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Simple quantification of lansoprazole and rabeprazole concentrations in human serum by liquid chromatography/tandem mass spectrometry

Takanori Hishinuma [a], Kaori Suzuki [b], Hiroaki Yamaguchi [b], Hatsushi Yamagishi [c], Tomoyuki Koike [c], Shuichi Ohara [c], Toru Shimosegawa [c], Nariyasu Mano [b], Junichi Goto [b,*]

[a] Division of Pharmacotherapy, Graduate School of Pharmaceutical Sciences, Tohoku University, 6-3, Aoba, Aramaki, Aoba-ku, Sendai 980-8578, Japan
[b] Department of Pharmaceutical Sciences, Tohoku University Hospital, 1-1 Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan
[c] Division of Gastroenterology, Tohoku University Graduate School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai, Japan

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ABSTRACT

A rapid, simple and highly sensitive method was developed for the quantitative determination of lansoprazole and rabeprazole concentrations in 20 μL of human serum using high-performance liquid chromatography/tandem mass spectrometry (LC/MS/MS). Analytes, along with an internal standard (lansoprazole deuterium derivatives), were separated using a mobile phase of acetonitrile/1 mM ammonium formate (140/60, v/v) on a C18 analytical column and analyzed in the selected reaction-monitoring (SRM) mode. The lower limit of quantification was 0.25 ng/mL. A good linear response was observed for each analyte (from 0.25 ng to 2.5 μg/mL). This method was useful for therapeutic drug monitoring and pharmacokinetic studies.

1. Introduction

Lansoprazole, 2-[[[3-methyl-4-(2,2,2-trifluoro-ethoxy)-2-pyridinyl]methyl]sulfinyl]-1H-benzimid-azole and rabeprazole, 2-[[[4-(3-methoxypropoxy)-3-methyl-2-pyridil]-methyl]sulfinyl]-1H-benzimidazole are proton pump inhibitors (PPIs) that inhibit gastric acid secretion via interaction with the (H^+/K^+)-ATPase in gastric parietal cells [1,2]. Like most compounds of this class, these compounds (particularly rabeprazole) are acid labile and are reversibly transformed to sulfenamides in acidic media [3,4].

Lansoprazole concentrations have previously been determined in solutions and serum by spectroscopy and high-performance liquid chromatography (HPLC) [5,6]. Many studies using HPLC with UV detection have had higher ranges for their limits of quantification (LOQ) (between 5.0 and 20 ng/mL) [7–11] and longer retention times (RT) 11 min [7,8]. Landes et al. [12] observed an LOQ of 2.0 ng/mL using an HPLC method with a loop column, but their RT was approximately 11 min and their method required double extraction and two evaporation steps with nitrogen. Oliveira et al. [13] had an LOQ of 2.5 ng/mL using HPLC coupled to tandem mass spectrometry (LC/MS/MS) and a double extraction method, but this study did not use isotopic labeling derivatives as an internal standard.

Several methods have also been employed for quantification of rabeprazole in plasma [14–20]. Nakai et al. [14] established a method using HPLC with UV detection, for the simultaneous determination of rabeprazole and its four metabolites in 1 mL human plasma. The LOQ was 5 ng/mL for rabeprazole and 20 ng/mL for each of its four metabolites, but the double extraction complicated the sample preparation. Nakai et al. [14] and Mano et al. [16] described gradient HPLC methods for determining rabeprazole in plasma, which required a long run time (>25 min) and had a higher LOQ (30 ng/mL). Moreover, the stability of rabeprazole was not determined. Recently, Zhong et al. [17] and Huang et al. [18] reported an LC/MS/MS method for quantification of rabeprazole in human plasma. Zhang et al. [17] used methanol as a mobile phase, but the sample was extracted with a mixture of *n*-hexane/dichloromethane/isopropanol (20/10/1, v/v/v). This process had an LOQ of 2.0 ng/mL and used 0.5 mL of plasma. Huang et al. [18] observed an LOQ of 0.2 ng/mL using methanol/water (50/50, v/v) containing 0.1% formic acid as a mobile phase and methanol for precipitation of the plasma proteins. Although this method was simple and sensitive, its background was not as clear as the LLE method, which may have affected its sensitivity and might cause column damage with long-term usage. El-Gindy et al. [19] used spectrophotometric and chromatographic methods to investigate

* Corresponding author. Tel.: +81 22 717 7565; fax: +81 22 717 7545.
E-mail address: jun1goto@mail.tains.tohoku.ac.jp (J. Goto).

