Simultaneous Determination of Omeprazole and Domperidone in Dog Plasma by LC–MS Method

Zhan Li[2,3], Jing Yao[1,*], Ziqiang Zhang[1,2], and **Luyong Zhang[3]**
[1]Department of pharmaceutics, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China; and [2]Nanjing Changao Pharmaceutical Science and Technology Limited Company, Nanjing 210022, China; and [3]New Drug Screening Center, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China.

Abstract

A liquid chromatography coupled with mass spectrometry method was developed and validated to determine omeprazole and domperidone simultaneously in dog plasma. Chromatographic separation was performed on a Narrow Bore C_{18} column using methanol–10 mM ammonium acetate (68:32, v/v) as the mobile phase at the flow rate of 0.2 mL/min. The chromatographic separation was achieved in less than 7 min. Diazepam was used as the internal standard. Quantitative analysis was achieved by mass spectrometry detection using a mass spectrometer equipped with an electrospray ionization interface and operated in selected ion monitoring and positive ion mode using target ions at m/z 346 for omeprazole, m/z 426 for domperidone, and m/z 285 for internal standard, respectively. The lower limit of quantification was 1 ng/mL and a linear range of 1–200 ng/mL. The intra- and inter-day relative standard deviation was both less than 10%, and the accuracy values were higher than 90%. The method is sensitive and repeatable enough to be used in pharmacokinetic and bioavailability studies.

Introduction

Omeprazole, a well-studied proton pump inhibitor, inhibits the gastric parietal cell proton pump, dose-dependently reducing basal and stimulated gastric secretion and raising intragastric pH (1). Domperidone is a dopamine antagonist that produces extrapyramidal reactions. It stimulates gastro-intestinal motility and is used as an antiemetic for the short-term treatment of nausea and vomiting of various aetiologies (2). Recently, a combination of a proton pump inhibitor and other agents was reported for the treatment of gastric diseases (3–5). The combined therapy with omeprazole and domperidone in adult asthmatics with gastroesophageal reflux may be beneficial by reducing asthma symptoms, rescuing medication use, and improving pulmonary function (5).

Several analytical methods were published for the determination of omeprazole or domperidone in biological samples. Most of them were high-performance liquid chromatography (HPLC) (6–7) or liquid chromatography and mass spectrometry (LC–MS) methods (8–10). Some of them had relatively low sensitivity (6,7,9), and the others required a longer analysis time (6,10). An HPLC method with fluorescence detection was described to determine the domperidone in rat plasma with a limit of detection of 1 ng/mL using 1 mL of plasma (11). LC–tandem mass spectrometry (MS/MS) methods to quantify omeprazole or domperidone in human plasma have been published in recent years (12,13). Unfortunately, the required equipment is not available in some laboratories. To our knowledge, no LC–MS method for simultaneous quantification of omeprazole and domperidone in dog plasma has been reported.

In this study, a simple, sensitive, and rapid LC–MS method was developed and validated for the simultaneous quantification of omeprazole and domperidone in dog plasma obtained from a pharmacokinetic study. The method was applied for omeprazole and domperidone quantitation to support a pharmacokinetics study of omeprazole and domperidone sustained-release capsules (OD-Cap.) after oral administration in dogs. The structures of the analytes are shown in Figure 1.

Experimental

Chemicals and regents

Omeprazole (99.02% purity) was purchased from Shanghai Dazong Pharmaceutical Factory (Shanghai, China). Domperidone (99.86% purity) was purchased from Shanxi Baotai Pharmaceutical Co. Ltd (Jinzhong, China). Diazepam

Figure 1. Molecular structures of omeprazole, A; domperidone, B; and diazepam, C.
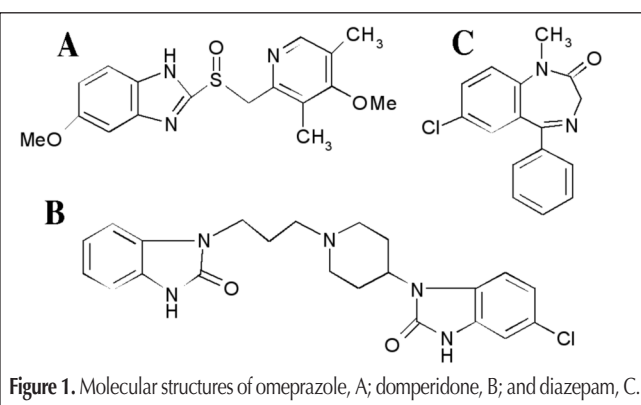
* Author to whom correspondence should be sent: email yaoj3@163.com.

