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# Highly sensitive method for the determination of omeprazole in human plasma by liquid chromatography–electrospray ionization tandem mass spectrometry: application to a clinical pharmacokinetic study

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**ABSTRACT: A highly sensitive, rapid assay method has been developed and validated for the estimation of omeprazole (OPZ) in human plasma with liquid chromatography coupled to tandem mass spectrometry with electrospray ionization in the positive-ion mode. The assay procedure involves alkalinization of plasma followed by simple liquid–liquid extraction of OPZ and lansoprazole (internal standard, IS) from human plasma with acetonitrile. Chromatographic separation was achieved with 0.01 M ammonium acetate:acetonitrile (40:60, v/v) at a flow rate of 0.25 mL/min on an Inertsil ODS 3 column with a total run time 2.5 min. The MS/MS ion transitions monitored were 346.1 → 198.1 for OPZ and 370.1 → 252.1 for IS. Method validation and clinical sample analysis were performed as per FDA guidelines and the results met the acceptance criteria. The lower limit of quantitation achieved was 0.05 ng/mL and the linearity was observed from 0.05 to 10.0 ng/mL. The intra-day and inter-day precisions were in the ranges 2.09–8.56 and 5.29–8.19%, respectively. This novel method has been applied to a pharmacokinetic study of OPZ in humans. Copyright © 2008 John Wiley & Sons, Ltd.**

**Keywords:** omeprazole; LC-MS/MS; method validation; human plasma; pharmacokinetics

## Introduction

Omeprazole (OPZ; CAS no. 73590-58-6; Fig. 1) is the first of the class of the drugs known as proton pump inhibitors. It inhibits the gastric acid secretion in the stomach by inhibiting gastric parietal cell proton pump ($H^+/K^+$-ATPase) and used in the treatment of gastroesophageal reflux disease (GERD), Zollinger–Ellison syndrome and other conditions caused by excess stomach acid secretion. OPZ is also used to promote healing of erosive esophagitis (Andersson, 1996). OPZ is a prodrug and acid labile compound. Its absorption is rapid and peak plasma concentrations occur within 0.5–3.5 h following oral administration of enteric coated tablet/granules. Following a single-dose, oral administration of 40 mg of drug, peak plasma levels ($C_{max}$) attained were 300–600 ng/mL but the area under the plasma concentration–time curve (AUC) and $C_{max}$ showed large inter-individual variations due to the genetically different activities of CYP2C19, which metabolizes OPZ. The elimination half-life in plasma is reported to be 40–60 min. The $C_{max}$ and AUC of OPZ are approximately proportional in doses up to 40 mg, but because of a saturable first-pass effect, a greater than linear response in $C_{max}$ and AUC occurs with doses greater than 40 mg. Absolute bioavailability (compared with intravenous administration) is about 30–40% at doses of 20–40 mg, due in large part to pre-systemic metabolism (Andersson, 1996).

Several LC-MS/MS bioanalytical methods have been reported for estimation of OPZ alone or along with its metabolites in human biological samples (Woolf and Matuszewski, 1998; Stenhoff *et al*., 1999; Kanazawa *et al*., 2002; Yin *et al*., 2004; Frerichs *et al*., 2005; Wang *et al*., 2005; Hofmann *et al*., 2006; Song and Naidong, 2006; Hultman *et al*., 2007; Martens-Lobenhoffer *et al*., 2007). Although the reported LC-MS/MS methods are sensitive enough, the reported lowest limit of quantitation (LLOQ) amongst them was 0.4 ng/mL (Frerichs *et al*., 2005). In the present paper we are presenting an LC-MS/MS method with an LLOQ of 0.05

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**Abbreviations used:** AUC, area under the plasma concentration–time curve; CE, collision energy; CXP, collision exit potential; DP, declustering potential; EP, entrance potential; GERD, gastroesophageal reflux disease; OPZ, omeprazole.



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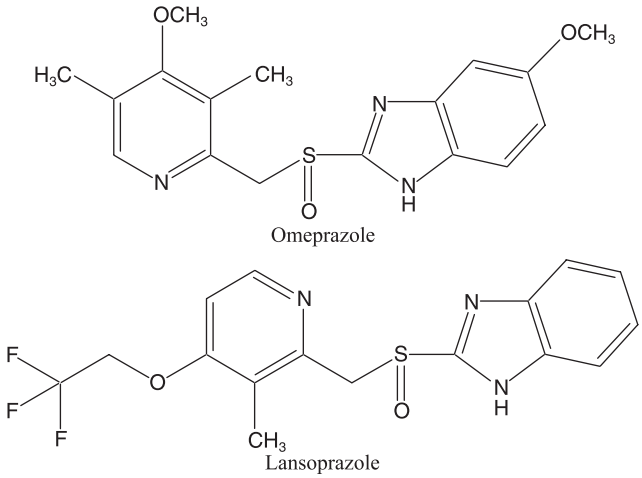


**Figure 1.** Structural representation of omeprazole (OPZ) and lansoprazole (IS).

ng/mL, which is 8-fold lower than the lowest reported LLOQ for OPZ. In addition, our method involves simple sample processing and has a very short run time (2.5 min); hence it gives high throughput. The newly developed LC-MS/MS method was successfully used in a human pharmacokinetic study following administration of 10 mg of OPZ tablet.