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Table 1
SRM conditions for the analytes analyzed in positive ion mode

Compound	SRM transition (*m*/*z*)	Retention time (min)	DP	EP	CE	CXP
Lansoprazole-d_4	374 > 252	2.92	81	10	19	24
Lansoprazole	370 > 252	2.95	116	10	19	8
Rabeprazole	360 > 242	2.53	16	10	19	22

DP: declustering potential, EP: entrance potential, CE: collision energy, CXP: collision cell exit potential

Table 2
Tandem mass spectrometer main working parameters

Parameters	Value
Collision gas (CAD)	4.5
Curtain gas (CUR)	40
Ion source gas 1 (GS1)	40
Ion source gas 2 (GS2)	60
Ionspray voltage (IS) (V)	5500
Probe temperature (TEM) (°C)	550
Interface heater (ihe)	on
Dwell time per transition (ms)	330

the stability of rabeprazole in solution under acidic and oxidative conditions and during photodegradation. They found that rabeprazole was rapidly degraded in acidic media, but was more stable in alkaline solutions. Thus, the mobile phases used by Huang et al. [18] may have adversely affected the stability of rabeprazole. Feng et al. [20] reported an LC/MS method for the quantification of rabeprazole in 0.1 mL of dog plasma, which had an LOQ of 1 ng/mL. However, their selected ion monitoring (SIM) method lacked selectivity and their gradient elution mode had unstable ionization.

Recently, the use of LC/MS/MS in the quantification of drug concentrations in blood from patients has become widespread in the clinical field. Moreover, the practice of personalized medicine is now regarded as important in the prescription of medication. Even if the PPI in particular has the same effect, the structure and formulation of the compound is characteristic. Therefore, it is necessary to understand the individual differences of the curative effect of these drugs in order to promote individualized medical care. Through development of highly sensitive methods of quantification using a very small amount of sample, it is possible to avoid high-volume operations and reduce foreign elements. Furthermore, simple and easy operations in quantification of drug concentrations will be beneficial for pharmacists in clinical practice in the future.

In this work we describe a simple, rapid, sensitive and selective method for the quantitation of lansoprazole and rabeprazole using LC/MS/MS. The current method offers a number of advantages over existing methods, including shorter analysis time, smaller required sample volumes (20 μL serum), a less extensive sample clean up procedure, and the inclusion of lansoprazole-d_4 as an internal standard. This method was applied to a pharmacokinetic study of serum lansoprazole and rabeprazole concentrations, after administration of 30 mg of lansoprazole and 10 mg of rabeprazole to 14 and 8 healthy volunteers, respectively.

2. Experimental methods

2.1. Chemicals and reagents

Lansoprazole and lansoprazole deuterium derivatives were provided by Takeda Pharmaceutical Co., Ltd. (Osaka, Japan). Rabeprazole was obtained from Eisai Co., Ltd. (Tokyo, Japan). Acetonitrile (LC/MS grade) was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Ultra-pure water was purified using a Purelab-Ultra-Analytic (Organo Corp., Tokyo, Japan). Analytical grade ammonium formate was obtained from Wako Pure Chemical Industries (Osaka, Japan). Blank serum was purchased from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan). Stock solution of lansoprazole, rabeprazole and internal standard (each 1 mg/mL) were prepared in acetonitrile and kept in glass tubes at −20 °C. Stock solutions were used for the preparation of calibration standards and quality control samples.

2.2. Sample preparation

Aliquots of frozen human serum samples (20 μL) were thawed at room temperature prior to use, then 40 μL of internal standard solution (25 pg/μL lansoprazole-d_4 in acetonitrile) was added to each sample. The tubes were briefly vortexed, sonicated for 60 s and centrifuged at 10,000 rpm for 5 min at 4 °C to precipitate solids. The supernatant then was filtrated and transferred to an autosampler vial, where 15 μL was injected for LC/MS/MS.

2.3. Chromatographic conditions

A Nanospace SI-2 HPLC system (Shiseido, Tokyo, Japan) was used. Chromatography was performed on a SunfireTM C18 3.5 μm (2.1 mm i.d. × 150 mm) analytical column (Waters, Milford, MA, USA) and a C18 Capcell Pak MGII 5 μm (2.0 mm i.d. × 10 mm) guard column (Shiseido, Tokyo, Japan) operated at 40 °C. The mobile phase was an isocratic elution with acetonitrile/1 mM ammonium formate (70/30, v/v) at a flow-rate of 200 μL/min. Under these conditions, RTs were typically 2.92 min for lansoprazole-d_4, 2.95 min for lansoprazole and 2.53 min for rabeprazole. Column effluent was introduced into the mass spectrometer using a fused silica capil-

Table 3
Accuracy and precision of the determination of lansoprazole and rabeprazole in human serum

