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Determination of omeprazole in human plasma by liquid chromatography–electrospray quadrupole linear ion trap mass spectrometry

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Abstract

An analytical method for the determination of omeprazole in human plasma has been developed based on liquid chromatography mass spectrometry. The analyte and internal standard sildenafil are extracted from plasma by liquid–liquid extraction using diethyl ether:dichloromethane (60:40, v/v) and separated by reversed phase high-performance liquid chromatography (HPLC) using acetonitrile:methanol:10 mM ammonium acetate (37.5:37.5:25, v/v/v) as mobile phase. Detection is carried out by multiple reaction monitoring on a Q TRAP™ LC/MS/MS system (Q TRAP™). The method has a chromatographic run time of 3.5 min and is linear within the range 0.50–800 ng/mL. Intra- and inter-day precision expressed as relative standard deviation ranged from 0.4 to 8.5% and from 1.2 to 6.8%, respectively. Assay expressed as relative error was <5.7%. The method has been applied in a bioequivalence study of two capsule formulations of omeprazole.

Keywords: Omeprazole; LC–MS–MS

1. Introduction

Omeprazole (5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulphinyl]-1*H*-benzimidazole) is the first of the class of drugs known as proton-pump inhibitors. It acts to inhibit gastric acid secretion in the stomach, and is used to treat various acid-related gastrointestinal disorders [1]. Omeprazole is in fact a prodrug, which is converted at low pH to a reactive sulfenamide intermediate which binds to thiol groups of the H^+–K^+ ATPase in gastric parietal cells.

Omeprazole has been determined in formulations and biological fluids by a variety of methods [2–10,13] such as high-performance liquid chromatography (HPLC) with UV detection [2–5] and liquid chromatography coupled with tandem mass spectrometry (LC–MS–MS) [6–8]. These methods suffer from a number of limitations such as complex sample preparation [9], uneconomical solid-phase extraction (SPE) [13], inconvenient chromatography [10] or the need for a relatively large volume of plasma (2 mL) [8]. HPLC with UV detection [5] is relatively insensitive (limit of quantification (LOQ), 10 ng/mL) compared to liquid chromatography–mass spectrometry (LOQ, 0.5 ng/mL), but the latter requires a more complex SPE procedure and a large volume of plasma. In addition, the use of selected-ion monitoring (SIM) was associated with a loss of specificity. Woolf and Matuszewski [6] validated an assay using tandem mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) interface but the sensitivity was insufficient for pharmacokinetic studies and the run time of 11 min was rather long.

In this paper, we describe a rapid, sensitive and selective liquid chromatography–electrospray mass spectrometry (LC–ESI–MS) method for the quantitation of omeprazole. The method was applied to a bioequivalence study of two oral formulations of omeprazole.

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2. Experimental

2.1. Instrumentation

Chromatography was performed using a Hewlett-Packard Model 1100 series (Hewlett-Packard, Palo Alto, CA, USA) HPLC coupled to an Applied Biosystems/MDS SCIEX Q TRAP™ linear ion trap mass spectrometer (Concord, Ont., Canada). Applied Biosystems/MDS SCIEX Analyst software (Version 1.4) was used for data acquisition and processing.

2.2. Chemicals and reagents

An omeprazole standard was provided by Jilin Province Northeast Asia Pharmaceutical Co. Ltd. (>99% Dunhua, China). The internal standard (IS), sildenafil, was provided by Peking University First Hospital (Beijing, China). Acetonitrile and methanol were HPLC grade purchased from Fisher Scientific (Fair Lawn, NJ, USA). All other chemicals were of analytical grade and used without further purification. Blank (drug free) human plasma was obtained from Changchun Blood Donor Service (Changchun, China). Distilled water, prepared from demineralized water, was used throughout the study.

2.3. Calibration standards and quality controls

A stock solution of omeprazole was prepared by dissolving 10 mg in 5 mL methanol followed by dilution to 25 mL with 0.1 M Na_2CO_3. This solution was stored frozen at −20 °C in 5 mL portions for up to 3 months. The stock solution (100 μg/mL) of internal standard was prepared by weighing 1 mg of sildenafil into a 10 mL volumetric flask and filling the flask to volume with methanol. This solution was stored at 4 °C. The internal standard solution containing sildenafil at a concentration of 1 μg/mL was prepared by pipetting 0.25 mL the stock solutions into a 25 mL volumetric flask and filling the flask to volume with methanol. Calibration curves were prepared using blank plasma spiked at concentrations of 0.5, 1.00, 2.00, 6.00, 20.0, 60.0, 200 and 800 ng/mL. Quality control (QC) samples were prepared in blank plasma at concentrations of 1.00, 20.0 and 640 ng/mL. Care was taken to protect omeprazole solutions and QC samples from direct sunlight [2]. In each analytical run, calibration standards, QC samples and unknowns were extracted together.