(internal standard, IS) was purchased from National Institute for the Control of Pharmaceutical and Biological Products (NICPBP). Methanol (HPLC-grade) was purchased from Merck Corporation (Darmstadt, Germany). Diethyl ether (HPLC-grade) was purchased from Shanghai Experiment Reagent Co., Ltd. (Shanghai, China). Omeprazole and domperidone sustained release capsules (OD-Cap.) were obtained from Nanjing Changao Pharmaceutical Science and Technology Co. Ltd (Nanjing, China). All other reagents were obtained from commercial sources.

Figure 2. Product ion mass spectra of omeprazole (A), domperidone (B), and IS (C) in positive electrospray ionization mode. The target ions were selected at *m/z* 346 for omeprazole, *m/z* 426 for domperidone, and *m/z* 285 for IS, respectively.

LC–MS apparatus and conditions

An Agilent 1200 HPLC system (Agilent, Waldbronn, Germany) equipped with degasser, binary pump, auto-sampler, thermostatic column compartment, and G1956 mass selective detector equipped with an electrospray ion source was used for the LC–electrospray ionization (ESI)-MS analysis.

Chromatographic separation was achieved on a Narrow Bore C_{18} column (150 mm × 2.1 mm, 5 μm, Agilent). The mobile phase consisted of a mixture of methanol–10 mM ammonium acetate (68:32, v/v) with a total run time of less than 7 min. The flow rate was 0.2 mL/min, and the column temperature was maintained at 30ºC.

Electrospray parameters were as follows: drying gas temperature 350°C, capillary voltage 4000 V, nebulizer pressure 60 psig, drying gas (nitrogen) flow at 5 L/min, and fragmentor at 70 V. The LC–ESI-MS was performed in the selected ion-monitoring (SIM) mode. The ESI in positive ion mode was adopted for the analytes quantitation with the following parameters: *m/z* 285 for diazepam, *m/z* 346 for omeprazole, and *m/z* 426 for domperidone.

Sample preparation

A 10-μL aliquot of IS working solution (2.0 μg/mL in methanol), 100 μL of 0.1 M sodium hydroxide, and 5 mL of diethyl ether were added to 500 μL of dog plasma samples. The mixtures were vortex-mixed for 3 min and centrifuged at 4000 × g for 10 min. The organic phase was transferred to a clean test tube and evaporated to dryness under a gentle nitrogen stream at 40°C. The residue was reconstituted in 20 μL of the mobile phase. A 2.0-μL aliquot of the supernatant was injected into the LC–MS system.

Assay validation

The method has been validated using selectivity, sensitivity, linearity, precision, accuracy, and stability. Stock solutions of omeprazole, domperidone and diazepam were separately prepared in methanol and stored at –20°C. Working standard solutions of omeprazole and domperidone were then serially diluted to obtain the desired concentrations with methanol. The concentration of working IS was 2.0 μg/mL by dilution from the stock solution of diazepam.

Working solutions of omeprazole and domperidone were spiked into 0.5 mL blank plasma to obtain final drug concentrations of 1–200 ng/mL.

Peak area ratio of analyte/IS were plotted versus nominal concentration, and a weighted least-squares linear regression model ($1/x^2$) was applied to obtain the calibration curves. The calibration standards were prepared and assayed in duplicate in each batch on three separate days.

The quality control (QC) samples were prepared in the blank plasma at the concentration of 5, 50, 200 ng/mL, respectively. They were prepared independently of the calibration standards and analyzed with processed test samples at intervals in each run.