Compounds	Added	Intra-day (*n* = 3)			Inter-day (*n* = 4)		
		Found mean ± S.D.	C.V. (%)	Accuracy (%)	Found mean ± S.D.	C.V. (%)	Accuracy (%)
	0.5 pg	0.52 ± 0.041	7.8	105	0.50 ± 0.028	5.6	101
	5 pg	5.5 ± 0.13	2.5	109	5.5 ± 0.10	1.8	111
	50 pg	51 ± 0.41	0.81	101	51 ± 1.3	2.6	102
Lansoprazole	0.5 ng	0.51 ± 0.0057	1.1	102	0.51 ± 0.0076	1.5	102
	5 ng	5.1 ± 0.022	0.43	101	5.1 ± 0.040	0.78	101
	25 ng	26 ± 0.14	0.55	102	26 ± 0.15	0.58	103
	50 ng	51 ± 0.19	0.37	101	51 ± 0.49	1.0	102
	0.5 pg	0.49 ± 0.010	2.0	98.7	0.51 ± 0.018	3.5	102
	5 pg	5.2 ± 026	5.0	105	5.4 ± 0.13	2.4	107
	50 pg	51 ± 0.99	1.9	103	51 ± 1.3	2.6	102
Rabeprazole	0.5 ng	0.50 ± 0.0085	1.7	101	0.50 ± 0.011	2.2	99.5
	5 ng	5.1 ± 0.0094	0.19	101	5.0 ± 0.052	1.0	99.7
	25 ng	25 ± 0.14	0.57	100	25 ± 0.33	1.3	101
	50 ng	49 ± 0.66	1.3	97.9	50 ± 0.69	1.4	100

lary between 1.9 and 4.0 min after injection. The temperature of the autosampler was kept 4 °C and the run time was 5 min.

2.4. Mass spectrometric conditions

An API-5000 triple-quadrupole mass spectrometer (Applied Biosystems/MDS SCIEX, Foster City, CA/Concord, Ontario, Canada) was equipped with an electrospray source, operating in the positive ion mode. Data were collected and processed using Sciex Analyst 1.4.1 data collection and integration software on an IBM compatible computer. Using selected reaction monitoring (SRM), transitions of m/z 374.1 > 252.2, m/z 370.2 > 252.1 and m/z 360.3 > 242.1 were used for quantitation of lansoprazole-d_4, lansoprazole and rabeprazole, respectively. Fig. 1 shows the full scan (upper trace) and enhanced product ion (lower trace) spectra obtained for (A) lansoprazole-d_4, (B) lansoprazole and (C) rabeprazole. In order to optimize the MS parameters, a standard solution of analytes and IS was infused into the mass spectrometer using a syringe pump. The optimized parameters were: curtain gas, gas 1 and gas 2 (nitrogen) 40, 40 and 60 units, respectively; dwell time 330 ms; source temperature 550 °C; ionspray voltage 5500 V. Unit mass resolution was set in both mass-resolving quadrupole Q1 and Q3. The transitions to monitor each analyte and its labeled internal standard are listed in Table 1. The main working parameters for the mass spectrometer are summarized in Table 2. Data were acquired on a Dell Precision 370 workstation and were processed using the Analyst 1.4.1 software package (MDS Sciex).

Table 4
Pharmacokinetics parameters of (A) lansoprazole and (B) rabeprazole after oral administration of 30 mg lansoprazole or 10 mg rabeprazole

Parameters	Lansoprazole (n = 14)	Rabeprazole (n = 9)
T_{max} (h)	2.7 ± 0.8	4.2 ± 1.1
C_{max} (ng/mL)	1044 ± 323	120 ± 61
AUC (ng h/mL)	3667 ± 1768	251 ± 155

2.5. Stability

Stability quality control serum samples were subjected to short-term (6 h) room temperature, three freeze/thaw (−20 to 25 °C) cycles and 6 h autosampler (4 °C) stability tests. Subsequently, the

