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Quantification of rabeprazole in human plasma by liquid chromatography–tandem mass spectrometry

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Abstract

An analytical method based on liquid chromatography coupled with tandem mass spectrometry detection has been developed and validated for the determination of rabeprazole in human plasma using omeprazole as the internal standard. The analyte and internal standard was extracted with *n*-hexane–dichloromethane–isopropanol (20:10:1, v/v) and chromatographed isocratically on a Diamonsil C_{18} analytical column. Methanol was used as the mobile phase to avoid decomposition of rabeprazole. The drug was detected in the selected reaction monitoring mode using an atmospheric pressure chemical ionization source. The method was linear within the range 2.0−800 ng/ml. The lower limit of quantification was 2.0 ng/ml. The intra- and inter-day precision, calculated from quality control (QC) samples, was less than 9.8%. The accuracy was within ±1.1%. The method herein described was employed in a pharmacokinetic study after an oral administration of 20 mg rabeprazole to 18 healthy volunteers.

Keywords: Liquid chromatography−tandem mass spectrometry; Rabeprazole; Human plasma

1. Introduction

Rabeprazole,2-[[4-(3-methoxypropoxy)-3-methylpyridine-2-yl]methylsulfinyl]-1*H*-benzimidazole, is an anti-ulcer agent which possesses inhibitory activity against gastric acid secretion as a consequence of inhibition of H^+, K^+-ATPase [1]. Like most compounds of this class, it is acid labile and reversibly transformed in acidic media to a sulfenamide [2,3]. Therefore, developing a reliable method for the quantification of rabeprazole in a biological fluid, its stability must be considered during sample collection and handling, after long-term and short-term storage, and after sample preparation and the analytical process.

Several methods have been employed for the quantification of rabeprazole in plasma [4−6]. Nakai et al. determined rabeprazole and its metabolites in human plasma using an Inertisil C_8 column at 40 °C with acetonitrile−pH 7 phosphate buffer (28:72, v/v) as a mobile phase, where the stability of rabeprazole in human plasma after short-term and long-term storage was investigated, but the stability after sample preparation and during the analytical process was not reported. The method provided a lower limit of quantification (LLOQ) of 5 ng/ml using a 1-ml aliquot of plasma, but the sample preparation was complex with double extraction. Takakuwa et al [5] and Mano et al. [6] described a gradient HPLC method for determination of rabeprazole in plasma which required a long chromatographic run time (>25 min) and the LLOQ (30 ng/ml) was high, where the stability of rabeprazole was not described. El-Gindy et al. [7] used spectrophotometric and chromatographic methods to investigate the stability of rabeprazole in solution towards acidic, oxidative and photo degradation processes. It was found that rabeprazole was rapidly degraded in acid medium and was more stable in alkaline.

In this work, we described a liquid chromatographic−tandem mass spectrometric (LC−MS−MS) method for the quantification of rabeprazole in human plasma using omepra-

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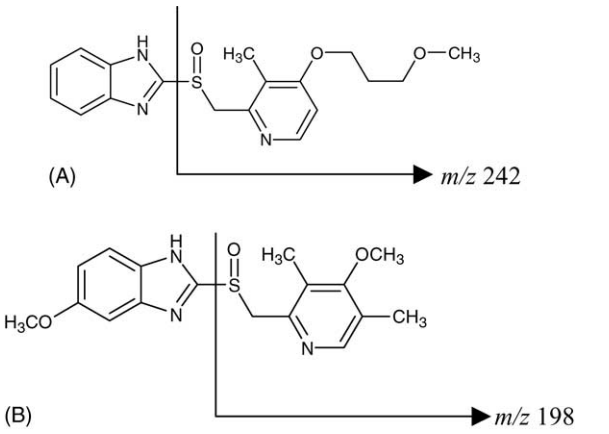

Fig. 1. Structures of rabeprazole (A) and omeprazole (B, internal standard).

zole as the internal standard (Fig. 1). And herein the stability of rabeprazole in plasma and solution samples was investigated under a variety of storage and process conditions. The method was applied to a pharmacokinetic study of rabeprazole after an administration of 20 mg rabeprazole to 18 healthy volunteers.

2. Experimental

2.1. Chemicals and reagents

Rabeprazole and omeprazole (internal standard (IS)) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol was of HPLC-grade, and other chemicals used were of analytical grade and purchased from Yuwang (Shandong, China). Blank (drug free) human plasma was obtained from Shenyang Blood Donor Service (China). Distilled water, prepared from demineralized water, was used throughout the study.