2.4. Sample preparation

Frozen plasma samples were thawed at ambient temperature and centrifuged at 3000 × *g*, 4 °C for 5 min. An aliquot of plasma (500 μL) was placed in a glass tube followed by 100 μL 0.1 M Na_2CO_3, 100 μL of IS solution (1 μg/mL in methanol) and 3 mL diethyl ether:dichloromethane (60:40, v/v). The mixture was vortex-mixed for 30 s, centrifuged at 3500 × *g*, room temperature (about 18 °C) for 5 min. The organic layer was transferred to another clean glass tube and evaporated at 25 °C with nitrogen. The dry residues were reconstituted with 100 μL acetonitrile:methanol (50:50, v/v) containing 0.1 M Na_2CO_3 and vortex-mixed for 15 s. A 20 μL aliquot of reconstituted sample was injected into the LC–MS system.

2.5. Chromatographic conditions

HPLC was carried out on a ZORBAX Extend-C_{18} 5 μm (4.6 mm × 150 mm i.d.) analytical column (from Agilent Technologies) operated at 40 °C. The mobile phase was acetonitrile:methanol:10 mM ammonium acetate (37.5:37.5:25, v/v/v) at a flow-rate of 1.0 mL/min. An approximately 1:1 split of the column eluant was included so that only 0.5 mL/min entered the mass spectrometer. Under these conditions, retention times were typically 2.1 min for omeprazole and 2.9 min for sildenafil.

2.6. Mass spectrometer conditions

The Q TRAP™ LC–MS–MS system was equipped with an electrospray source operating in the positive ion mode. Using multiple reaction monitoring (MRM), the transitions m/z 346.2 → 198.2 and m/z 475.2 → 283.2 were used for quantitation of omeprazole and sildenafil, respectively. Fig. 1 shows enhanced product ion (EPI) spectra and fragmentation schemes [14,15] of omeprazole and sildenafil. In order to optimize MS parameters, a standard solution of analyte and IS was infused into the mass spectrometer using a syringe pump. Optimized parameters were as follows: curtain gas, gas 1 and gas 2 (nitrogen) 15, 50 and 50 units, respectively; dwell time 400 ms; source temperature 500 °C; IonSpray voltage 5500 V. Declustering potential (DP) and collision energy (CE) were, respectively, 27 V and 17 eV for omeprazole and 52 V and 46 eV for sildenafil.

2.7. Stability

Stability was assessed using QC samples stored for 6 h at room temperature and subjected to three freeze–thaw cycles (−20 to 25 °C). Omeprazole stock solution was stored at −20 °C and measured weekly through 1 month to determine the change in drug concentration.

2.8. Assay validation

Calibration curves were based on peak area ratios of omeprazole to IS for the eight calibration standards analysed in duplicate. Linearity was assessed by linear least-squares regression with a weighting index of $1/x^2$. Accuracy and precision were based on assay of six replicates of QC samples analysed on 3 different days and calculated using one-way ANOVA. Absolute recoveries of omeprazole and IS were determined by assaying QC samples and comparing peak areas with those obtained from direct injection of the compounds dissolved in the supernatant of the processed blank plasma.