## Experimental

### Chemicals and Reagents

OPZ and lansoprazole (IS, Fig. 1) were procured from Generics Division, Dr Reddy's Laboratories, Hyderabad, India. HPLC-grade acetonitrile and methanol were purchased from Rankem, Ranbaxy Fine Chemicals Limited, New Delhi, India. Analytical-grade ammonium acetate and sodium hydrogen carbonate were purchased from Himedia Laboratories, Mumbai and S.D. Fine Chemicals, Mumbai, India, respectively. Control $K_2$EDTA human plasma was obtained from Cauvery Diagnostics and Blood Bank, Secunderabad.

### HPLC Operating Conditions

An Agilent (Agilent Technologies, Waldbronn, Germany) 1100 series LC system equipped with a degasser (G1379A), quaternary pump (G1311A) and auto-sampler (G1367A) was used to inject 10 μL aliquots of the processed samples onto an Inertsil ODS 3 column (75 × 2.1 mm, 3 μm, GL Sciences Inc., Tokyo, Japan), which was kept at ambient temperature. The isocratic mobile phase, a mixture of 0.01 M ammonium acetate and acetonitrile mixture (40:60, v/v) was filtered through a 0.45 μm membrane filter [Millipore (XI5522050), USA or equivalent] and then degassed ultrasonically for 5 min and delivered at a flow rate of 0.25 mL/min into the mass spectrometer electrospray ionization chamber.

### Mass Spectrometry Operating Conditions

Quantitation was achieved by MS/MS detection in positive ion mode for analyte and IS using an MDS Sciex (Foster City, CA, USA) API 4000 mass spectrometer, equipped with a Turboionspray™ interface at 500°C. The common parameters, viz. curtain gas, nebulizer gas, auxillary gas and collision gas, were set at 10, 40, 45 and 6 psi, respectively. The compound parameters, viz. declustering potential (DP), collision energy (CE), collision exit potential (CXP) and entrance potential (EP), for OPZ and IS were 53, 20, 15, 10 V and 51, 18, 13, 10 V, respectively. Detection of the ions was performed in the multiple-reaction monitoring (MRM) mode, monitoring the transition of the *m/z* 346.1 precursor ion to the *m/z* 198.1 product ion for OPZ and *m/z* 370.1 precursor ion to the *m/z* 252.1 product ion for IS. Quadrupole Q1 was set on low resolution whereas Q3 was set on unit resolution. The analytical data were processed using Analyst software (version 1.4.1).

### Preparation of Stock and Standard Solutions

Primary stock solutions of OPZ for preparation of standard and quality control (QC) samples were prepared from separate weighing. The primary stock solutions were prepared in methanol (1000 μg/mL). The IS stock solution of 1000 μg/mL was prepared in methanol. The stock solutions of OPZ and IS were stored at 4°C, and were found to be stable for one month (data not shown) and successively diluted with methanol to prepare working solutions to prepare the calibration curve (CC). Another set of working stock solutions of OPZ were made in methanol (from primary stock) for preparation of QC samples. Working stock solutions were stored approximately at 4°C for a week (data not shown). Appropriate dilutions of OPZ stock solution were made in methanol to produce working stock solutions of 0.50, 1.00, 2.00, 5.00, 10.0, 20.0, 50.0 and 100 ng/mL. Working stocks were used to prepare plasma calibration standards. A working IS solution (100 ng/mL) was prepared in methanol. Calibration samples were prepared by spiking 270 μL of control human plasma with the appropriate working solution of the analyte (30 μL) and IS (10 μL) on the day of analysis. Samples for the determination of precision and accuracy were prepared by spiking control human plasma in bulk with OPZ at appropriate concentrations (0.50, 1.50, 40.0 and 80.0 ng/mL) and 300 μL aliquots were distributed into different tubes. All the samples were stored at −80 ± 10°C.

### Recovery

The efficiency of OPZ and IS extraction from human plasma was determined by comparing the responses of the analytes extracted from replicate QC samples ($n = 6$) with the response of analytes from post-extracted plasma standard sample at equivalent concentrations (Dams *et al*., 2003) by liquid–liquid extraction process. Recoveries of OPZ was determined at QC low and QC high concentrations, viz. 0.15 and 8.00 ng/mL, whereas the recovery of the IS was determined at a single concentration of 3.33 ng/mL.