2.2. Instrumentation

A Finnigan TSQ API II tandem mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) source (San Jose, CA, USA) and a Shimadzu LC-10AD pump (Kyoto, Japan) were used for LC−MS−MS analyses. Data acquisition was performed with Xcalibur 1.1 software (Finnigan, USA). Peak integration and calibration were performed using Finnigan LCQuan software (Finnigan, USA).

2.3. LC−MS−MS conditions

Chromatographic analyses were performed using a Diamonsil C_{18} analytical column (250 mm × 4.6 mm i.d.; 5 μm; Dikma, Beijing, China) and a SecurityGuard C_{18} guard column (4 mm × 3.0 mm i.d.; Phenomenex, Torrance, CA, USA)

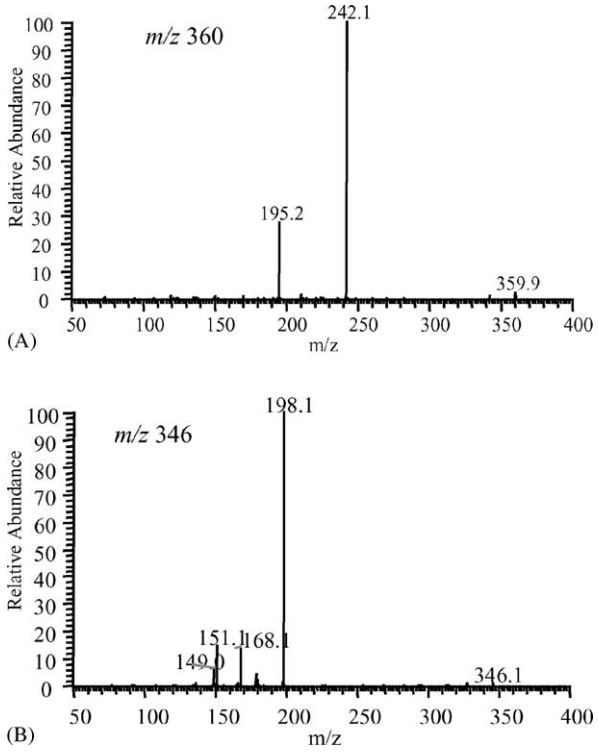

Fig. 2. Full-scan product ion spectra of $[M + H]^+$ of rabeprazole (A) and omeprazole (B).

operating at 20 °C. Methanol was used as the mobile phase and delivered at a flow-rate of 0.8 ml/min.

The mass spectrometer was operated in the positive ion detection mode. Quantification was performed using selected reaction monitoring (SRM) of the transitions of *m/z* 360 → *m/z* 242 for rabeprazole and *m/z* 346 → *m/z* 198 for omeprazole (IS). Fig. 2 shows the product ion spectra of $[M + H]^+$ of rabeprazole and omeprazole.

In order to optimize MS parameters, a standard solution (1 μg/ml) of the analyte and IS was infused into the mass spectrometer. For both rabeprazole and omeprazole, the following optimized parameters were obtained: the corona discharge current was 4.0 μA, the temperatures of the vaporizer and heated capillary were 450 °C and 250 °C, respectively. Nitrogen was used as the sheath (0.6 MPa) and auxiliary gas (3 l/min). Argon was used as the collision gas at a pressure of about 1.9 Pa. The optimized collision energy of 17 eV was used for rabeprazole and IS. Scan time was 0.3 s per transition.

2.4. Sample preparation

To a 0.5-ml aliquot of plasma sample, 100 μl of IS (1 μg/ml omeprazole in methanol) and 200 μl of 40 mM $NH_4H_2PO_4$ buffer (pH 7.0 adjusted by 0.1 M NaOH) were added. The samples were briefly mixed and 3 ml of a mixture of *n*-hexane−dichloromethane−isopropanol (20:10:1, v/v) was added. The mixture was vortex-mixed for approximate

1 min and shaked on a mechanical shaker for 10 min. After centrifugation at 2000 × *g* for 5 min, the upper organic layer was removed and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The dry residues were reconstituted to 100 μl with methanol and vortex-mixed. A 20-μl aliquot of the resulting solution was injected onto the LC−MS−MS system for analysis.

2.5. Calibration standards and quality control samples

Stock solution of rabeprazole was prepared in methanol at the concentration of 400 μg/ml. Stock solution of IS was prepared in methanol at the concentration of 400 μg/ml and diluted to 1 μg/ml. Calibration curves of rabeprazole were prepared by spiking the blank plasma at concentrations of 2.0, 5.0, 16, 50, 200, 400 and 800 ng/ml and the analysis was carried out in duplicate for each concentration. The stock and diluted solutions of rabeprazole and IS were stored at 4 °C.