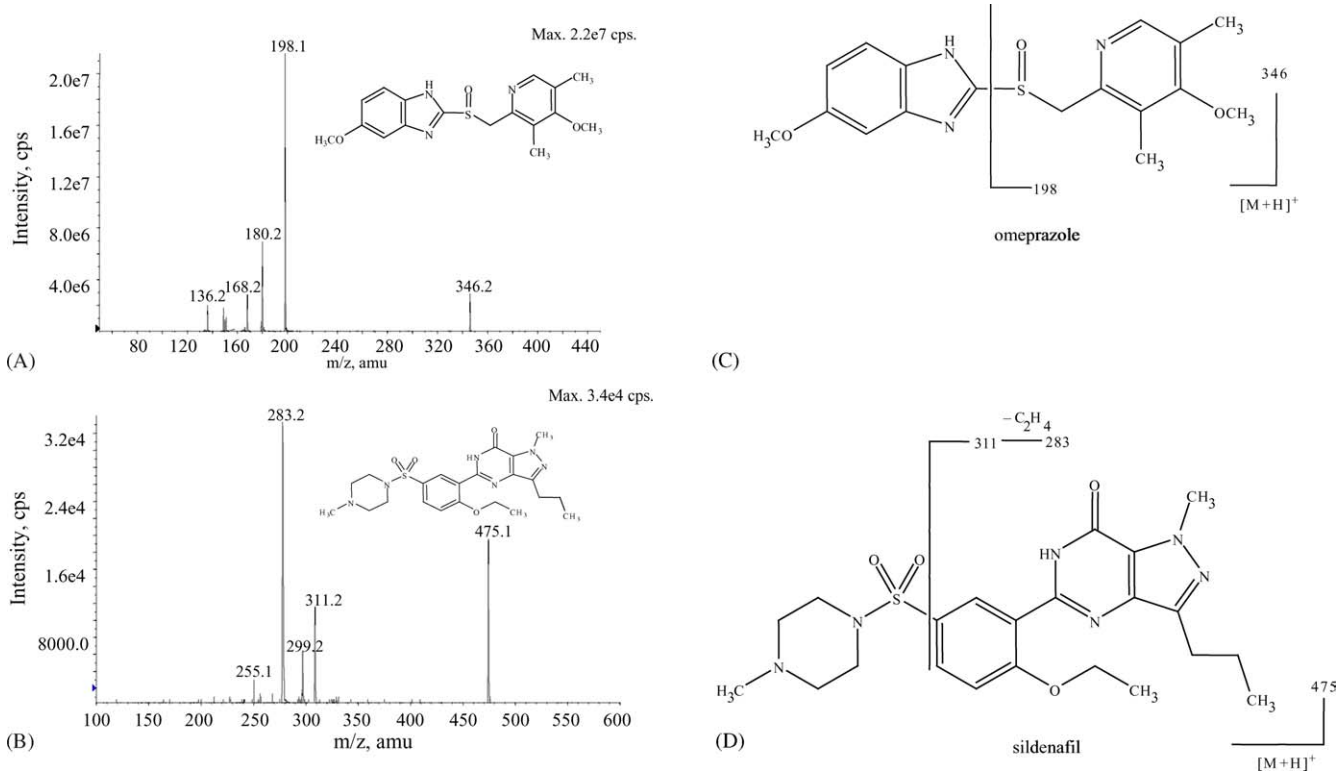

Fig. 1. Full-scan product ion spectra and fragmentation schemes of $[M+H]^+$ of: (A) omeprazole and (B) sildenafil.

2.9. Bioequivalence study

The method was applied to evaluate the bioequivalence of two capsule formulations of omeprazole in healthy volunteers. The formulations were an enteric-coated capsule (Jilin Province Northeast Asia Pharmaceutical Co. Ltd., China) and a standard reference formulation (AstraZeneca China Pharmaceutical Corp., Wuxi, China). Twenty healthy male volunteers received a single dose in a two-way randomized crossover design with a 2 weeks washout period between doses. Blood samples were collected before and at 0.5, 1.0, 1.5, 2.0, 2.5, 3, 4, 5, 6, 8, 12 and 16 h post-dose. Bioequivalence of the two formulations was assessed according to US-FDA methodology [11].

3. Results

As shown in Fig. 2A, no endogenous peaks were observed in the chromatogram of blank plasma. The chromatogram for the standard sample at the LOQ is shown in Fig. 2B. The retention times for omeprazole and IS were 2.1 and 2.9 min, respectively.

The calibration curves showed good linearity within the range 0.5–800 ng/mL. As shown in Table 1, the method gave good precision and accuracy. Intra- and inter-day precisions were below 8.5 and 6.8%, respectively. The accuracy ranged from −4.3 to 5.7%.

The recoveries of omeprazole at 1.00, 20.0 and 640 ng/mL (mean ± S.D., $n = 6$) were 80.6 ± 6.1, 83.4 ± 7.6 and 85.1 ± 8.9%, respectively. The recovery of the IS was 80.3 ± 4.3% at a concentration of 1000 ng/mL.

