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Journal of Chromatography B, 824 (2005) 238–243
JOURNAL OF CHROMATOGRAPHY B
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Determination of rabeprazole and its active metabolite, rabeprazole thioether in human plasma by column-switching high-performance liquid chromatography and its application to pharmacokinetic study

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Received 19 March 2005; accepted 16 July 2005
Available online 8 August 2005

Abstract

A new sensitive column-switching high-performance liquid chromatographic (HPLC) method with ultraviolet detection was developed for the simultaneous determination of rabeprazole, a proton pump inhibitor, and its active metabolite, rabeprazole thioether in human plasma. Rabeprazole, its thioether metabolite and 5-methyl-2-[{4-(3-methoxypropoxy)-3-methyl pyridin-2-yl} methyl sulfinyl]-1H-benzimidazole, as an internal standard were extracted from 1 ml of plasma using diethyl ether–dichloromethane (9:1, v/v) mixture and the extract was injected into a column I (TSK-PW precolumn, 10 μm, 35 mm × 4.6 mm I.D.) for clean-up and column II (C18 Grand ODS-80TM TS analytical column, 5 μm, 250 mm × 4.6 mm I.D.) for separation. The peak was detected with an ultraviolet detector set at a wavelength of 288 nm, and the total time for chromatographic separation was ∼25 min. Mean absolute recoveries were 78.0 and 88.3% for rabeprazole and rabeprazole thioether, respectively. Intra- and inter-day coefficient variations were less than 6.5 and 4.5% for rabeprazole, 3.6 and 5.3% for rabeprazole thioether, respectively, at the different concentration ranges. The validated concentration ranges of this method were 1–1000 ng/ml for rabeprazole and 3–500 ng/ml for rabeprazole thioether. The limits of quantification were 1 ng/ml for rabeprazole and 3 ng/ml for rabeprazole thioether. The method was suitable for therapeutic drug monitoring and was applied to pharmacokinetic study in human volunteers.

Keywords: Rabeprazole; Rabeprazole thioether; HPLC

1. Introduction

Rabeprazole, 2-[[[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl]-methyl] sulfinyl]-1H-benzimidazole, structurally related to omeprazole, is a substituted benzimidazole and is a proton pump inhibitor (PPI) that suppresses gastric acid secretion through an interaction with (H^+/K^+)-ATPase in gastric parietal cells. As are the other three PPIs (omeprazole, lansoprazole and pantoprazole), rabeprazole is effective in the treatment of various peptic diseases, including gastric and duodenal ulcer, gastroesophageal reflux disease (GERD), and Zollinger–Ellison syndrome [1,2].

Preclinical studies demonstrated that the efficacy of rabeprazole was dose dependent and hence depended on drug concentration [3,4]. Several studies have suggested that the drug concentration of rabeprazole influences the cure rate of gastric-acid-related disorders including eradication rate of *Helicobacter pylori* (*H. Pylori*) and GERD [5–7]. Rabeprazole is metabolized mainly via nonenzymatic reduction to its thioether metabolite, which is pharmacologically active, and its effect inhibits the motility of *H. Pylori*, which colonizes the gastric mucosa and is closely associated with gastritis and peptic ulcers [8]. Therefore, it is clinically important to

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doi:10.1016/j.jchromb.2005.07.027

determine the concentration of rabeprazole and rabeprazole thioether, both of which reflect acid inhibition.

Several HPLC methods involving enantioselective assay and a LC–MS/MS method for the determination of rabeprazole concentrations have been previously published [9–18]. However, there are only a few publications of a simple HPLC method for the simultaneous determination of rabeprazole and rabeprazole thioether concentrations in human plasma, and there is no information describing the determination of rabeprazole thioether concentrations by a column-switching HPLC method after liquid–liquid extraction of human plasma. In addition, most of these HPLC methods have a high quantification limit (>10 ng/ml) for rabeprazole. Nakai et al. [9] have reported a simple liquid–liquid extraction procedure for rabeprazole and rabeprazole thioether concentrations, and achieved low quantification limit (5 ng/ml) for rabeprazole. However, the high quantification limit (20 ng/ml) of rabeprazole thioether in that method is not sufficiently sensitive to obtain precise pharmacokinetic parameters. In the present study, we describe a simple and sensitive column-switching HPLC method for the determination of rabeprazole and rabeprazole thioether in human plasma. The assay is suitable for pharmacokinetic studies or therapeutic drug monitoring.