The quality control (QC) samples were prepared using a different stock solution of rabeprazole to obtain the plasma concentrations of 2.0, 50 and 680 ng/ml, representing low, medium and high concentration of QC samples, respectively. The spiked plasma samples (standards and quality controls) were extracted on each analytical batch along with the unknown samples.

2.6. Method validation

Plasma samples were quantified using the ratio of the peak area of rabeprazole to that of IS as the assay parameter. Peak area ratios were plotted against rabeprazole plasma concentrations and standard curves in the form of $y = A + Bx$ were calculated using weighted ($1/x^2$) least squares linear regression.

To evaluate linearity, plasma calibration curves were prepared and assayed in duplicate on three separate days. Accuracy and precision were also assessed by determining QC samples using six replicate preparations of plasma samples at three concentration levels on three separate days using three separate sources of human plasma. The accuracy, i.e. percentage concentration deviation, was expressed by (mean observed concentration − spiked concentration)/(spiked concentration) × 100%, and the precision by relative standard deviation (R.S.D.).

The extraction recoveries of rabeprazole at three QC levels were evaluated by calculating the mean value of the response of each concentration and dividing the extracted sample mean by the unextracted (spiked blank plasma extract) sample mean of the corresponding concentration.

Rabeprazole stability in plasma was assessed by analyzing replicates ($n = 3$) of QC samples at concentrations of 5.0 and 680 ng/ml, respectively, exposed to different time and temperature conditions. The results were compared with those for QC samples freshly prepared, and the percentage concentration deviation was calculated. The short-term stability was evaluated after exposure of the plasma samples to room temperature for 2 h. The long-term stability was assessed after storage of the test samples at −20 °C for 3 months. The freeze-thaw stability was determined after three freeze-thaw cycles (−20 to 20 °C) on consecutive days. The bench-top storage stability was assessed by placing QC samples at room temperature for a fixed period of time after being extracted and analyzed. The stability of standard solutions was also tested at room temperature for 4 h and upon refrigeration (4 °C) for 1 week.

2.7. Pharmacokinetic study

The method was applied to determine the concentrations of rabeprazole in human plasma from healthy volunteers received an oral dosage Pariet tablet (containing rabeprazole 20 mg, Eisai Co., Ltd., Japan). Eighteen healthy male volunteers were selected for the study. Blood (3 ml) was removed by venepuncture prior to dosage and at 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0 and 12.0 h thereafter. Following centrifugation (2000 × *g* for 10 min) the plasma was removed and stored at −20 °C until analysis.

3. Results and discussion

3.1. Sample preparation

Liquid–liquid extraction with a mixture of *n*-hexane−dichloromethane−isopropanol (20:10:1, v/v) was chosen for a fast and easy sample preparation. It was reported that a buffer of pH 10.4 was added to plasma samples before liquid–liquid extraction [4,5], which made rabeprazole changed into sodium salt and avoided acidic degradation [7,8], but it needed twice extraction with ethyl acetate. In this paper, several pH values had been evaluated before extraction. It was found that the recovery was higher at pH 7 than that at pH 10 and the stability of rabeprazole did not decrease. Therefore, the $NH_4H_2PO_4$ buffer of pH 7 was added, which made it much easier to extract analyte from plasma samples.

3.2. LC−MS−MS analysis

Because rabeprazole has three nitrogens in its structure, the signal intensities obtained in the positive mode was much higher than that in the negative mode. It was also found that APCI spectra revealed higher signals for *m*/*z* 360 compared to ESI source. Further assay development was therefore limited to the APCI source. The collision-induced dissociation (CID) of protonated rabeprazole (*m*/*z* 360) results in the loss of the benzimidazole ring, leading to the main fragment ion at *m*/*z* 242. Other fragments showed relative intensities below 30% and are not likely to improve the sensitivity when used in SRM mode. A similar fragmentation pathway for omeprazole was also observed. The most abundant fragment was *m*/*z* 198. Additional tuning of the APCI source and CID parameters onto the transition *m*/*z* 360 → *m*/*z* 242 (rabeprazole) and *m*/*z* 346 → *m*/*z* 198 (omeprazole) further improved the sensitivity.

