaluated in vitro biotransformation of each enantiomer of **1** in human blank plasma. The chiral inversions of **1a** to **1b** and **1b** to **1a**, and the biotransformation to **2** or **3** from **1** were not observed for 1 week at −30 °C and at 37 °C in human plasma.Similarly to the previous results obtained from healthy subjects [5], the plasma concentrations of **1a** at any time point in renal transplant recipients were also consistently higher than those of **1b** (Fig. 4), and *R*/*S* AUC ratio of **1** was 13.5 ± 5.1, whereas there was no significant difference between the t_{max} of **1a** and **1b**. Further, the maximum concentration (C_{max}), the half-life ($t_{1/2}$), and oral clearance (CL/F) of **1a** and **1b** in the present study were also in good agreement with reported values [5]. However, the t_{max} value in patients (3.7 h) was much longer than that in healthy subjects (2.0 h) who not take any drugs and foods for 12 h before the experiments. These data suggest that the absorption of lansoprazole may be greatly affected by meal ingestion, because lansoprazole is enteric courted product [12]. Although food delayed the t_{max} value, no effect of food on half-life ($t_{1/2}$) was observed.

We have developed a HPLC method for simultaneous quantification of lansoprazole enantiomers and their metabolites, 5-hydroxylansoprazole and lansoprazole sulfone, in human plasma. This method is a simple, sensitive, precise, and applicable to routine analysis of these analytes in plasma.

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