2. Experimental

2.1. Chemicals and reagents

Rabeprazole sodium (purity 99.47%), its metabolite rabeprazole thioether (purity 99.37%), and 5-methyl-2-[{4-(3-methoxypropoxy)-3-methyl pyridin-2-yl} methyl sulfinyl]-1H-benzimidazole (purity 99.56%), as an internal standard (IS) (Fig. 1) were kindly provided by Eisai Co. Ltd. (Tokyo, Japan). Sodium dihydrogen phosphate, di-sodium hydrogen phosphate, acetonitrile, methanol, diethyl ether, dichloromethane and diethylamine 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. Preparation of stock and working solutions

Standard solution (0.1% diethylamine in methanol) of rabeprazole was prepared by dissolving appropriate amount of rabeprazole sodium, and rabeprazole thioether and IS were prepared by dissolving an appropriate amount of each compound to yield concentrations of 1.0 mg/ml for generating standard curves. Diethylamine was added in the standard solutions because these compounds are fragile in acidity [9–15]. Working standard solutions of rabeprazole and rabeprazole thioether (100, 10, 1 and 0.1 μg/ml) were prepared by serial dilution with 0.1% diethylamine in methanol. Internal standard solution (5 μg/ml) was prepared by 200 times diluting standard solution (1.0 mg/ml) with 0.1% diethylamine in methanol.

2.3. Preparation of calibration standards and quality control samples

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 and 25 μl of 1% diethylamine solution to yield the final concentrations of rabeprazole (1, 5, 10, 50, 200, 500 and 1000 ng/ml plasma) and rabeprazole thioether (3, 10, 50, 200 and 500 ng/ml plasma). Standard curves were prepared daily and constructed by linear regression analysis of the compounds/internal standard peak-area ratio versus the respective concentration of rabeprazole and rabeprazole thioether. Stock solutions (0.1% diethylamine in methanol) of rabeprazole, rabeprazole thioether and IS were prepared for quality controls in the same manner as for standard curves. Quality control samples were obtained by spiking 10–50 μl of working plasma solutions in 1 ml of blank plasma and 25 μl of

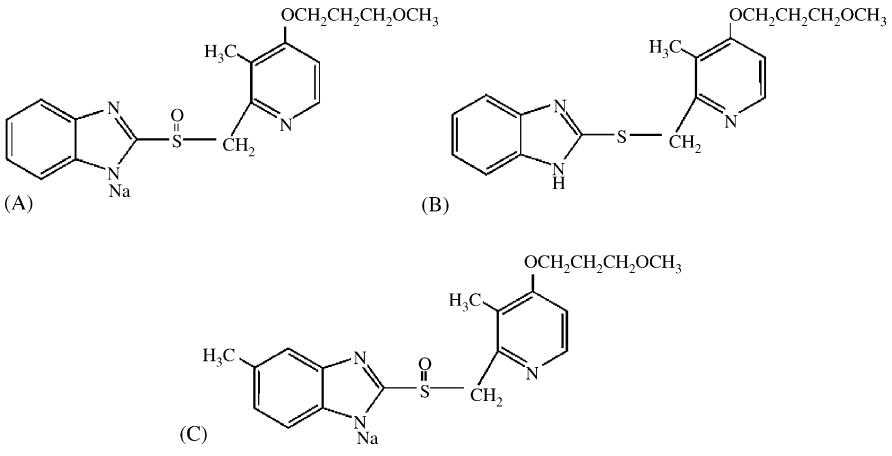

Fig. 1. Chemical structures of rabeprazole (A), rabeprazole thioether (B) and the internal standard (C).

1% diethylamine solution (final volume) to yield the final concentrations range of 2, 50, 500 and 800 ng/ml plasma for rabeprazole, 5, 50, 250 and 400 ng/ml plasma for rabeprazole thioether, and stored at −20 °C until analysis. All standard curves were checked using these quality control samples.