The intra-day and inter-day precision and accuracy were calculated by determining the concen-
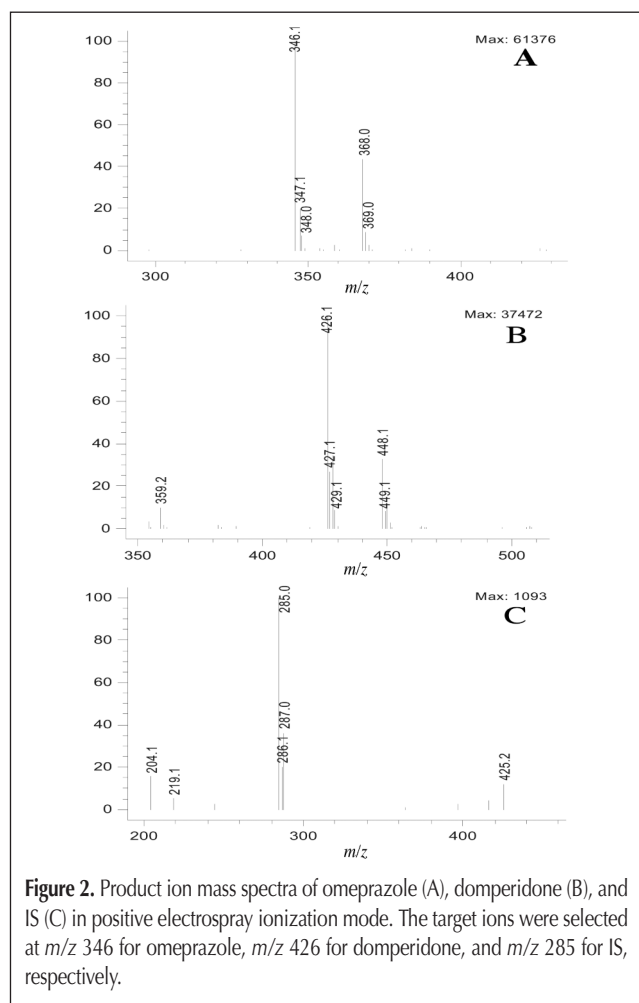
Table I. Precision and Accuracy for the Analysis of Omeprazole and Domperidone in Dog Plasma by LC–MS Method (*n* = 5)

Components	Spiked concentration (ng/mL)	Detected concentration (mean ± SD) (ng/mL)	Intra-day RSD (%)	Inter-day RSD (%)	Accuracy (%)
Omeprazole	5.0	4.82 ± 0.302	8.64	7.34	96.4 ± 0.6
	50	50.7 ± 0.397	7.56	6.57	101.4 ± 0.8
	200	202.3 ± 8.14	5.23	4.35	101.2 ± 4.1
Domperidone	5.0	4.63 ± 0.413	7.89	7.43	92.6 ± 8.3
	50	51.13 ± 0.597	7.21	6.41	102.3 ± 1.2
	200	201 ± 7.63	5.54	4.36	100.5 ± 3.8

tration of the omeprazole or domperidone in QC samples at three concentration levels (5, 50, and 200 ng/mL). The concentrations of omeprazole and domperidone in samples were calculated from the linear regression equation obtained from the same batch. Precision was calculated as the relative standard deviation (RSD) within a single run and between runs, and the accuracy was calculated as the percentage of deviation between nominal and measured concentrations.

The lower limit of quantification of omeprazole and domperidone were experimentally defined as the lowest concentration in plasma samples at which the within- and between-run precision were < 20% and the accuracy varied between 80–120%.

Results and Discussion

LC–MS optimization

Omeprazole, domperidone, and diazepam were ionized under positive electrospray ionization (ESI+) because of the amide group in their structures. In general, ESI produced greater sensitivity and exhibited less interference than atmospheric pressure chemical ionization (APCI) sources, which is consistent with the results from the study on the protodioscin by Wang et al. (14). The full-scan mass spectra for omeprazole, domperidone, and diazepam were predominately protonated by the molecules [M+H]+. No solvent adduct ion was observed. The major product ion included m/z 285 for diazepam, m/z 346 for omeprazole, and m/z 426 for domperidone. A typical SIM spectra of omeprazole, domperidone, and diazepam is shown in Figure 2.