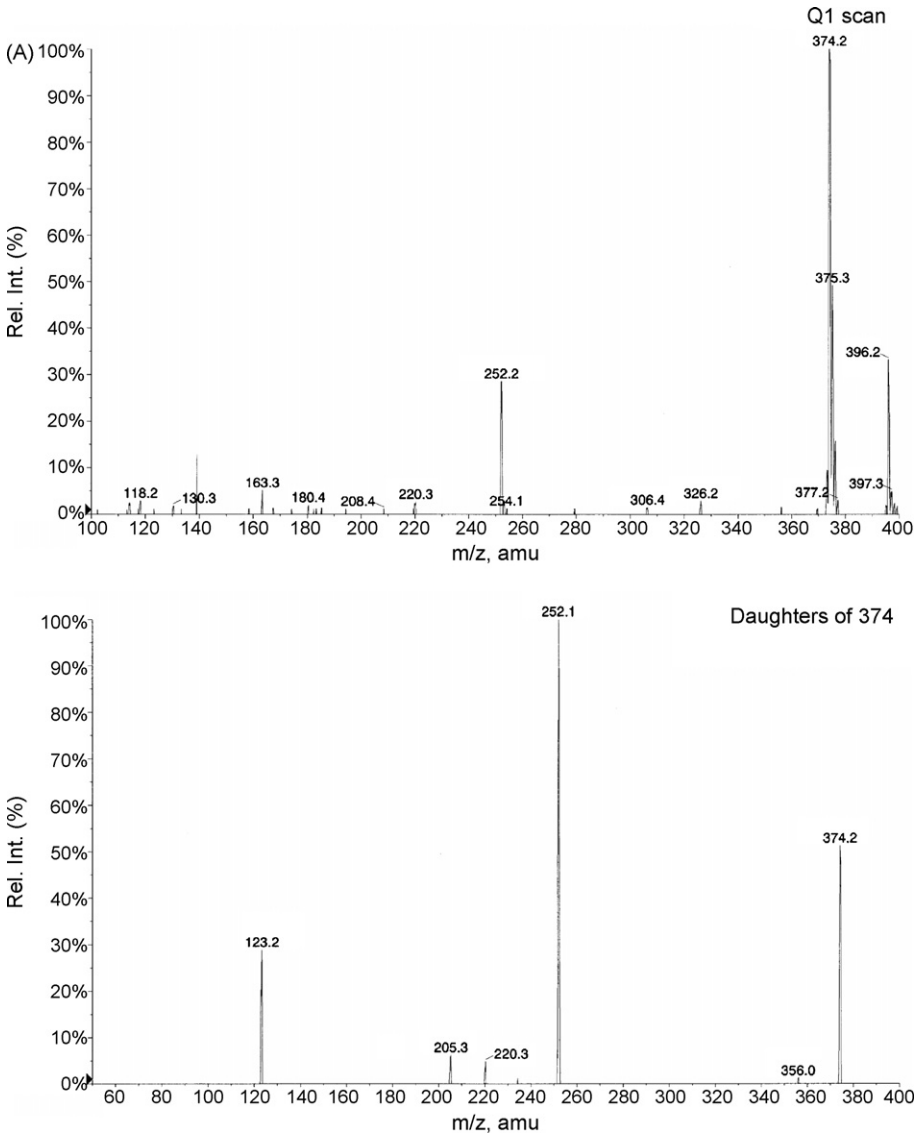

Fig. 1. Traces show full scan mass (upper trace) and product ion (lower trace) spectra. (A) lansoprazole-d_4, (B) lansoprazole and (C) rabeprazole.

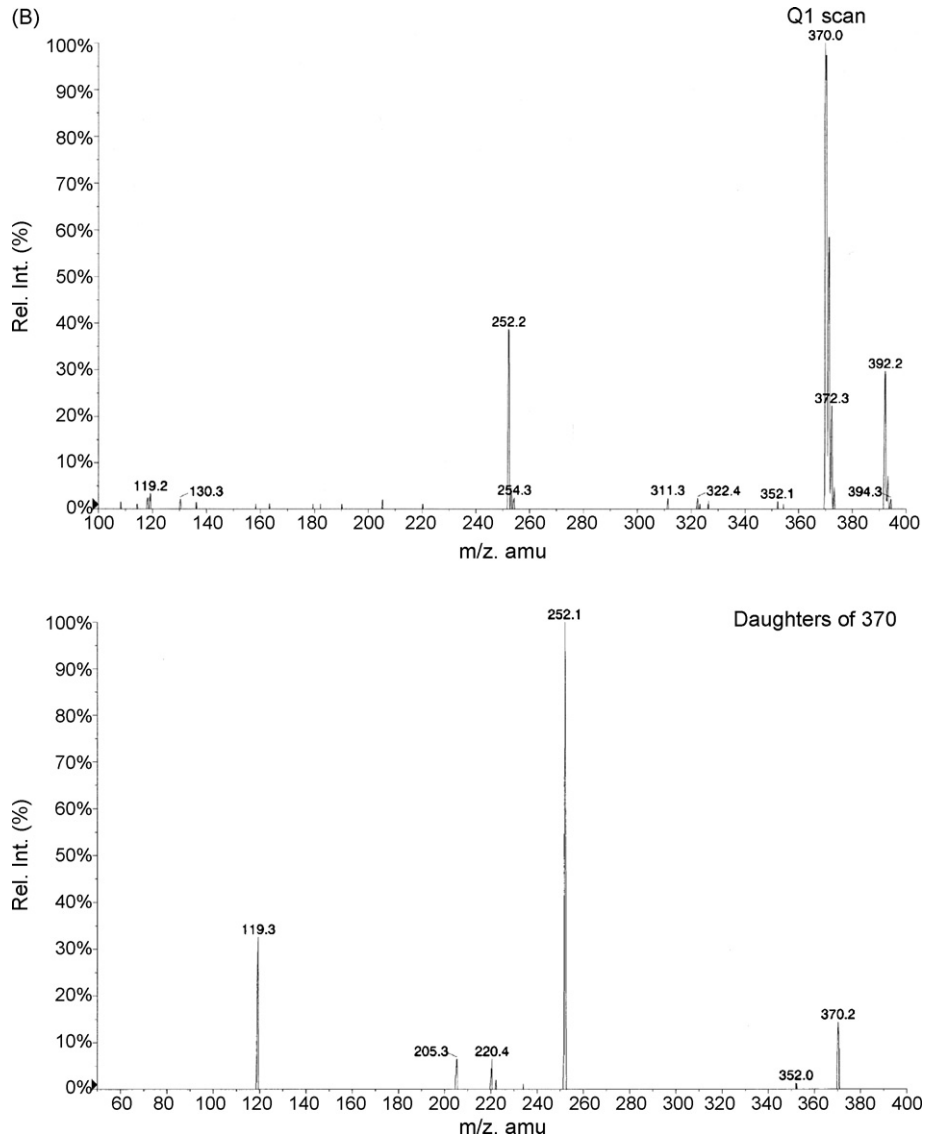

Fig. 1. (*Continued*)

lansoprazole and rabeprazole concentrations were measured compared with freshly prepared samples and the significance of the results obtained was analyzed by Student's *t*-test ($P < 0.05$).