2.4. Sample preparation

Internal standard solution (0.1% diethylamine in methanol solution) 20 μl of 5 μg/ml and 1 ml of 0.05 M phosphate buffer adjusted to pH 10.40 using 2.5 M NaOH were added to 1 ml of plasma. The tubes were vortex-mixed for 10 s and 4 ml of diethyl ether–dichloromethane (90:10, v/v) was added as extraction solvent. After 10 min of vortex-mixing, the mixture was centrifuged at 2500 × *g* for 10 min at 4 °C (KUBOTA 5910, Kubota, Tokyo, Japan), and the organic phase (3.5 ml) was evaporated in vacuo at 40 °C to dryness (TAITEC VC-960, Shimadzu, Kyoto, Japan). The residue was dissolved in 100 μl of 0.1% diethylamine in methanol and a 30 μl aliquot was injected onto the column.

2.5. Instrumentation

The column-switching HPLC system consisted of two Shimadzu LC-10AT high-pressure pumps (for eluents A and B), a Shimadzu CTO-10AVP column oven and a Shimadzu Work station CLASS-VP chromatography integrator (Kyoto, Japan), 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 C18 Grand ODS-80TM TS as an analytical column (column II; 250 mm × 4.6 mm I.D., particle size 5 μm; MASIS Inc., Aomori, Japan) were used.

2.6. Chromatographic condition

Column-switching chromatographic condition was set based on our previous report [19]. A 30 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 6.0 min after the sample injection, the assay agents were separated from the interfering substances present in the extract on column I with a mobile phase (eluent A) of phosphate buffer (0.05 M, pH 7.0) and acetonitrile (88:12, v/v). Between 6.0 and 7.0 min after the injection, rabeprazole, rabeprazole thioether and IS retained on column I were eluted with a mobile phase (eluent B) consisting of phosphate buffer (0.05 M, pH 7.0) and acetonitrile (50:50, v/v), and effluent from column I was switched to column II. Then rabeprazole, rabeprazole thioether and IS were separated on column II by eluting with eluent B (between 7.0 and 25 min). The flow-rates were 0.8 ml/min for 0–17 min and 1.4 ml/min for 17–25 min for eluent B, respectively. The temperatures of columns I and II were 40 °C, respectively. The peak was detected by an ultraviolet detector set at a wavelength of 288 nm. The retention times of rabeprazole, rabeprazole thioether and IS were 11.7, 16.3 and 12.8 min, respectively. The peak area was used for the quantification of rabeprazole and rabeprazole thioether.

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)
0.0–6.0	A	1.2	B	0.8
6.0–7.0	B	0.8	B	0.8
7.0–17.0	A	1.2	B	0.8
17.0–25.0	A	1.2	B	1.4

2.7. Pharmacokinetic study design

Twelve Japanese healthy volunteers (six males and six females) who were *H. pylori*-negative were enrolled in this study. Their mean age was 25.7 ± 4.7 years and mean body weight was 54.1 ± 8.0 kg. The Ethics Committee of Hirosaki University School of Medicine approved this study protocol, and written informed consent had been obtained from each participant before any examinations. The purpose of this pharmacokinetic study was to determine how precisely pharmacokinetic parameters of rabeprazole and rabeprazole thioether could be defined in healthy subjects with this method.

Two tablets containing 10 mg each of rabeprazole (Pariet, Eizai Co. Ltd., Tokyo, Japan) were orally administered to each of 12 healthy volunteers. Blood samples were obtained before and at 1–6, 8, 10, 12 and 24 h after the dosing. Blood samples of 10 ml were collected in heparinized tubes and centrifuged immediately at 2500 × *g* for 10 min. The isolated plasma samples (4 ml) were placed in covered storage tubes containing a 1% diethylamine solution (100 μl) and were stored at −20 °C for a month until analysis.

3. Results and discussion

3.1. Chromatographic optimization

This paper describes a new method for the simultaneous determination of rabeprazole and rabeprazole thioether in human plasma by column-switching HPLC. Initially, our goal was to develop a more sensitive HPLC method for measuring pharmacokinetic parameters of rabeprazole and rabeprazole thioether than had previously been reported. In the present study, there were no interfering peak of endogenous substances with a retention time similar to the peaks of rabeprazole and rabeprazole thioether in each blank plasma sample