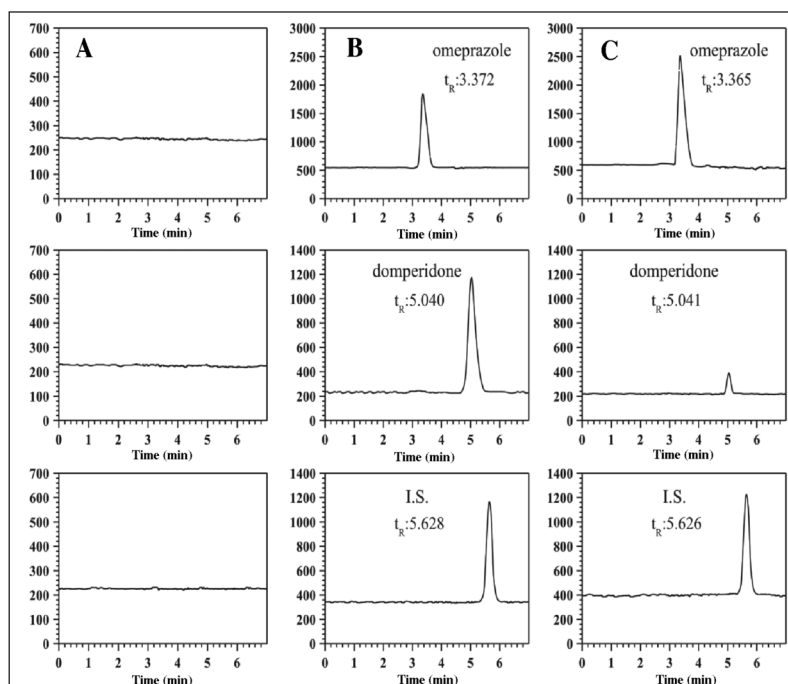The composition of the mobile phase was a critical factor for achieving good chromatographic peak shape and resolution. In the present study, 10 mM ammonium acetate in the mobile phase is used and can avoid the decomposition of omeprazole at acidic pH. Finally, the good separation of target compounds was obtained with a mobile phase of the mixture of methanol–10 mM ammonium acetate (68:32, v/v). Under the present chromatographic conditions, the run time of each sample was less than 7 min. For dog plasma samples at each batch, the regions of the analytes and IS were found to be free of interference.

Assay specificity

The selectivity was assessed by comparing the chromatograms of six different batches of the blank plasma with the corresponding spiked plasma. Figure 3 shows the typical chromatograms of the blank plasma, a spiked plasma sample with omeprazole, domperidone, and IS, and the plasma sample at 2 h after an oral administration. No significant endogenous interfering peaks were observed at or near the retention time of omeprazole, domperidone, and IS. The retention times were about 3.4 min for omeprazole, 5.0 min for domperidone, and 5.6 min for IS.

Linearity

The calibration equation was as follows: $y = 0.0356x + 0.046$ for omeprazole and $y = 0.0378x + 0.0012$ for domperidone, where y is the peak area ratio of analyte and IS, and x is the plasma concentration of the compounds. Good linearity ($r^2 = 0.9988$ for omeprazole and $r^2 = 0.9978$ for domperidone) was observed in the concentration ranges of 1–200 ng/mL. The lower limit of the quantification was 1 ng/mL for both omeprazole and domperidone in dog plasma.

Precision and accuracy

The results of inter- and intra-day precision and accuracy of omeprazole and domperidone in dog plasma are presented in Table I. In this assay, the intra-day and inter-day precision of omeprazole and domperidone in plasma were less than 10%, and accuracy values were higher than 90%. So, the precision and accuracy of the assay for plasma were acceptable as defined in the State Food and Drug Administration (SFDA) guidelines (15).

Stability

Freeze-thaw stability of the samples was obtained over three freeze-thaw cycles by thawing at room temperature and refreezing at –20ºC for at least 24 h. No significant changes in the omeprazole and domperidone concentrations were measured after three freeze–thaw cycles. Short-term stability was examined by keeping plasma samples at ambient temperature for 24 h. Long-term stability was determined by analyzing the aliquots of three concentrations stored at –20ºC for eight weeks. The results in Table II showed that no significant degradation of omeprazole and domperidon was observed under the tested conditions. Each stability test was determined at 5, 50, and 200 ng/mL in three replicates.