3.3. Chromatographic conditions

To achieve maximum peak responses and symmetric chromatographic peaks, the mobile phase containing varying percentages of organic phase and pH modifiers was tested. At the same time, the stability of rabeprazole in the mobile phase was also examined. Furthermore, it was found necessary to reconstitute the residues with the mobile phase, otherwise the chromatographic behaviors of the analytes would be seriously deteriorated at low flow-rate.

In positive ion mode, the presence of a low amount of formic acid in the mobile phase can improve the MS response of rabeprazole and omeprazole, but it decreased significantly the stability of rabeprazole. After the reconstitution of residue of plasma extract with the mobile phase consisting of methanol−water−formic acid (80:20:1, v/v), rabeprazole degraded more than 20% within only 5 min, and about 40% within 10 min. Using the mobile phase of methanol−water (80:20, v/v), rabeprazole degraded more than 20% within 10 min. The stability of rabeprazole could be improved using the mobile phase containing 5 mM ammonia acetate buffer (pH 7), but the MS response of rabeprazole was reduced significantly. Therefore, 100% methanol was used as the mobile phase, which shortened analytical time and avoided the degradation of rabeprazole. Fig. 3 shows selected blank and spiked blank chromatograms as well as a real plasma sample. As shown in Fig. 3A, no endogenous interference was observed in the blank plasma. The chromatogram for the standard LLOQ sample is shown in Fig. 3B, in which the retention times for rabeprazole and omeprazole (IS, 200 ng/ml) were both 3.8 min. Chromatogram of blank plasma spiked with rabeprazole (50 ng/ml) and IS is shown in Fig. 3C. Chromatogram of a volunteer plasma sample is shown in Fig. 3D. The total run time was 4.3 min.

3.4. Linearity, precision and accuracy

Visual inspection of the plotted duplicate calibration curves and correlation coefficients >0.99 confirmed that the calibration curves were linear over the concentration ranges 2.0–800 ng/ml for the analyte. Typical standard curve was $y = -3.334 \times 10^{-3} + 4.125 \times 10^{-3} x$. The lower limit of quantification, defined as the lowest concentration at which both precision and accuracy were less than 15%, was 2.0 ng/ml.

Table 1 shows the intra- and inter-assay precision and accuracy for rabeprazole from QC samples. The results were

Table 1
Accuracy and precision for the analysis of rabeprazole in human plasma (in prestudy validation, six replicates per day)

Added C (ng/ml)	Found C (ng/ml)	Intra-run R.S.D. (%)	Inter-run R.S.D. (%)	Relative error (%)
2.00	1.99	9.2	2.9	−0.5
50.0	49.4	9.8	3.2	−1.2
680	672.7	9.4	4.1	−1.1