2.6. Recovery

The recovery was evaluated by calculating the mean of the response of each concentration and dividing the extracted sample mean by the unextracted (spiked blank serum extract) sample mean of the corresponding concentration. Comparison with the unextracted samples, spiked on serum residues, was done in order to eliminate matrix effects, giving a true recovery. The matrix effect experiments were carried out using the ratio between spiked mobile phase solutions and unextracted samples, spiked on serum residues.

2.7. Calibration curves

Calibration curves were generated from the SRM chromatograms of a range of lansoprazole and rabeprazole standards, spiked with constant levels of the internal standard (lansoprazole-d_4). Linear calibration curves were constructed using least-squares regression of quantities versus peak area ratio to lansoprazole-d_4 with a weighting index of $1/x$.

2.8. Validation

To examine the accuracy and precision of our method, serum spiked with seven concentrations were prepared. Intra-day precision and accuracy were evaluated by analyzing each control once a day for 4 days, while inter-day precision and accuracy were evaluated by analyzing the spiked controls three times a day in a random order. Precision was evaluated at each concentration by comparing the values for the coefficients of validation (C.V.), which were determined by calculating the standard deviations (S.D.) as a percentage of the average calculated concentrations. The accuracy of the method was estimated for each spiked control by comparing the nominal concentration with the assayed concentration. The results are shown in Table 3.

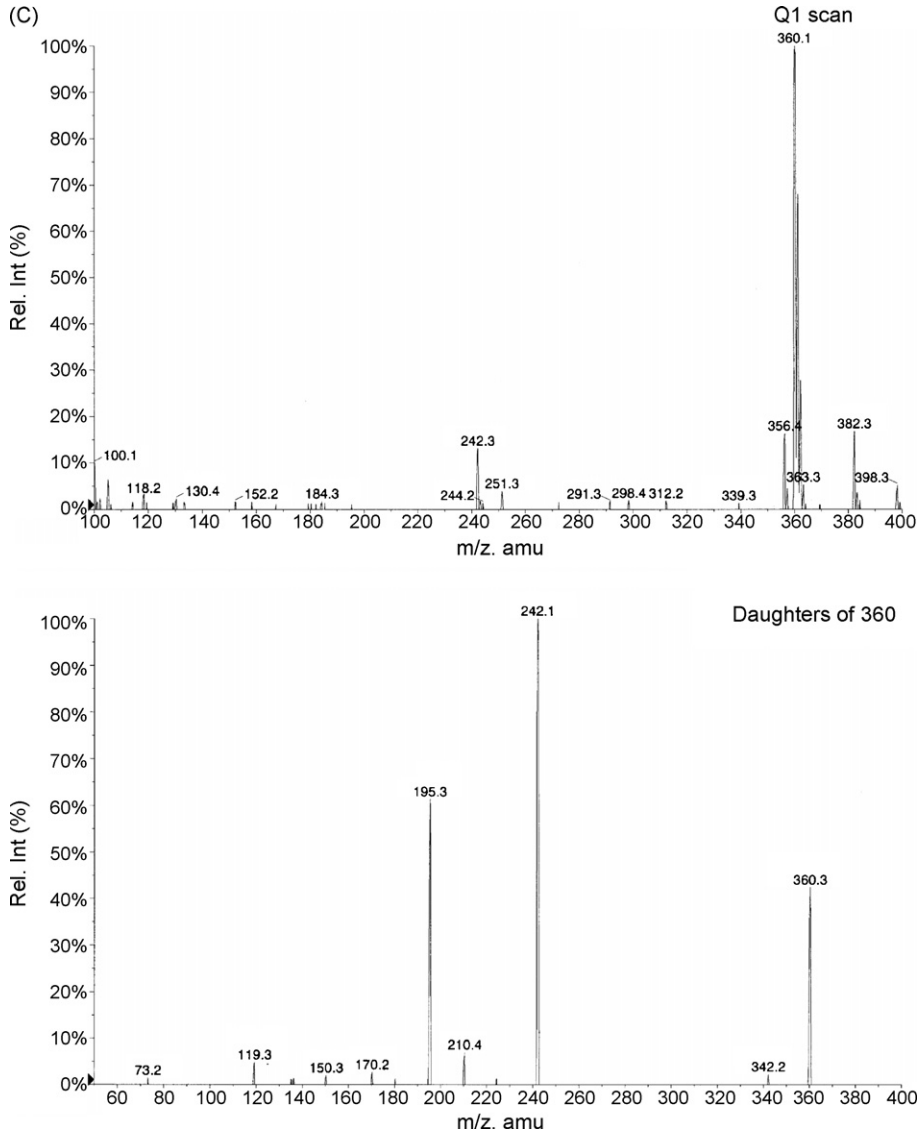

Fig. 1. (*Continued*).

