ejpmr, 2016,3(6), 26-30

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

A VALIDATED REVERSED PHASE HPLC ASSAY FOR THE DETERMINATION OF OMEPRAZOLE IN HUMAN PLASMA

Rajaa F. Hussein, Nada H. Binhashim, Syed N. Alvi and Muhammad M. Hammami*

Clinical Studies and Empirical Ethics Department King Faisal Specialist Hospital & Research Center MBC-03, P.O. Box 3354, Riyadh 11211, Kingdom of Saudi Arabia.

***Corresponding Author: Muhammad M. Hammami**
Clinical Studies and Empirical Ethics Department King Faisal Specialist Hospital & Research Center MBC-03, P.O. Box 3354, Riyadh 11211, Kingdom of Saudi Arabia.

Article Received on 24/03/2016 **Article Revised on 14/04/2016** **Article Accepted on 04/05/2016**

ABSTRACT

A simple and precise reversed-phase high performance liquid chromatographic (HPLC) assay for the measurement of omeprazole level in human plasma, using lansoprazole as an internal standard (IS), was developed and validated. Plasma samples containing omeprazole were spiked with the IS then extracted with *tert.* butyl methyl ether and reconstituted in mobile phase. The compounds of interest were efficiently separated on Atlantis dC_{18} column at room temperature and detected with photodiode array detector set at 302 nm. The mobile phase consisted of 0.05 M dibasic sodium phosphate (pH = 7.0, adjusted with phosphoric acid) and acetonitrile (60:40, v:v) and was delivered at a flow rate of 1.0 ml/min. The relationship between omeprazole concentration in plasma and peak height ratio of omeprazole/ IS was linear ($R^2 \geq 0.9992$) in the range of 0.01 – 1.20 µg/ml and the intra- and inter-day coefficient of variations (CV) and accuracy were ≤5.0% and ≤10.1%, ≥90% and ≥92%, respectively. Mean extraction recovery of omeprazole and the IS from plasma samples both was 91% and 91%, respectively. In processed samples, omeprazole was stable for at least 24 hours at room temperature (≥91%) and 48 hours at -20 º C (≥89%). In unprocessed samples it was stable at least 12 weeks at -20ºC (≥96%) and after 3 cycles of freeze and thaw (≥96%). Further, it was successfully applied to measure omeprazole level in samples obtained from a healthy volunteer.

KEYWORDS: Omeprazole, Lansoprazole, Human plasma, HPLC.

INTRODUCTION

Omeprazole (CAS: 73590-58-6), 5-methoxy-2-[(4-methoxy-3,5-dimethylpyridine-2-yl) methylsulfinyl]-1-H- benzimidazole, belongs to proton-pump inhibitors (PPI) that decrease the amount of acid produced in the stomach. It is widely used in the treatment of gastroesophageal reflux disease and in peptic ulcers.[1-2] Omeprazole is absorbed rapidly with a peak plasma concentration (0.4- 1.1 µg/ml) within 1-2 hours after ingestion of single oral therapeutic dose of 20 mg. Its bioavailability is about 30-40%, which generally increases slightly with repeated administration.[3-4]

Several analytical methods for the determination of omeprazole in pharmaceutical preparations and biological matrixes have been reported. They includes high-performance liquid chromatography (HPLC),[5-12] high-performance thin layer chromatography (HPTLC),[13] polarography,[14] capillary electrophoreses (CE),[15] voltammetry,[16] and liquid chromatography-tandem mass spectrometry (LCMS/MS).[17] The most commonly used assays for pharmacokinetic and bioequivalence studies are LCMS/MS and HPLC based. Although LCMS/MS assays have several advantages over HPLC assays, many laboratories prefer HPLC assays because of low cost and availability.

The present paper describes a simple, precise, and rapid HPLC assay that requires 1.0 ml human plasma and is based on liquid-liquid extraction. The method was validated and used to determine omeprazole level in plasma samples from a healthy volunteer and to determine stability of omeprazole under various laboratory conditions.

MATERIAL AND METHODS

Apparatus

Chromatography was performed on a Waters Alliance HPLC 2695 (Waters Associates Inc, Milford, MA, USA) consisting of a quaternary pump, autosampler, column thermostat, and photodiode array detector. A reversed-phase Atlantis dC_{18} column (4.5 x 150 mm, 5-µm) and a guard pak pre-column module with a Nova-pak C18 (4-µm) insert were used for the separation. Data were collected with a Pentium IV computer using Empower Chromatography Manager Software.