### Sample Preparation

A simple liquid–liquid extraction method was followed for extraction of OPZ from human plasma. To an aliquot of 300 μL plasma sample, 30 μL of 0.1 M $NaHCO_3$ was added and mixed thoroughly then IS solution (10 μL of 100 ng/mL) was added and mixed for 15 s on a cyclomixer (Remi Instruments, Mumbai, India). After the addition of 3 mL of acetonitrile, the mixture was vortexed for 3 min, followed by centrifugation for 4 min at 3200 rpm on Multifuge 3 $_{SR}$ (Heraus, Germany). The organic layer (2.7 mL) was separated and evaporated to dryness at 50°C using a gentle stream of nitrogen (Turbovap®, Zymark®, Kopkinton, MA, USA). The residue was reconstituted in 200 μL of the mobile phase and 10 μL was injected onto LC-MS/MS system.



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### Validation Procedures

A full validation according to the FDA guidelines (US DHHS *et al*., 2001) was performed for the assay in human plasma.

**Specificity and selectivity.** The specificity of the method was evaluated by analyzing human plasma samples from at least six different lots to investigate the potential interferences at the LC peak region for analyte and IS.

**Matrix effect.** The effect of human plasma constituents over the ionization of OPZ and IS was determined by comparing the responses of the post-extracted plasma standard QC samples ($n = 6$) with the response of analytes from neat standard samples (30 μL of required working stock sample spiked into 270 μL of methanol instead of blank plasma) at equivalent concentrations (Hubert *et al*., 1999; Dams *et al*., 2003). The matrix effect was determined at low and high concentrations, viz. 0.15 and 8.00 ng/mL, whereas the matrix effect over the IS was determined at a single concentration of 3.33 ng/mL.

**Calibration curve.** The eight-point calibration curve (0.05, 0.10, 0.20, 0.50, 1.00, 2.00, 5.00 and 10.0 ng/mL) was constructed by plotting the peak area ratio of OPZ:IS against the nominal concentration of calibration standards in human plasma. Following the evaluation of different weighing factors, the results were fitted to linear regression analysis with the use of $1/x^2$ ($x$-concentration) weighting factor. The calibration curve had to have a correlation coefficient ($r$) of 0.99 or better. The acceptance criteria for each back-calculated standard concentration were ±15% deviation from the nominal value except at the LLOQ, which was set at ±20% (US DHHS *et al*., 2001).

**Precision and accuracy.** The intra-assay precision and accuracy were estimated by analyzing six replicates containing OPZ at four different QC levels, i.e. 0.05, 0.15, 4.00 and 8.00 ng/mL. The inter-assay precision was determined by analyzing the four levels of QC samples on four different runs. The criteria for acceptability of the data included accuracy within ±15% deviation (SD) from the nominal values and a precision of within ±15% relative standard deviation (RSD) except for LLOQ, where it should not exceed ±20% of SD (US DHHS *et al*., 2001).

**Stability experiments.** The stability of OPZ and IS in the injection solvent was determined periodically by injecting replicate preparations of processed samples for up to 18 h (in the auto sampler at 4°C) after the initial injection. The peak areas of the analyte and IS obtained at initial cycle were used as the reference to determine the stability at subsequent points. The stability of OPZ in the biomatrix during 6 h (bench-top) was determined at ambient temperature (25 ± 2°C) at two concentrations (0.15 and 8.00 ng/mL) in six replicates. Freezer stability of OPZ in human plasma was assessed by analyzing the low QC and high QC samples stored at −80 ± 10°C for at least 30 days. The stability of OPZ in human plasma following three freeze–thaw cycles was assessed using QC samples spiked with OPZ. The samples were stored at −80 ± 10°C between freeze–thaw cycles. The samples were thawed by allowing them to stand (unassisted) at room temperature for approximately 2.00 h. The samples were then returned to the freezer. The samples were processed using the same procedure as described in the Sample Preparation section. Samples were considered stable if assay values were within the acceptable limits of accuracy (i.e. ±15% SD) and precision (i.e. ±15% RSD).

**Dilution effect.** The dilution effect was investigated to ensure that samples could be diluted with blank matrix without affecting the final concentration. OPZ-spiked human plasma samples prepared at two concentrations (1.5 and 800 ng/mL) of OPZ were diluted with pooled human plasma at dilution factors of 10 and 100 in six replicates and analyzed. The six replicates should have a precision of ≤15% and an accuracy of 100 ± 15%.

### Human Pharmacokinetic Study

A pharmacokinetic study was performed in healthy ($n = 3$) male subjects. The ethics committee approved the protocol and the volunteers provided written informed consent. Blood samples were obtained following administration of 10 mg OPZ into polypropylene tubes containing $K_2$EDTA solution as an anti-coagulant at pre-dose and 0.5, 1, 2, 3, 5, 8, 12, 24, 48 and 72 h. Plasma was harvested by centrifuging the blood using Biofuge (Hereaus, Germany) at 1760***g*** for 5 min and stored frozen at −80 ± 10°C until analysis. Plasma (300 μL) samples were spiked with IS and processed as described above. Along with clinical samples, QC samples at low, medium and high concentration were assayed in duplicate and were distributed among calibrators and unknown samples in the analytical run; not more than 33% of the QC samples were greater than ±15% of the nominal concentration. Plasma concentration–time data of OPZ was analyzed by non-compartmental method using WinNonlin Version 5.1 (Pharsight Corporation, Mountain View, CA, USA).