2.9. Application to pharmacokinetic studies

The method was used to determine the concentrations of lansoprazole and rabeprazole in the serum of 14 healthy male volunteers who received an oral dosage of Takepron (containing 30 mg lansoprazole, Takeda Pharmaceutical Co., Ltd.) or Pariet (containing 10 mg rabeprazole, Eisai Co., Ltd.) tablet formulations. They received a single dose in the same meal condition. Blood samples were collected before and 0.5, 1.0, 1.5, 2.0, 3.5, 6, 9 and 12 h post-dose.

3. Results

Lansoprazole and rabeprazole each have three nitrogen atoms in their structures, and therefore much higher signal intensities were obtained in the positive mode than in the negative mode. The collision-induced dissociation (CID) of protonated lansoprazole (m/z 370) and rabeprazole (m/z 360) resulted in a loss of the benzimidazole ring, leading to the main fragment ions at m/z 252 and 242. A proposed fragmentation for a compound of similar structure (omeprazole) has already been described [21]. As shown in Fig. 2, chromatograms of blank serum samples did not contain endogenous peaks. The chromatograms for the standard LOQ samples showed that the RTs for lansoprazole and rabeprazole were 2.95 and 2.53 min, respectively (Fig. 3).

Linearity, precision and accuracy were determined to assess the performance of the method. The calibration curves showed good linearity within the range from 0.25 ng to 2.5 μg/mL. The recoveries observed were more than or equal to 80.3%. No significant matrix effect (<10%) was observed. The LOQ was determined to be 0.25 ng/mL (as defined by the lowest concentration at which both precision and accuracy were less than or equal to 7.8%). Table 3 shows the intra-day and inter-day assay validation quality report. Stability analysis was carried out with serum quality control samples. All samples showed no significant degradation under the conditions previously described in the Section 2.

The serum chromatograms of healthy volunteers, 12 h after oral administration of 30 mg lansoprazole or 10 mg rabeprazole are shown in Figs. 4 and 5. Mean serum concentration–time profiles

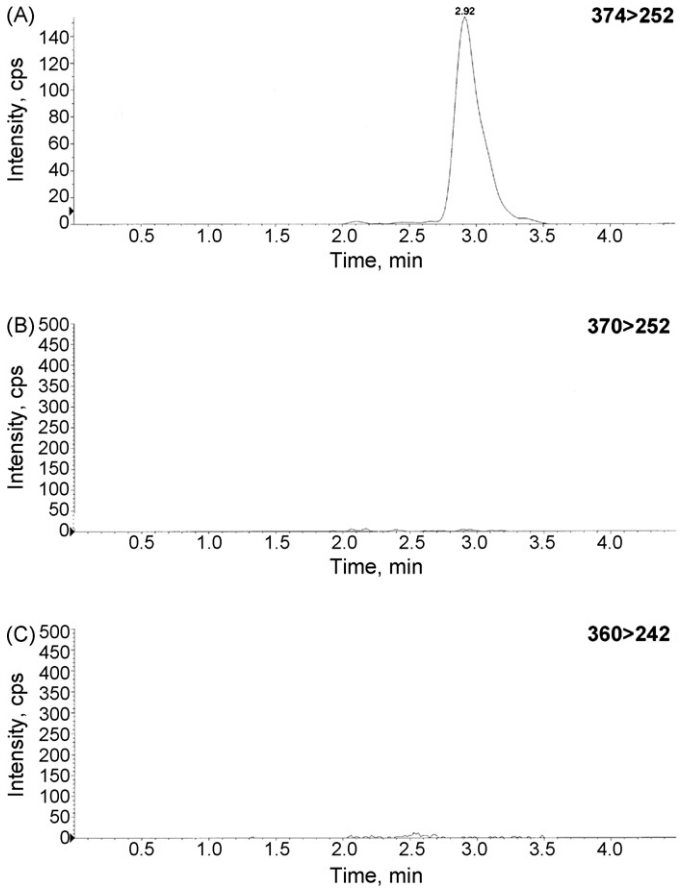

Fig. 2. SRM chromatograms show blank normal human serum samples. (A) lansoprazole-d_4, (B) lansoprazole and (C) rabeprazole.