The lower limit of quantification, defined as the lowest concentration at which both precision and accuracy were less than or equal to 20%, was 0.5 ng/mL.

In terms of stability, QC samples showed no significant degradation when stored at room temperature for 6 h (Table 2) or after three freeze–thaw cycles (Table 3).

The geometric mean and respective 90% confidence interval (CI) of Omeprazol/Losec percent ratios were 93.1% (81.7–105.6%) for C_{max}, 94.8% (87.9–102.4%) for AUC_{0-t} and 94.7% (87.8–102.3%) for $AUC_{0-\infty}$.

Table 1
Summary of precision and accuracy from QC samples of human plasma extracts ($n = 3$ days, six replicates per day)

Added concentration (ng/mL)	Found concentration (ng/mL)	Intra-run R.S.D. (%)	Inter-run R.S.D. (%)	Relative error (%)
1.00	0.97 ± 0.06	8.49	5.90	−2.61
20.0	21.1 ± 1.4	5.27	6.79	5.69
640.0	612.6 ± 6.9	0.40	1.18	−4.29

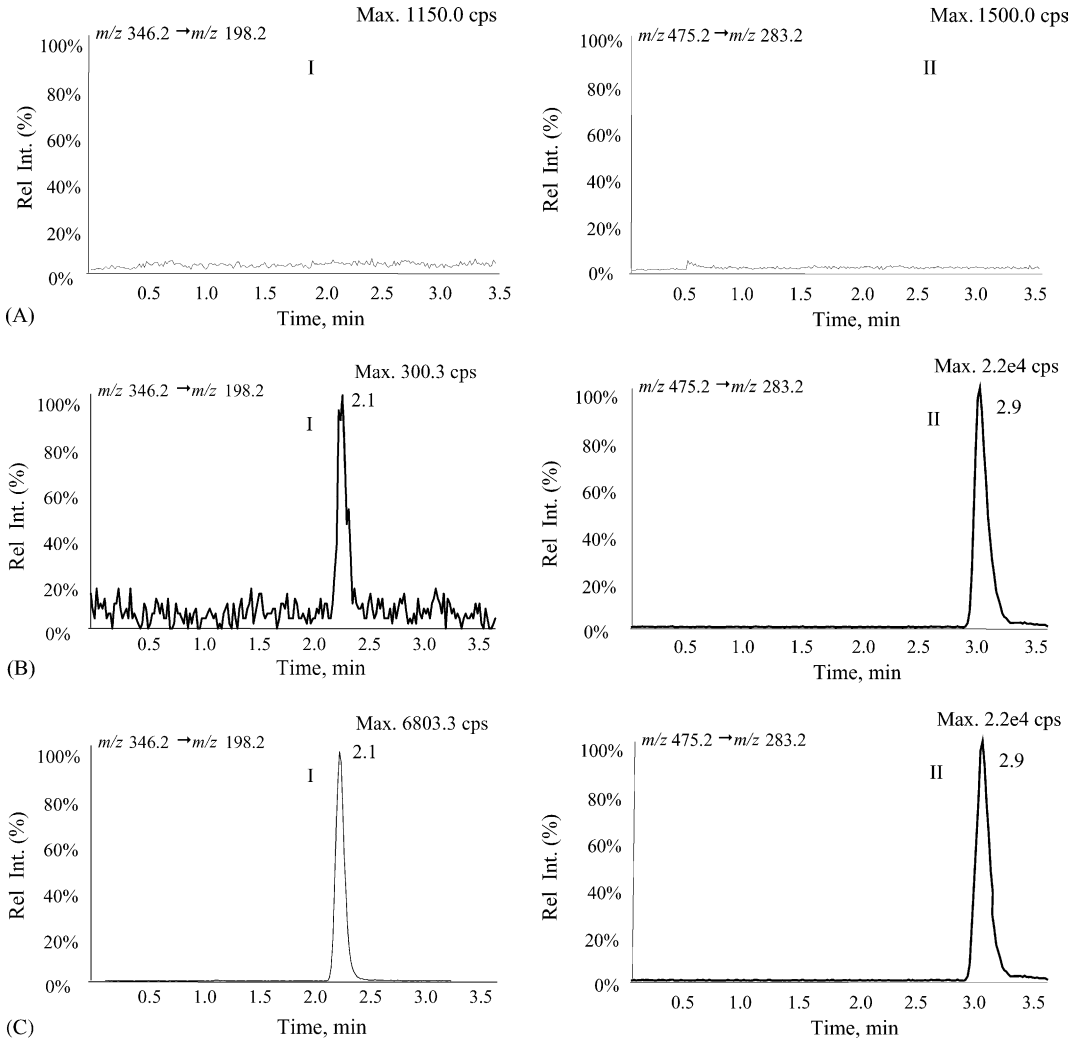

Fig. 2. MRM chromatograms of omeprazole and sildenafil in human plasma: (A) blank plasma; (B) blank plasma spiked with 0.5 ng/mL omeprazole (I) and 1000 ng/mL sildenafil (II); (C) plasma from a human volunteer 1.5 h after oral administration of 20 mg omeprazole.

Table 2
Results for stability samples at room temperature for 6 h

Spiked concentration (ng/mL)	Mean calculated concentration (ng/mL)	No. of replicates	CV (%)
1.0	0.93	4	−7.4
20.0	19.3	4	−3.4
640.0	634.1	4	−0.9

Table 3
Results for stability samples to three freeze–thaw cycles (−20 to 25 °C)

Spiked concentration (ng/mL)	Mean calculated concentration (ng/mL)	No. of replicates	CV (%)
1.0	0.94	4	−6.2
20.0	19.1	4	−4.7
640.0	628.7	4	−1.8

4. Discussion

Full-scan positive mode spectra of omeprazole and sildenafil contained predominant molecular ions at *m*/*z* 346.2 and 475.1, respectively. The product ion mass spectra of these protonated molecular ions (Fig. 1) showed the presence of one major product ion at *m*/*z* 198.2 and 283.2 for omeprazole and sildenafil, respectively.