## Results

### Liquid Chromatography

Feasibility of various mixture(s) of solvents such as acetonitrile and methanol using different buffers such as ammonium acetate, ammonium formate and formic acid along with altered flow-rates (in the range of 0.1–0.5 mL/min) were tested for complete chromatographic resolution of OPZ and IS (data not shown). The resolution of peaks was achieved with 0.01 M ammonium acetate:acetonitrile (40:60, v/v) with a flow rate of 0.25 mL/min, on an Inertsil ODS 3 column (75 × 2.1 mm, 3 μm, GL Sciences Inc., Tokyo, Japan) and was found to be suitable for the determination of electrospray response for OPZ and IS. As OPZ is unstable below pH 8.0 (Mathew *et al*., 1995), we have used $NaHCO_3$ to get the alkaline conditions during sample processing and the optimized mobile phase composition having alkaline pH was found to maintain the stability of OPZ during analysis. Earlier Wang *et al*. (2005) also adopted a similar approach.

### Mass Spectroscopy

In order to optimize ESI conditions for OPZ and IS, quadrupole full scans were carried out in positive ion detection mode. During a direct infusion experiment, the mass spectra for OPZ and IS revealed peaks at *m/z* 346.10 and 370.10, respectively as protonated molecular ions, $[M + H]^+$. Following detailed optimization of mass spectrometry conditions (provided in Instrumentation and Chromatographic Conditions section), the *m/z* 346.1 precursor ion to the *m/z* 198.1 was used for the quantification for OPZ. Similarly, for the IS the *m/z* 370.1 precursor ion to the *m/z* 252.1 was used for quantification purposes. As the earlier publications (Kanazawa *et al*., 2002; Wang *et al*., 2005; Hofmann *et al*., 2006)



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have discussed extensively for the fragmentation pattern of OPZ, we are not presenting the data pertaining to this.

### Recovery

A simple liquid–liquid extraction with acetonitrile following alkalization proved to be robust and provided cleanest samples. The results of the comparison of neat standards versus plasma-extracted standards were estimated for OPZ at 0.15 and 8.00 ng/mL and the mean recovery was found to be 70.98 ± 6.84 and 73.92 ± 8.02%, respectively. The recovery of IS at 3.33 ng/mL was 78.10 ± 4.84%.

### Matrix Effect, Specificity and Selectivity

Average matrix factor values (matrix factor = response of post spiked concentrations/response of neat concentrations) obtained were +3.02 (CV: 6.06%, $n = 6$) and +2.16 (CV: 7.22%, $n = 6$) for OPZ in human plasma at low QC (0.15 ng/mL) and high QC (8.00 ng/mL) concentrations, respectively. No significant peak area differences were observed. The matrix effect on IS was found to be +5.06 (CV: 4.84%, $n = 6$) at tested concentration of 3.33 ng/mL. Overall it was found that the plasma extract has a small impact on the ionization of analyte and IS.

Figure 2 shows a typical overlaid chromatogram for the control human plasma (free of analyte and IS), human plasma spiked with OPZ at LLOQ and IS and an *in vivo* plasma sample obtained at 3.0 h after oral administration of OPZ. No interfering peaks from endogenous compounds were observed at the retention times of analyte and IS in the matrix. The retention time of OPZ and IS was ~1.22 and 1.40 min, respectively. The total chromatographic run time was 2.5 min.

### Calibration Curve

The plasma calibration curve was constructed using eight calibration standards (viz. 0.05–10.0 ng/mL). The calibration standard curve had a reliable reproducibility over the standard concentrations across the calibration range. The calibration curve was prepared by determining the best fit of peak-area