Chemical and reagents

All reagents were of analytical-reagent grade unless stated otherwise. Omeprazole (USP reference standard) was purchased from US Pharmacopeia, Rockville, Maryland, USA. Lansoprazole and dibasic sodium phosphate were purchased from Sigma-Aldrich Co., St. Louis, MO, USA. Acetonitrile and phosphoric acid (all HPLC grade) were purchased from Fisher Scientific, Fairlawn, NJ, USA. HPLC grade water was prepared by reverse osmosis and was further purified by passing through a Synergy Water Purification System (Millipore, Bedford, MA, USA). Drug-free human plasma was obtained from the blood bank of King Faisal Specialist Hospital & Research Centre (KFSHRC) Riyadh, Saudi Arabia. The study was approved by the Research Ethical Committee of King Faisal Specialist Hospital and Research Centre.

Chromatographic conditions

The mobile phase was composed of 0.05 M dibasic sodium phosphate (pH 7.0, adjusted with phosphoric acid) and acetonitrile (60:40, v:v). Before delivering into the system, it was filtered through 0.45 µm polyetersulfone membrane and sonicated under vacuum for 5 minutes. The analysis was carried out under isocratic conditions using a flow rate 1.0 ml/min at room temperature with the sample compartment temperature being maintained at 8ºC. A photodiode array detector set at 302 nm was used for recording chromatograms.

Preparation of standard and quality control samples

Stock solutions (1.0 mg/ml) of omeprazole and lansoprazole (internal standard, IS) were prepared in methanol. They were diluted with blank human plasma or mobile phase, respectively, to produce working solutions of 10 µg/ml. Nine calibration standards in the range of 0.01 – 1.20 µg/ml were prepared in human plasma. Four quality control (QC) samples concentrations (0.01, 0.03, 0.60 and 1.08 µg/ml) were prepared in human plasma. QC samples were vortexed for one minute, and then 1.0 ml aliquots were transferred into Teflon-lined, screw-capped, borosilicate (13 x 100 mm) glass culture tubes and stored at -20 ºC until used.

Sample preparation

Aliquots of 1.0 ml of calibration curve, quality control, or volunteer samples were allowed to equilibrate to room temperature. To each tube, 200 µl of the IS working solution were added and the mixture was vortexed for 10 seconds. After the addition of 5.0 ml of *tert.* butyl methyl ether, samples were vortexed again for 5 min and then centrifuged for 15 min at 6000 rpm at ambient temperature. The organic layer was carefully collected and dried under a gentle stream of nitrogen at 40ºC and the residue was reconstituted in 250 µl mobile phase and centrifuged at 3500 rpm for 2 min. The supernatant was transferred into auto-sampler vials, and 100 µl were injected into the chromatograph with a run time of 9 min.

Stability studies

Two QC samples (0.03 and 1.08, µg/ml) were used for stability studies, five aliquots of each QC sample were extracted and immediately analyzed (baseline), five aliquots were allowed to stand on the bench-top for 24 hours at room temperature before being processed and analyzed, five aliquots were stored at –20°C for twelve weeks before being processed and analyzed and five aliquots were processed, reconstituted, and stored at room temperature for 24 hours or 48 hours at –20°C before analysis. Finally, fifteen aliquots of each QC sample were stored at –20°C for 24 hours. They were then left to completely thaw unassisted at room temperature. Five aliquots of each sample were extracted and analyzed and the rest returned to –20°C for another 24 hours. The cycle was repeated three times (freeze-thaw stability).

Method validation

The method was validated according to standard procedures described in the US Food and Drug Administration (FDA) bioanalytical method validation guidance.[18] The validation parameter included: specificity, linearity, accuracy, precision, recovery and stability.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

Optimal experimental conditions, consisted of a the mobile phase composed of 0.05 M dibasic sodium phosphate (pH=7.0) and acetonitrile (60:40, v:v) and a flow rate of 1.0 ml/min. Omeprazole, lansoprazole and components of plasma exhibited a well-defined separation within nine minutes run. The retention times of omeprazole and lansoprazole were around 4.4 and 7.0 minutes, respectively.

Specificity

In order to confirm method specificity, we screened six batches of blank plasma and eight frequently used medications (aspirin, acetaminophen, ranitidine, nicotinic acid, ascorbic acid, caffeine, ibuprofen and diclofenac) for potential interference. No interference was found in plasma and none of the drugs co-eluted with omeprazole or the IS. **Figure 1** depicts a representative chromatogram of drug free human plasma used in preparation of standard and QC samples.