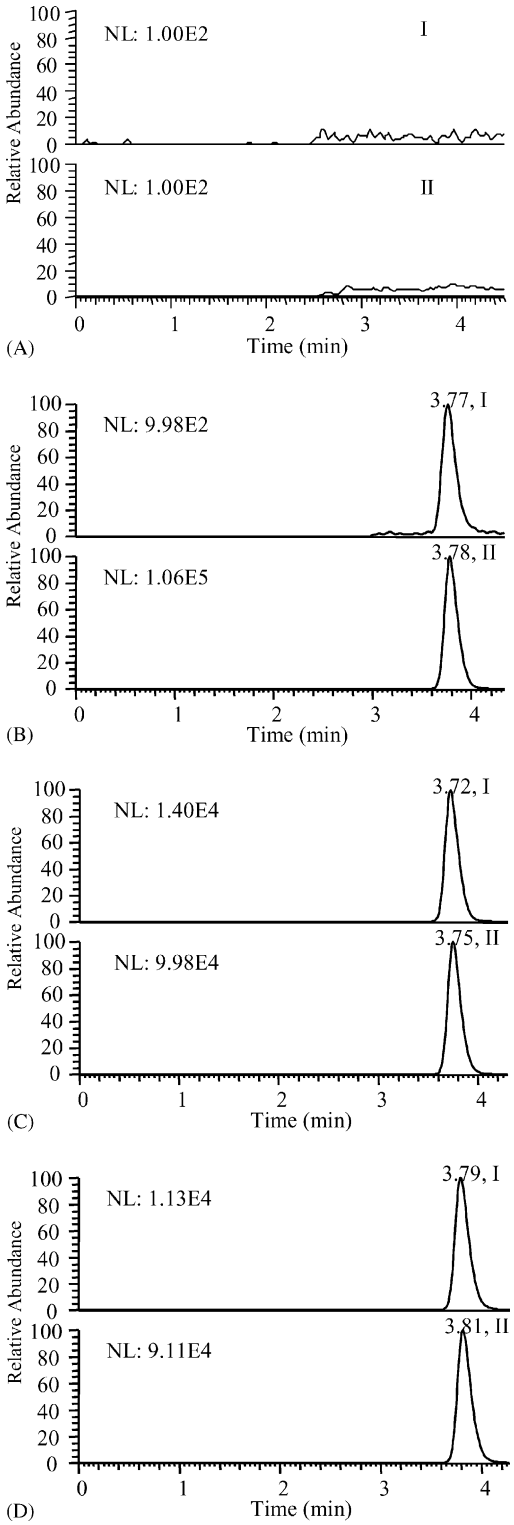

Fig. 3. SRM chromatograms of rabeprazole (I) and omeprazole (IS, II) in human plasma samples, (A) Blank plasma sample; (B) plasma sample spiked with rabeprazole (2.0 ng/ml) and IS (200.0 ng/ml); (C) plasma sample spiked with rabeprazole (50.0 ng/ml) and IS (200.0 ng/ml); (D) a volunteer plasma sample 8 h after oral dose of 20 mg rabeprazole.

calculated using one-way ANOVA. The intra- and inter-assay precision were measured to be below 10% and 3%, respectively. The inter-assay accuracy ranged from −1.2% to −0.5%.

3.5. Extraction recovery and storage stability

The extraction recoveries of rabeprazole were 62.5 ± 3.4%, 63.8 ± 4.5% and 68.7 ± 2.1% at concentrations of 5.0, 50 and 680 ng/ml, respectively (n = 6). The extraction recovery of IS was 68.1 ± 3.8%.

Acceptable analyte stability was demonstrated for all phases of storage and processing. The accuracy values of low (5.0 ng/ml) and high (680 ng/ml) concentrations of rabeprazole in human plasma were 108% and 93.4% at room temperature for 2 h, 91.2% and 103% after three freeze-thaw cycles, and 88.6% and 92.1% at −20 °C for 3 months. These stability results were similar to those reported in the literature [4]. In addition, the stability of processed plasma sample was also tested. It was found that rabeprazole was stable at room temperature for 4 h and at 4 °C for 12 h after the reconstitution of residue of plasma extract with methanol (the accuracy varied between 94.2% and 104%), but it degraded more than 30% at room temperature after 12 h, suggesting that plasma samples after extraction should be analyzed within 4 h if left at room temperature or within 12 h if left at 4 °C. The standard solutions of rabeprazole in methanol allowed to stand at 4 °C for a week. The coefficient of variation for each QC levels was within 8.2%. The methanolic solution of IS (1 μg/ml) was proved stable at room temperature for more than 8 h and at 4 °C for a week. The coefficient of variation was within 5.9%.

3.6. Pharmacokinetic study

The method was applied to determine the plasma concentrations of rabeprazole after an oral administration of 20 mg rabeprazole to eighteen volunteers. The mean plasma concentration−time curve of rabeprazole is shown in Fig. 4. Because of the relatively short chromatographic run time and straightforward sample preparation procedure, a sample throughput of 100 per day is routinely achieved. This simple and selective method for the determination of rabeprazole in human plasma was readily applicable to the clinical pharmacokinetic study for rabeprazole.

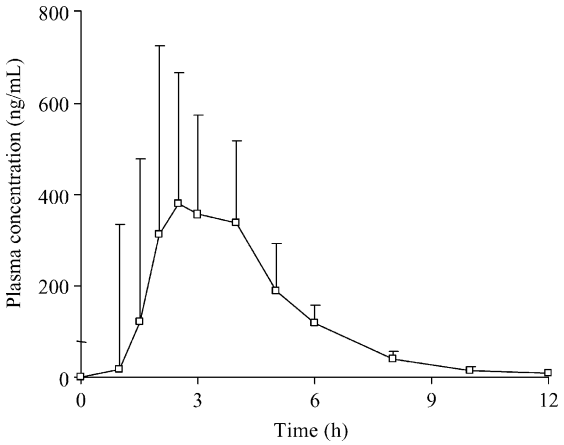

Fig. 4. Mean plasma concentration–time curve of rabeprazole after an oral administration of 20 mg rabeprazole to 18 healthy volunteers (n = 18, $\bar{x}$ and S.D.).

4. Conclusion

A sensitive LC−MS−MS method for the quantification of rabeprazole in human plasma was developed and validated. The method satisfied the requirements of high sensitivity, selectivity and high throughput for pharmacokinetic studies.

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