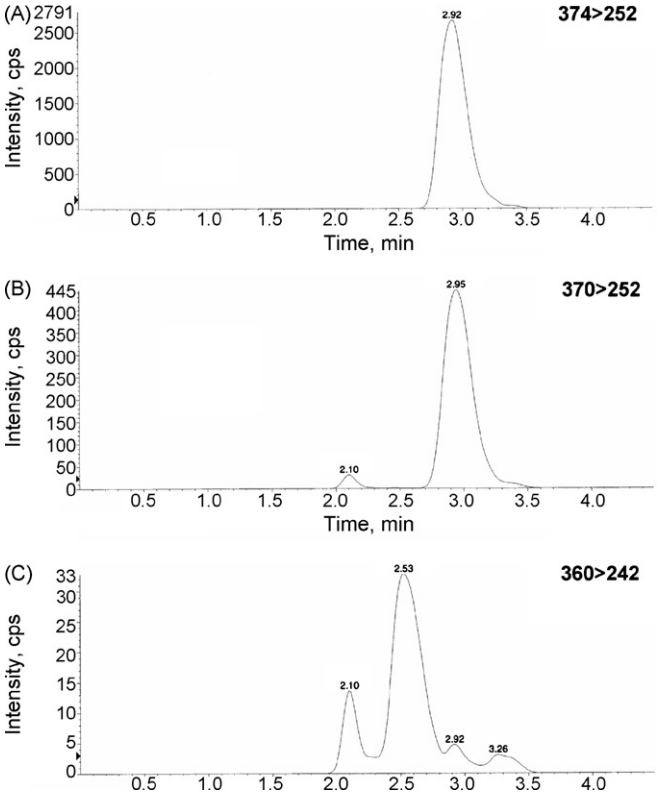

Fig. 3. SRM chromatograms show the LOQ standard samples (0.25 ng/mL) for (A) lansoprazole-d_4, (B) lansoprazole and (C) rabeprazole.

of lansoprazole and rabeprazole are shown in Fig. 6 and pharmacokinetic parameters are summarized in Table 4.

4. Discussion

Full-scan positive mode spectra of lansoprazole and rabeprazole contained predominant molecular ions at m/z 370 and 360, respectively. The product ion mass spectra of these protonated molecular ions (Fig. 1) showed the presence of major product ion at m/z 252 and 242 for lansoprazole and rabeprazole, respectively.

Microdetermination methods for lansoprazole or rabeprazole using LC/MS/MS have been reported previously [13,17–19]. However, these methods were inadequate because they could not simultaneously quantify lansoprazole and rabeprazole, they required large sample volumes, and they did not use isotopic labeling derivatives for their IS. By using isotopic labeling derivative for their IS, we demonstrated a broad fixed-quantity range and good validation.

Although it is well known that lansoprazole and rabeprazole are unstable at low pH, the presence of the acid was necessary in order to improve their detection in positive electrospray. In the positive ion mode, a small amount of formic acid present in the mobile phase improved the MS response of lansoprazole and rabeprazole, but also significantly decreased their stability. The stability of lansoprazole and rabeprazole in plasma has been reported previously [9,10]. Consequently, although it slightly reduced their MS response, we chose 1 mM ammonium formate

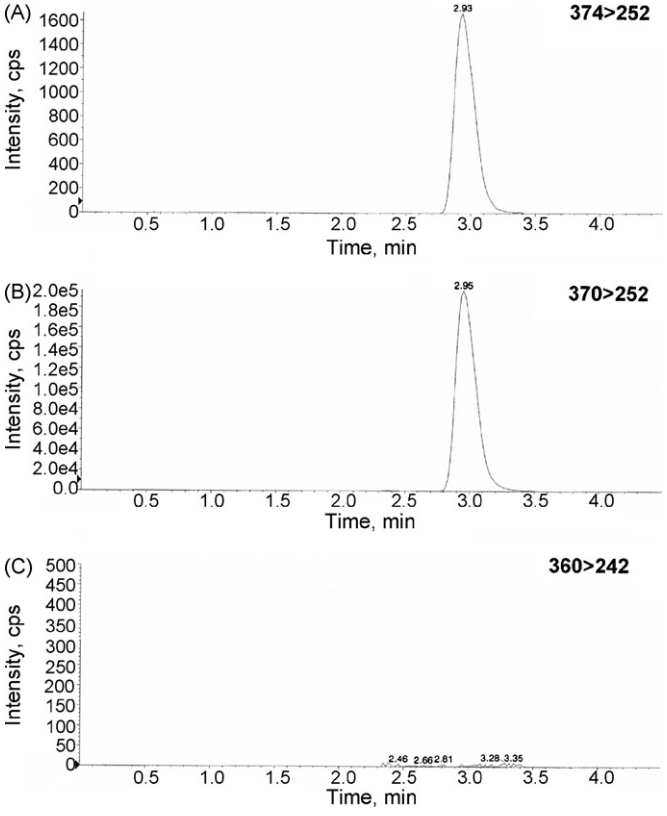

Fig. 4. SRM chromatograms show serum from a healthy volunteer, 12 h after oral administration of 30 mg lansoprazole (A) lansoprazole-d_4, (B) lansoprazole and (C) rabeprazole.