In relation to the chromatographic system, it was necessary to take into account the instability of omeprazole in solution at pH < 8 [12]. It was found that a mobile phase containing 25% 10 mM ammonium acetate as the aqueous component gave a good response with no evidence of degradation of analyte despite its pH of 7.3. This is probably the result of the short run time of only 3.5 min. In further consideration of analyte instability in solution, residues from the liquid–liquid extraction were reconstituted in a solution containing sodium carbonate. This was shown to maintain the stability of omeprazole in autosampler vials. Finally, in all

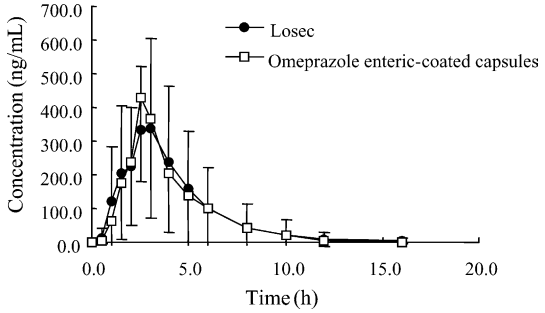

Fig. 3. Mean plasma concentration vs. time curves for two omeprazole capsule formulations in healthy volunteers ($n = 20$).

manipulations involving omeprazole care must be taken to exclude exposure to direct sunlight [2].

A number of compounds were evaluated as internal standard including diazepam, diphenhydramine, huperzine and sildenafil. Sildenafil was best not only because of its satisfactory peak shape and good resolution from omeprazole but also because it gave a relatively high recovery on solvent extraction. In contrast, the retention times of diazepam, diphenhydramine and huperzine were either too long or too short under the chromatographic conditions employed. Although isotope-labeled omeprazole or analogue would be the ideal IS, the extraction recovery of sildenafil is relative high and close to that of omeprazole, and it did not affect the good performance of the assay. Ultimately, sildenafil was chosen as the internal standard excluding variation in extraction and reconstitution recoveries.

After oral administration of omeprazole, the C_{max} and t_{max} values were similar to those reported in the literature [2,5]. In addition, the two capsule formulations were found to be equivalent (Fig. 3) with calculated 90% confidence intervals for omeprazole/Losec ratios of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ within the 80–125% interval required by the US Food and Drug Administration [11].

5. Conclusions

A method based on HPLC with electrospray quadrupole linear ion trap mass spectrometric detection has been developed for the quantification of omeprazole in human plasma. The precision, accuracy, sensitivity and selectivity of the method make it suitable for human pharmacokinetic studies. The run-time of the assay is shorter than that of other published assays allowing increased sample throughput.

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