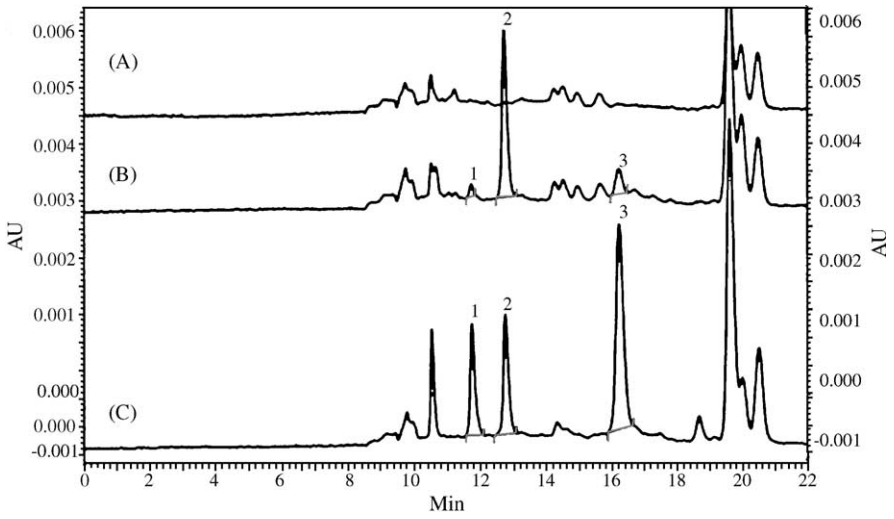

Fig. 2. The chromatogram of an extracted blank plasma sample (A), an extracted sample spiked with 5 ng/ml of rabeprazole, 10 ng/ml of rabeprazole thioether and I.S. (B), extracted plasma samples obtained from one volunteer at 5 h after receiving 20 mg rabeprazole; the plasma concentrations of rabeprazole and rabeprazole thioether were 133.4 and 190.4 ng/ml (C). Peaks: (1) rabeprazole, (2) I.S. and (3) rabeprazole thioether.

from three healthy subjects (Fig. 2A). Nakai et al [9] reported that the recovery of rabeprazole thioether was overestimated or underestimated because an interference peak was present at the same time as rabeprazole thioether. We overcame the limits of quantification of 1 ng/ml for rabeprazole and 3 ng/ml for rabeprazole thioether by using a different organic solvent (diethyl ether–dichloromethane) for a liquid–liquid extraction and an automated column-switching system for plasma sample clean-up. This method using a longer C_{18} analytical column (250 mm × 4.6 mm I.D) might also be effective for separation from endogenous interferences. Therefore, this study showed a more sensitive method enabling the monitoring of rabeprazole and its metabolite plasma concentrations up to 24 h after an administration of rabeprazole 20 mg in all volunteers.

3.2. Linearity

Calibration curves were linear over the concentrations range from 1 to 1000 ng/ml ($r = 0.9999$, $n = 6$) for rabeprazole, and from 3 to 500 ng/ml ($r = 0.9998$, $n = 6$) for rabeprazole thioether (Table 2). *F*-test on lack of fit at 99% confidence level was performed and it exhibited non-significance (Table 2).

3.3. Specificity and sensitivity

The chromatogram of an extracted blank plasma sample is shown in Fig. 2A, while the chromatogram of an extracted sample spiked with 5 ng/ml of rabeprazole, 10 ng/ml rabeprazole thioether and I.S. is shown in Fig. 2B.

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

Analyte	Curve	Slope	Intercepts	*r*	*F*-test for lack of linearity		Added (ng/ml)	Mean found	S.D.	Accuracy (%)	CV (%)	Relative error (%)	*n*
					F	*p*-value							
Rabeprazole	1	0.0109	0.0098	0.9998	28255.72	<0.001	1	0.98	0.07	98.21	7.27	−1.79	5
	2	0.0129	−0.0091	1	310561.12	<0.001	5	4.69	0.17	93.82	3.59	−6.18	5
	3	0.0116	0.0102	0.9999	229626.32	<0.001	10	10.04	0.76	100.43	7.59	0.43	5
	4	0.0138	0.0071	0.9999	124622.57	<0.001	50	51.53	2.29	103.05	4.44	3.05	5
	5	0.0111	−0.0021	0.9998	37464.83	<0.001	200	200.07	4.48	100.03	2.24	0.03	5
	Mean	0.0122	0.0022	0.9999	–	–	500	494.02	7.75	98.80	1.57	−1.20	5
	S.D.	0.0012	0.0081	0.0001	–	–	1000	987.60	9.43	98.76	0.95	−1.24	5
Rabeprazole thioether	1	0.0246	0.0057	0.9998	22726.05	<0.001	3	3.01	0.15	100.27	4.99	0.27	5
	2	0.0246	−0.0041	0.9999	36629.42	<0.001	10	10.24	0.64	102.40	6.28	2.40	5
	3	0.0250	0.0101	0.9999	78539.45	<0.001	50	50.12	4.07	100.23	8.13	1.77	5
	4	0.0244	0.0098	0.9997	16901.93	<0.001	200	204.49	5.35	102.25	2.61	2.25	5
	5	0.0271	−0.0169	0.9997	22195.41	<0.001	500	495.65	7.92	99.17	1.60	−0.87	5
	Mean	0.0251	−0.0014	0.9998	–	–							
	S.D.	0.0009	0.0103	0.0001	–	–							