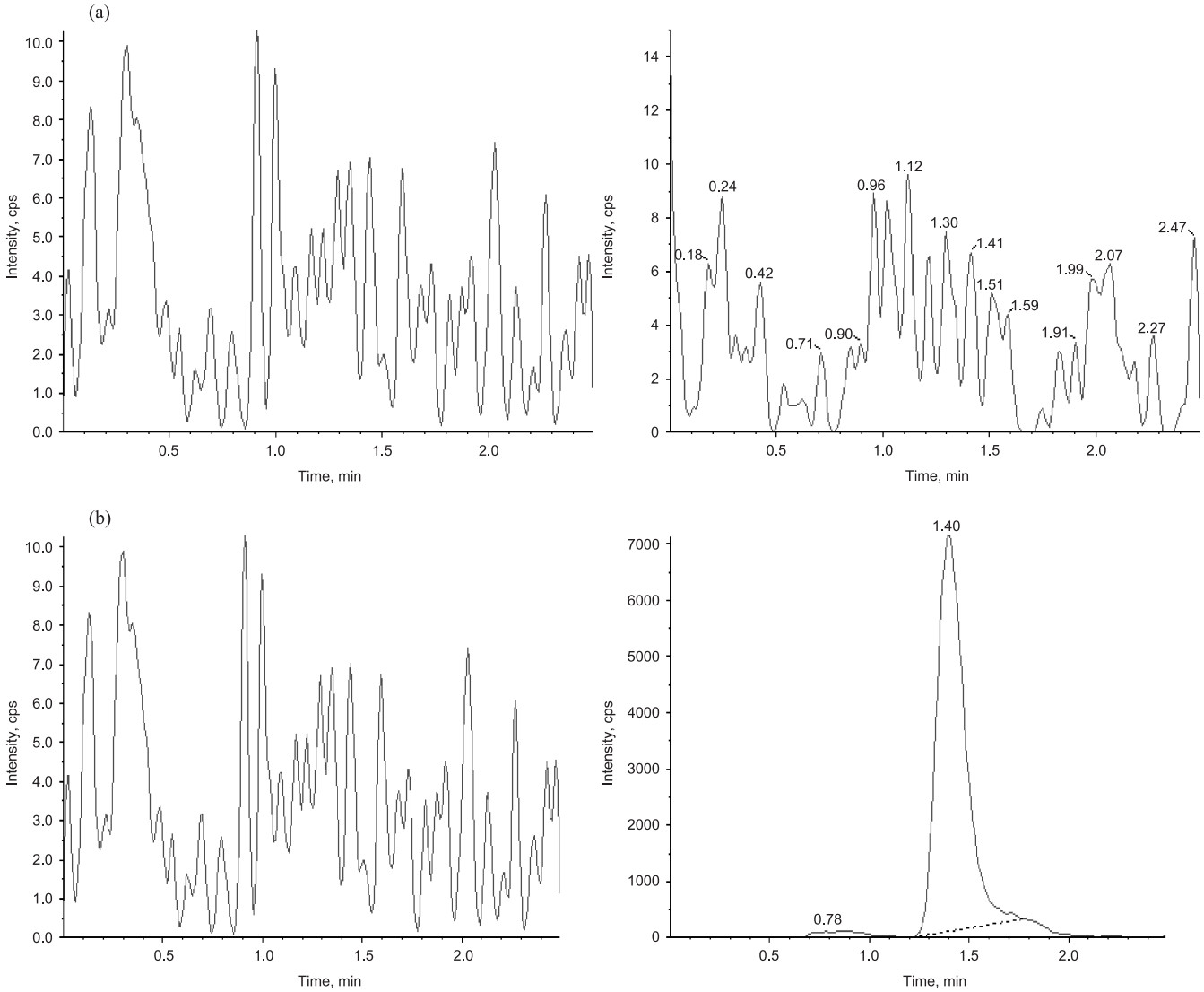


**Figure 2.** Typical MRM chromatograms of OPZ (left panel) and IS (right panel) in (a) human blank plasma, (b) human plasma spiked with IS, (c) human plasma spiked with OPZ at LLOQ (0.05 ng/mL) and IS and (d) a 3.00 h plasma sample showing an OPZ peak obtained following an oral dose of OPZ tablet to a healthy volunteer along with IS.