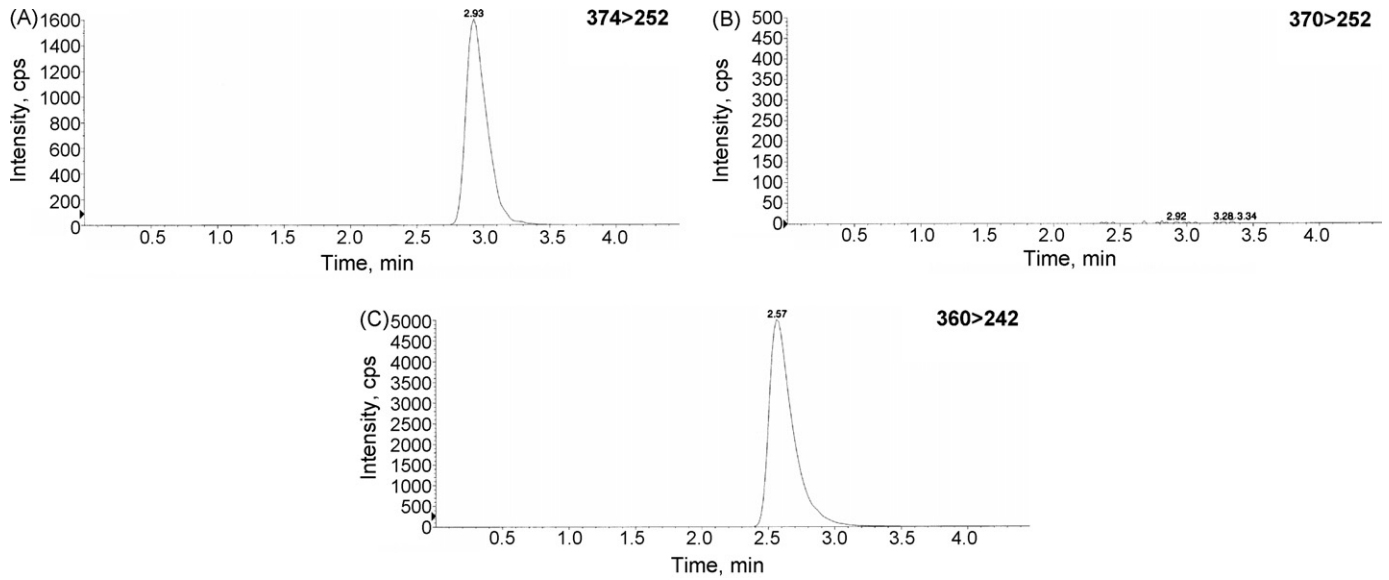

Fig. 5. SRM chromatograms show serum from a healthy volunteer, 12 h after oral administration of 10 mg rabeprazole (A) lansoprazole-d_4, (B) lansoprazole and (C) rabeprazole.

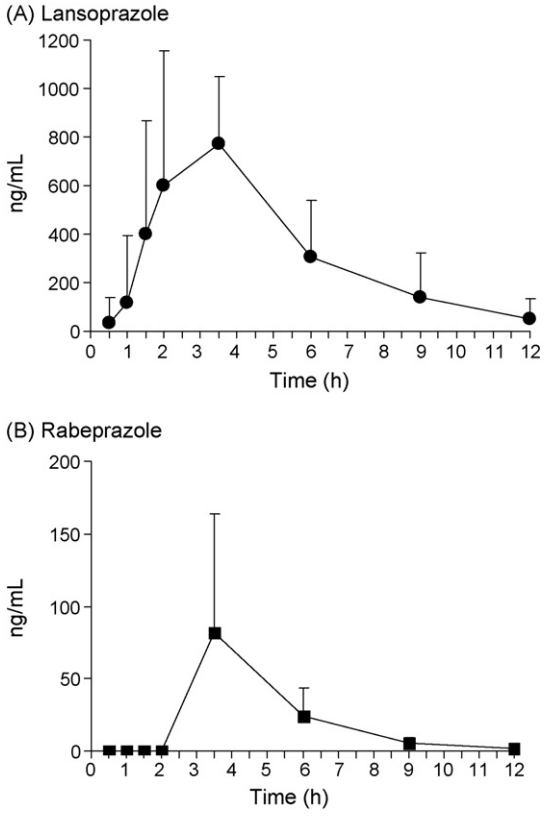

Fig. 6. Mean serum concentration–time profiles for (A) lansoprazole and (B) rabeprazole tablet formulations in healthy volunteers.

as the mobile phase buffer to improve the stability of lansoprazole and rabeprazole. Moreover, given their instability in solution, our simple procedure is preferable because it shortens the analytical time.

Our method required only 20 μL of serum sample, and assays that require small sample volumes are very useful for routine patient drug monitoring. Furthermore, it seems possible that our procedure will be sensitive enough to assay serum samples smaller than 20 μL.

In the present study, we applied our method to the determination of serum concentrations of lansoprazole and rabeprazole in a single dose. Pharmacokinetic parameters obtained in our study showed similar result reported previously [13,17,18,20]. The method we have developed is very useful for the monitoring of lansoprazole and rabeprazole levels in clinical.

5. Conclusion

A fast and sensitive LC/MS/MS method was developed and validated for the quantification of lansoprazole and rabeprazole in human serum. This method had the high sensitivity and specificity and the rapid sample throughput required to satisfy the requirements for pharmacokinetic studies. Additionally, because this method required lower volumes of serum than other previous methods, this method is very useful on making clinical application.

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