The chromatograms of extracted plasma samples obtained from one volunteer at 5 h after receiving 20 mg rabeprazole did not show interference peaks (Fig. 2C). There were no peaks resulting from interfering endogenous substances with a retention-time similar to those of rabeprazole and rabeprazole thioether.

The limit of detection was defined as an analyte response that was at least five times greater than the response compared to blank response (signal-to-noise ratio = 5), and 0.5 and 1.5 ng/ml for rabeprazole and rabeprazole thioether, respectively. The lowest standard on the calibration curve was defined as the limit of quantification by which the analyte peaks for both compounds were identifiable, discrete and reproducible with a precision of 20% and accuracy of 80–120%. The limits of quantification were 1 ng/ml for rabeprazole and 3 ng/ml for rabeprazole thioether

Potential interference from co-administered drugs was investigated (by determining their retention times) in this system. In plasma samples from a subject taking clarithromycin and amoxicillin given for *H. pylori* eradicative therapy, no peaks were observed to interfere the peaks of rabeprazole, rabeprazole thioether, and IS.

3.4. Recovery (extraction efficiency) from matrix

Recovery from plasma was calculated by comparing the peak areas of pure standards prepared in working solution, and injected directly into the analytical column,with those of extracted plasma samples containing the same amount of the test compounds ($n = 6$ each). Mean absolute recoveries of rabeprazole were 78.4, 78.6 and 77.0% for 2, 500 and 800 ng/ml, respectively, and their CV values were 1.2, 0.5 and 0.6%, respectively. In rabeprazole thioether, mean absolute recoveries and CV values were 89.6 and 1.1%, 87.6 and 0.5%, and 87.7 and 0.9% for 5, 250, and 400 ng/ml, respectively.

3.5. Accuracy and precision

Intra- and inter-day precision and accuracy were evaluated by assaying quality controls with different concentrations of rabeprazole and rabeprazole thioether. Intra- and inter-day precisions were assessed by analyzing six quality control samples at each concentration on the same day and mean values of a quality control for six days, respectively. The precision determined at each concentration level should not exceed 15% of the CV except for the lower limit of quantification (LLOQ), where it should not exceed 20% of the CV [19]. These extracts underwent the same column-switching procedure. Intra- and inter-day relative standard deviations were less than 6.5 and 4.5% for rabeprazole, 3.6 and 5.3% for rabeprazole thioether, respectively. Accuracy was expected as percent error (relative error) [(measured concentration − spiked concentration)/spiked concentration] × 100 (%), while precisions were quantified by calculating intra- and inter-CV values (Table 3).

3.6. Stability

The stock solutions (0.1% diethylamine in methanol solution) of rabeprazole, rabeprazole thioether and IS, and spiked rabeprazole and rabeprazole thioether in the blank plasma with 1% diethylamine (1000/25 for plasma/1% diethylamine, v/v), were stable at −20 °C for 3 months or at −80 °C for 6 months. Plasma samples from the pharmacokinetic study were stored at −20 °C and analyzed within a month after sampling. Rabeprazole, rabeprazole thioether and IS in extracts from plasma samples reconstituted in 0.1% diethylamine in methanol were stable at 4 °C for 48 h in the autosampler.

3.7. Pharmacokinetic analysis

The peak concentration (C_{max}) and concentration peak time (T_{max}) were obtained directly from the original data. Pharmacokinetic analyses were conducted using a one-compartment model. The terminal rate constant (k_e) used for the extrapolation was determined by regression analysis of the log-linear part of the concentration-time curve for each subject. The elimination half-life ($T_{1/2}$) was determined by $0.693/k_e$. The area under the plasma concentration-time curve (AUC (0–24)) was calculated with use of the trapezoidal rule.