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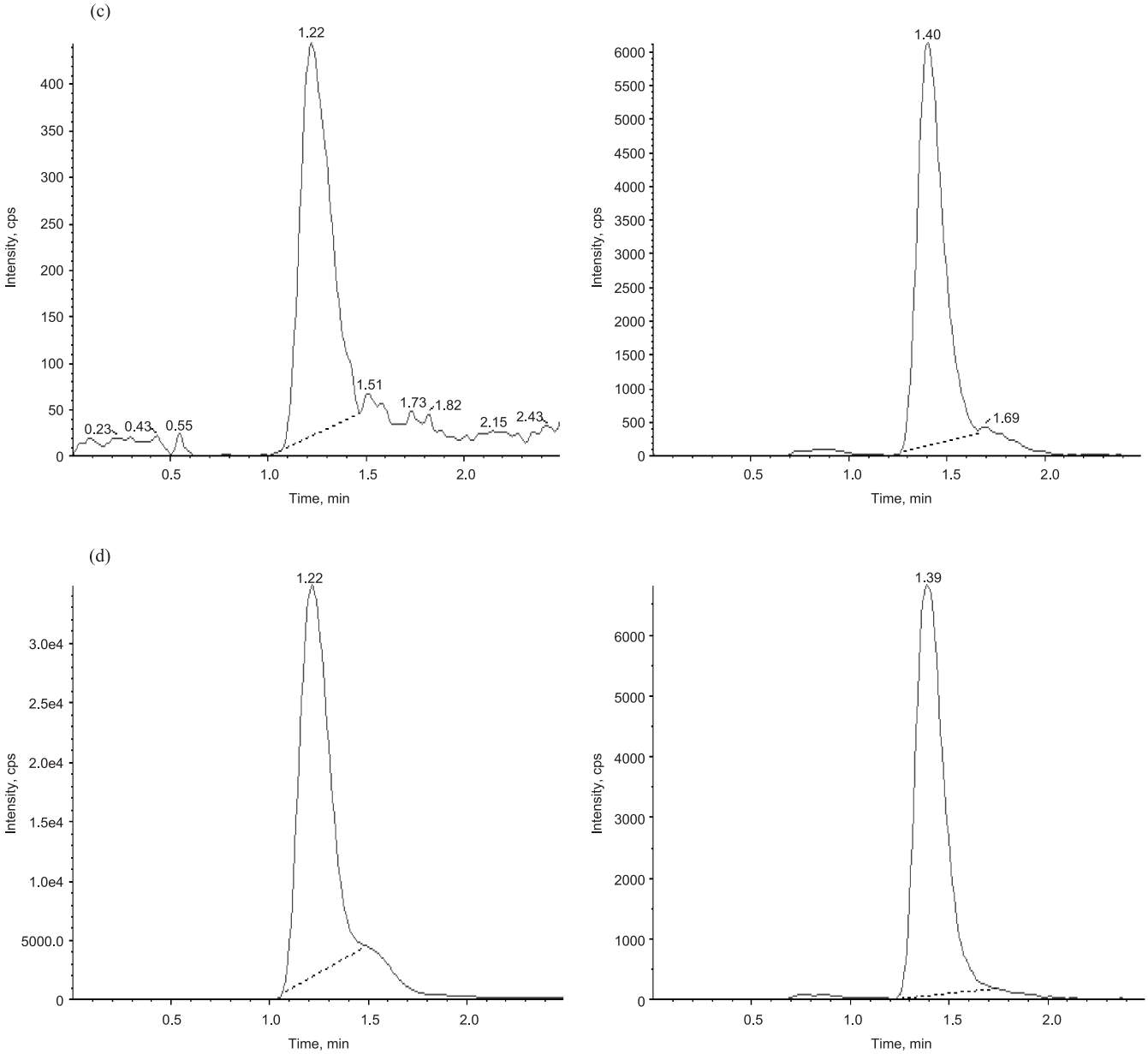


**Figure 2.** (*Continued*)

ratios (peak area analyte/peak area IS) vs concentration, and fitted to the $y = mx + c$ using weighing factor ($1/x^2$). The average regression ($n = 4$) was found to be ≥0.995. The lowest concentration with the RSD <20% was taken as LLOQ and was found to be 0.05 ng/mL. The percentage accuracy observed for the mean of back-calculated concentrations for four calibration curves for OPZ was within 92.9–110; while the precision (% CV) values ranged from 1.68 to 8.81.

### Accuracy and Precision

Accuracy and precision data for intra- and inter-day plasma samples are presented in Table 1. The assay values on both the occasions (intra- and inter-day) were found to be within the accepted variable limits.

### Stability

The predicted concentrations for OPZ at 0.15 and 8.00 ng/mL samples deviated within ±15% of the nominal concentrations in a battery of stability tests, viz. in-injector (18 h), bench-top (6 h), repeated three freeze–thaw cycles and freezer stability at −80 ± 10°C for at least for 30 days (Table 2). The results were found to be within the assay variability limits during the entire process.

### Dilution Effect

The standard curve can be extended up to 800 ng/mL without affecting the final concentrations. The results have shown that the precision and accuracy for six replicates of diluted samples were within the acceptance range (data not shown).



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**Table 1.** Intra- and inter-day precision of determination of OPZ in human plasma

| Theoretical concentration (ng/mL) | Run | Measured concentration (ng/mL) | | | |
|---|---|---|---|---|---|
| | | Mean | SD | RSD | Accuracy (%) |
| *Intra-day variation (six replicates at each concentration)* | | | | | |
| 0.05 | 1 | 0.05 | 0.00 | 6.29 | 107 |
| | 2 | 0.05 | 0.00 | 3.59 | 106 |
| | 3 | 0.05 | 0.00 | 8.56 | 99.2 |
| | 4 | 0.06 | 0.00 | 4.03 | 114 |
| 0.15 | 1 | 0.15 | 0.00 | 2.37 | 100 |
| | 2 | 0.16 | 0.01 | 3.72 | 106 |
| | 3 | 0.16 | 0.01 | 8.35 | 105 |
| | 4 | 0.14 | 0.01 | 5.49 | 94.8 |
| 4.00 | 1 | 3.57 | 0.14 | 3.92 | 89.1 |
| | 2 | 3.96 | 0.13 | 3.36 | 99.0 |
| | 3 | 4.27 | 0.13 | 3.03 | 107 |
| | 4 | 3.94 | 0.30 | 7.72 | 98.4 |
| 8.00 | 1 | 7.31 | 0.30 | 4.14 | 91.4 |
| | 2 | 8.10 | 0.17 | 2.09 | 101 |
| | 3 | 8.60 | 0.23 | 2.64 | 107 |
| | 4 | 8.22 | 0.32 | 3.88 | 103 |
| *Inter-day variation (24 replicates at each concentration)* | | | | | |
| 0.05 | | 0.05 | 0.00 | 6.71 | 107 |
| 0.15 | | 0.15 | 0.01 | 5.29 | 101 |
| 4.00 | | 3.93 | 0.32 | 8.19 | 98.3 |
| 8.00 | | 8.02 | 0.59 | 7.31 | 100 |

RSD, relative standard deviation (SD × 100/mean).