Figure 3. Typical chromatograms of omeprazole, domperidone, and internal standard (IS) in dog plasma: (A) blank plasma, (B) plasma sample spiked with omeprazole (50 ng/mL), domperidone (50 ng/mL), and IS (1000 ng/mL) and (C) plasma sample obtained from a dog at 2 h after an oral administration with a single dose of omeprazole (1 mg/kg) and domperidone (0.5 mg/kg).

Method application

The method described here was successfully applied to simultaneously determine the concentrations of omeprazole and domperidone in dog plasma up to 24 h after orally administration of OD-Cap. Four six-month-old male beagle dogs weighing approximately 10 kg were used for this pharmacokinetics study. After an overnight fast, each dog was administered orally with one OD-Cap (containing 10 mg omeprazole and 5 mg domperidone). Blood samples were collected into heparinized tubes through a forelimb cephalic vein at 0 (predose), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12, and 24 h after administration. Plasma was obtained by centrifugation at 4000 × g for 10 min, and samples were stored at –20°C until analysis. The mean plasma concentration-time curves after oral administration are shown in Figure 4.

Conclusion

A simple, rapid, and sensitive LC–MS method has been developed and validated for simultaneous determination of omeprazole and domperidon in dog plasma. The method had a good sensitivity and specificity for simultaneous determination of omeprazole and domperidon in dog plasma. No significant interference caused by endogenous compounds was observed. The results showed this method presented here fulfilled the general requirement for bioanalytical assays and was suitable for the pharmacokinetic study of OD-Cap.

Table II. Stability Data of Omeprazole and Domperidone in Dog Plasma Under Various Storage Conditions ($n = 3$)

Compounds	Added concentration (ng/mL)	Detected concentration (mean ± SD) (ng/mL)	RSD (%)	Remaining (%)
Omeprazole				
Freeze-thaw stability	5	4.84 ± 0.343	7.09	96.8
	50	49.35 ± 1.323	2.68	98.7
	200	195.6 ± 5.966	3.05	97.8
Short-term stability	5	4.72 ± 0.356	7.54	94.4
	50	48.39 ± 1.519	3.14	96.8
	200	194.3 ± 6.354	3.27	97.2
Long-term stability	5	4.83 ± 0.348	7.20	96.6
	50	48.58 ± 1.856	3.82	97.2
	200	193.7 ± 7.128	3.68	96.9
Domperidone				
Freeze-thaw stability				
	5	4.74 ± 0.451	9.51	94.8
	50	47.64 ± 0.983	2.06	95.3
	200	201.65 ± 6.53	3.24	100.8
Short-term stability	5	4.75 ± 0.359	7.56	95.0
	50	48.34 ± 1.223	2.53	96.7
	200	194.63 ± 6.95	3.57	97.3
Long-term stability	5	4.84 ± 0.343	7.09	96.8
	50	49.35 ± 1.321	2.68	98.7
	200	192.97 ± 7.16	3.71	96.5
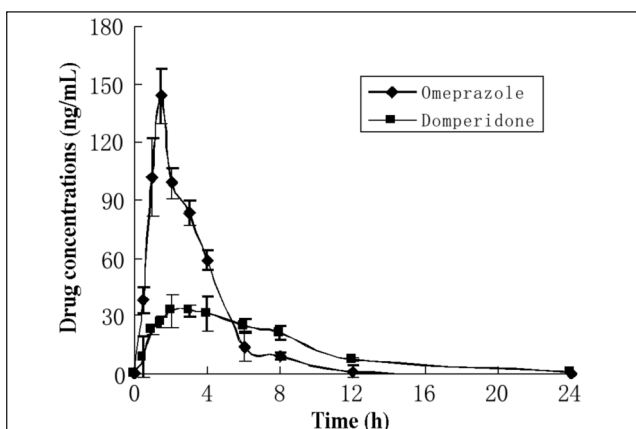
Figure 4. Mean plasma concentration-time profiles of omeprazole and domperidone after oral administration of omeprazole (1 mg/kg) and domperidone (0.5 mg/kg) sustained-release capsules (OD-Cap) to dogs ($n = 4$).