Fig. 3 shows concentration versus time curves obtained after an oral administration of rabeprazole (20 mg). The mean kinetic parameters of rabeprazole and rabeprazole thioether are summarized in Table 4. The plasma concentrations at all sampling points were measurable for both compounds. There

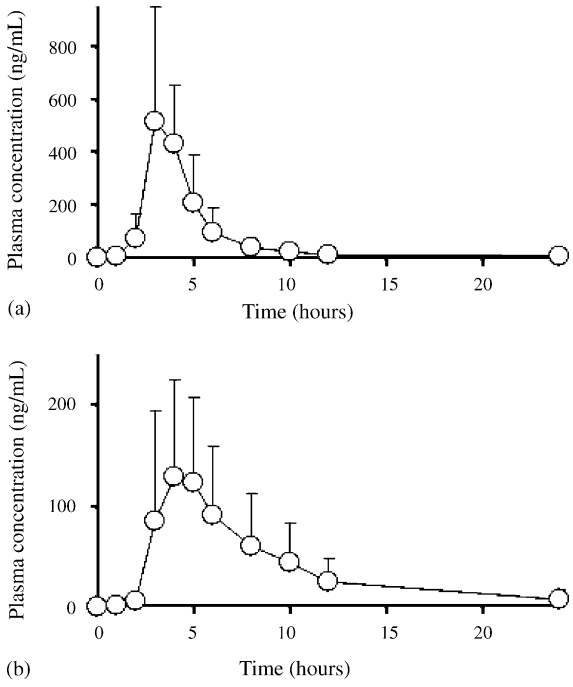

Fig. 3. Plasma concentration–time curves (mean ± S.D.) of rabeprazole (a) and rabeprazole thioether (b) from 0 to 24 h in 12 healthy volunteers after a single-oral dose of rabeprazole 20 mg.

Table 3
Presicion and accuracy for determination of analytes in spiked plasma ($n = 6$)

Analyte	Concentration added (ng/mL)	Found (mean ± S.D.) (ng/mL)	Accuracy (%)	Between-day		Found (mean ± S.D.) (ng/ml)	Accuracy (%)	Within-day	
				CV (%)	Relative error (%)			CV (%)	Relative error (%)
Rabeprazole	2	1.97 ± 0.13	98.74	6.46	−1.26	1.99 ± 0.02	101.23	1.16	−0.29
	50	49.97 ± 0.09	99.94	0.21	−0.06	48.98 ± 1.38	97.96	2.82	−2.03
	500	499.71 ± 1.38	99.94	0.28	0.06	499.50 ± 13.29	99.90	2.66	−0.10
	800	783.16 ± 28.08	97.90	3.58	−2.10	800.07 ± 35.69	100.01	4.46	0.01
Rabeprazole thioether	5	4.78 ± 0.17	95.60	3.60	−4.40	4.91 ± 0.18	98.15	3.64	−1.85
	50	49.60 ± 1.14	99.20	2.30	−0.79	50.12 ± 2.07	100.24	4.14	0.23
	250	252.87 ± 7.09	101.15	2.80	1.15	249.91 ± 4.30	99.97	2.15	−0.03
	400	405.21 ± 5.07	100.01	1.25	1.30	399.59 ± 10.63	99.89	5.32	−0.10

Table 4
Pharmacokinetic parameters of rabeprazole and rabeprazole thioether receiving rabeprazole 20 mg dose in 12 healthy subjects

Parameters	Mean	CV (%)
Rabeprazole		
C_{max} (ng/mL)	619.1	49.4
T_{max} (h)	3.4	14.7
$T_{1/2}$ (h)	3.1	61.3
$AUC_{0–24}$ (ng h/mL)	1590.2	59.6
Rabeprazole thioether		
C_{max} (ng/mL)	144.1	67.9
T_{max} (h)	4.2	19.0
$T_{1/2}$ (h)	5.6	44.6
$AUC_{0–24}$ (ng h/mL)	909.6	79.8

were inter-individual variabilities in not only plasma concentrations but also pharmacokinetic parameters for rabeprazole and rabeprazole thioether.

4. Conclusion

The HPLC procedure described for determination of rabeprazole and rabeprazole thioether is suitable for pharmacokinetic study in human volunteers 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 rabeprazole and its metabolite.

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