**Table 2.** Stability data OPZ quality controls in human plasma

| Nominal concentration (ng/mL) | Stability | Mean ± SD[a] $n = 6$ (ng/mL) | Accuracy (%)[b] | Precision (% CV) |
|---|---|---|---|---|
| 0.15 | 0 h (for all) | 0.16 ± 0.01 | 105 | 8.35 |
| | Third freeze–thaw | 0.15 ± 0.01 | 97.5 | 6.19 |
| | 6 h (bench-top) | 0.14 ± 0.01 | 91.6 | 10.1 |
| | 18 h (in-injector) | 0.16 ± 0.01 | 99.0 | 3.60 |
| | 30 days at − 80°C | 0.15 ± 0.01 | 93.4 | 8.30 |
| 8.00 | 0 h (for all) | 8.60 ± 0.23 | 107 | 2.64 |
| | Third freeze–thaw | 7.67 ± 0.42 | 89.2 | 5.50 |
| | 6 h (bench-top) | 7.91 ± 0.68 | 92.0 | 8.59 |
| | 18 h (in-injector) | 8.15 ± 0.17 | 95.0 | 2.06 |
| | 30 days at − 80°C | 8.55 ± 0.53 | 99.4 | 6.21 |

[a] Back-calculated plasma concentrations; [b] (mean assayed concentration/mean assayed concentration at 0 h) × 100.

### Pharmacokinetic Study

The sensitivity and specificity of the assay were found to be sufficient for accurately characterizing the plasma pharmacokinetics of OPZ in healthy volunteers. Profiles of the mean plasma concentration vs time are shown in Fig. 3. Maximum concentration in plasma ($C_{max}$ 499.61 ± 37.03 ng/mL) was achieved at 3.00 ± 0.00 h ($T_{max}$). The half-life ($t_{1/2}$) of OPZ was 3.88 ± 0.18 h, while the AUC $_{(0-\infty)}$ was 3152.36 ± 302.90 ng h/mL. The higher sensitivity of this method compared with the currently existing methods in literature facilitates the quantitation of OPZ at lower concentrations with high turnover.

## Conclusion

A method using LC-ESI-MS/MS for the determination of OPZ in human plasma employing simple liquid–liquid extraction was developed. The method is rapid, simple, specific and sensitive and additionally demonstrates good accuracy and precision. Compared with the published methods, the present method features high selectivity and sensitivity with an LLOQ of 0.05 ng/mL. We believe that this method could provide a useful tool for the determination of OPZ in plasma. The established method was successfully applied to a human pharmacokinetic study.



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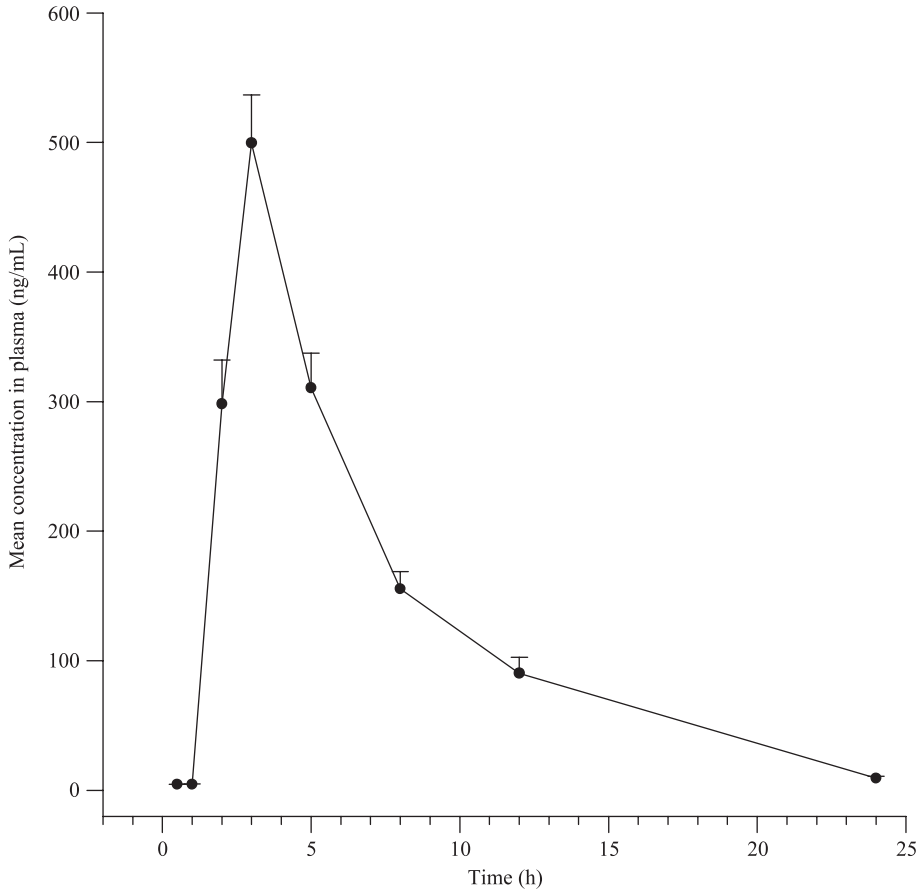