References

1. H.D. Langtry and M.I. Wilde. Omeprazole: A review of its use in Helicobacter pylori infection, gastro-oesophageal reflux disease and peptic ulcers induced by nonsteroidal anti-inflammatory drugs. **Drugs** 56: 447–486 (1998).
2. J.E.F. Reynolds, Part I, Martindale, The Extra Pharmacopoeia, 31st ed., Royal Pharmaceutical Society of Great Britain, London, 1996.
3. S.P. Jiang, R.Y. Liang, Z.Y. Zeng, Q.L. Liu, Y.K. Liang, and J.G. Li. Effects of antireflux treatment on bronchial hyper-responsiveness and lung function in asthmatic patients with gastroesophageal reflux disease. *World J. Gastroenterol.* **9:** 1123–1125 (2003).
4. G. Triadafilopoulos, B. Kaur, S. Sood, B. Traxler, D. Levine, and A. Weston. The effects of esomeprazole combined with aspirin or rofecoxib on prostaglandin E2 production in patients with Barrett's oesophagus. *Aliment Pharmacol. Ther.* **23:** 997–1005 (2006).
5. B. sharma, A. Sharma, M.K. Daga, G.K. Sachdev, and E. Bondi. Effect of omeprazole and domperidone on adult asthmatics with gastroesophageal reflux. *World J. Gastroenterol.* **13:** 1706–1710 (2007).
6. J. Huijuan, L. Wei, and Z. Kang. Determination of omeprazole in rat plasma by high-performance liquid chromatography without solvent extraction. *J. Chromatogr. B* **837:** 112–115 (2006).
7. K.H. Yuen, W.P. Choy, H.Y. Tan, J.W. Wong, and S.P. Yap. Improved high performance liquid chromatographic analysis of omeprazole in human plasma. *J. Pharm. Biomed. Anal.* **24:** 715–719 (2001).
8. J. Wang, Y. Wang, J.P. Fawcett, Y. Wang, and J. Gu. Determination of omeprazole in human plasma by liquid chromatography–electrospray quadrupole linear ion trap mass spectrometry. *J. Pharm. Biomed. Anal.* **39:** 631–635 (2005).
9. U. Hofmann, M. Schwab, G. Treiber, and U. Klotz. Sensitive quantification of omeprazole and its metabolites in human plasma by liquid chromatography-mass spectrometry. *J. Chromatogr. B* **831:** 85–90 (2006).
10. H. Kanazawa, A. Okada, Y. Matsushima, H. Yokota, S. Okubo, F. Mashige, and K. Nakahara. Determination of omeprazole and its metabolites in human plasma by liquid chromatography– mass spectrometry. *J. Chromatogr. A* **949:** 1–9 (2002).
11. K. Yamamoto, M. Hagino, H. Kotaki, and T. Iga. Quantitative determination of domperidone in rat plasma by high-performance liquid chromatography with fluorescence detection. *J. Chromatogr. B* **720:** 251–255 (1998).
12. V.A. Frerichs, C. Zaranek, and C.E. Haas. Analysis of omeprazole, midazolam and hydroxy-metabolites in plasma using liquid chromatography coupled to tandem mass spectrometry. *J. Chromatogr. B* **824:** 71–80 (2005).
13. M.J. Smit, F.C.W. Sutherland, H.K.L. Hundt, K.J. Swart, A.F. Hundt, and J. Els. Rapid and sensitive liquid chromatography–tandem massspectrometry method for the quantitation of domperidone in human plasma. *J. Chromatogr. A* **949:** 65–70 (2002).
14. T. Wang, Z. Liu, J. Li, M. Zhong, J. Li, X. Chen, and K. Bi. Determination of protodioscin in rat plasma by liquid chromatography-tandem mass spectrometry. *J. Chromatogr. B* **848:** 363–368 (2007).
15. X. Tong, J. Zhou, and Y. Tan. Determination of paclitaxel in rat plasma by LC-MS-MS. *J. Chromatogr. Sci.* **44:** 266–271 (2006)