**Figure 3.** Mean ± SD plasma concentration–time profile of OPZ in human plasma following oral dosing of OPZ tablet to healthy volunteers.

## References

Andersson T. Pharmacokinetics, metabolism and interactions of acid pump inhibitors. Focus on omeprazole, lansoprazole and pantoprazole. *Clinical Pharmacokinetics* 1996; **31**: 9–28.

Dams R, Huestis MA, Lambert WE and Murphy CM. Matrix effect in bio-analysis of illicit drugs with LC-MS/MS: influence of ionization type, sample preparation, and biofluid. *Journal of the American Society for Mass Spectrometry* 2003; **14**: 1290–1294.

Frerichs VA, Zaranek C and Hass CE. Analysis of omeprazole, midazolam and hydroxy-metabolites in plasma using liquid chromatography coupled to tandem mass spectrometry. *Journal of Chromatography B* 2005; **824**: 71–80.

Hofmann U, Schwab M, Treiber G and Klotz U. Sensitive quantification of omeprazole and its metabolites in human plasma by liquid chromatography–mass spectrometry. *Journal of Chromatography B* 2006; **831**: 85–90.

Hubert Ph, Chiap P, Crommen J, Boulanger B, Chapuzet E, Mercier N, Bervoas-Martin S, Chevalier P, Grandjean D, Lagorce P, Lallier M, Laparra MC, Laurentie M and Nivet JC. The SFSTP guide on the validation of chromatographic methods for drug bioanalysis Washington conférence to the laboratory. *Analytica Chimica Acta* 1999; **391**: 135–148.

Hultman Ia, Stenhoff H and Liljeblad M. Determination of esomeprazole and its two main metabolites in human, rat and dog plasma by liquid chromatography with tandem mass spectrometry. *Journal of Chromatography B* 2007; **848**: 317–322.

Kanazawa H, Okada A, Matsushima Y, Yokota H, Mashige F and Nakahara K. Determination of omeprazole and its metabolites in human plasma by liquid chromatography–mass spectrometry. *Journal of Chromatography A* 2002; **949**: 1–9.

Martens-Lobenhoffer J, Reiche I, Troger U, Monkemuller K, Malfertheiner P and Bode-Boger SM. Enantioselective quantification of omeprazole and its main metabolites in human serum by chiral HPLC–atmospheric pressure photoionization tandem mass spectrometry. *Journal of Chromatography B* 2007; **857**: 301–307.

Mathew M, Gupta VD and Bailey RE. Stability of omeprazoel solutions at various pH values as determined by high-performance liquid chromatography. *Drug Development and Industrial Pharmacy* 1995; **21**: 965–971.

Song O and Naidong W. Analysis of omeprazole and 5-OH omeprazole in human plasma using hydrophilic interaction chromatography with tandem mass spectrometry (HILIC-MS/MS)-eliminating evaporation and reconstitution steps in 96-well liquid/liquid extraction. *Journal of Chromatography B* 2002; **830**: 135–142.

Stenhoff H, Blomqvist A and Lagerstrom PO. Determination of the enantiomers of omeprazole in blood plasma by normal-phase liquid chromatography and detection by atmospheric pressure ionization tandem mass spectrometry. *Journal of Chromatography B* 1999; **734**: 191–201.

US DHHS, FDA and CDER. *Guidance for Industry: Bioanalytical Method Validation*. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Veterinary Medicine, 2001. Available at: http://www/fda.gov/cder/guidance/index.htm

Wang J, Wang Y, Fawcett JP, Wang Y and Gu J. Determination of omeprazole in human plasma by liquid chromatography–electrospray quadrupole linear ion trap mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis* 2005; **39**: 631–635.

Woolf EJ and Matuszewski BK. Simultaneous determination of omeprazole and 5′-hydroxyomeprazole in human plasma by liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A* 1998; **828**: 229–238.

Yin OO, Lam SS, Lo CM and Chow MS. Rapid determination of five probe drugs and their metabolites in human plasma and urine by liquid chromatography/tandem mass spectrometry: application to cytochrome P450 phenotyping studies. *Rapid Communications in Mass Spectrometry* 2004; **18**: 2921–2933.



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