Limit of detection & quantification and linearity

The limit of quantification (LOQ) was defined as the lowest concentration on the calibration curve that can be determined with acceptable precision and accuracy (i.e., coefficient of variation and bias ≤20%). The LOQ of omeprazole in human plasma was 0.01 µg/ml. The limit of detection (≥3 signal to noise ratio) was 0.005 µg/ml. Linearity of omeprazole was evaluated by analyzing ten curves of nine standard concentrations over the range of 0.01-1.20 µg/ml. **Figure 2** represents an overlay of chromatograms of extracts of 1.0 ml human plasma spiked with the IS and one of nine concentrations of

omeprazole. The peak area ratios were subjected to regression analysis. The mean regression equation was Y = 0.6143 X - 0.0026. The accuracy of the calibration curves was confirmed by back-calculating the concentration of omeprazole in human plasma from the calibration curves (**Table 1**). All calculated concentrations were well within the acceptable limits.

Accuracy and precision

Accuracy and precision were determined for four QC concentrations (0.01, 0.03, 0.60 and 1.20 µg/ml). The inter-day precision and accuracy of the assay were determined over three different days. The intra-day (n=10) and inter-day (n=20) precision were ≤5.0% and ≤10.1%, respectively. The intra-day and inter-day accuracy were in the range of 90-112% and 92-107%, respectively. The results are summarized in **Table 2**.

Recovery

The absolute recovery of omeprazole was assessed by direct comparison of absolute peak height of plasma and mobile phase samples, using five replicates of each of a three QC concentrations (0.01, 0.03, 0.60 and 1.08 µg/ml). Similarly, the recovery of the IS was determined by comparing the peak height of the IS in five aliquots of human plasma spiked with 100 µl of IS (10 µg/ml) with the peak areas of equivalent samples prepared in mobile phase. The results are presented in **Table 3.**

Stability

Omeprazole and IS stabilities in processed and unprocessed plasma samples were investigated. No significant change in chromatographic behavior of omeprazole or the IS were observed. Omeprazole in processed samples (0.03 and 1.08 µg/ml) was found to be stable for 24 hours at room temperature (91 - 110%) and 48 hours at -20°C (89% - 106%), respectively. Omeprazole in unprocessed plasma samples was stable for 24 hours at room temperature (93-109%), for at least twelve weeks at -20°C (96%) and after three freeze-and thaw cycles (96 and 97%). **Table 4** summarizes the results of stability studies.

Application to a volunteer sample

Figure 3 depicts an overlay chromatogram of samples collected from a volunteer before and 2.0 hours after ingestion of a single oral dose of 20 mg omeprazole. The measured concentrations of omeprazole were zero and 0.69 µg/ml, respectively.

Table 1: Back-calculated omeprazole concentrations from ten calibration curves

Nominal Level (µg/ml)	Calculated level (µg/ml)		CV (%)	Accuracy (%)
		Mean (SD)		
0.01		0.23 (0.001)	13.8	99
0.02		0.44 (0.002)	9.4	97
0.04		0.91 (0.004)	11.2	96
0.08		1.82 (0.008)	9.8	100
0.10		3.83 (0.009)	8.8	102
0.24		7.97 (0.017)	7.1	100
0.48		16.27 (0.020)	4.1	101
0.96		32.63 (0.016)	1.7	99
1.20		59.61 (0.013)	1.1	100

SD, standard deviation. CV, standard deviation divided by mean measured concentration x100
Accuracy, measured level divided by nominal level x 100.

Table 2: Intra and inter-day precision and accuracy of omeprazole assay

Nominal Level (µg/ml)	Measured level (µg/ml)		CV (%)	Accuracy (%)
	Mean	(SD)		
Intra-day (n=10)				
0.01	0.0095	(0.0002)	1.6	95
0.03	0.0307	(0.0060)	1.6	112
0.60	0.5599	(0.0219)	3.9	93
1.08	0.9745	(0.0485)	5.0	90
Inter-day (n=20)				
0.01	0.0101	(0.0009)	9.2	101
0.03	0.0320	(0.0032)	10.1	107
0.60	0.5658	(0.0203)	3.6	94
1.08	0.9938	(0.0593)	6.0	92

SD, standard deviation. CV, standard deviation divided by mean measured concentration x100
Accuracy, measured level divided by nominal level x 100.

Table 3: Recovery of omeprazole and the internal standard from 1.0 ml of human plasma

Concentration (µg/ml)	Human plasma*	Mobile phase*	Recovery** (%)
Omeprazole 0.01 0.03	76 (2.2) 233 (0.8)	88 (2.6) 272 (2.3)	86 86
0.60	5589 (44.9)	5901 (22.6)	95
1.08	9332 (34.9)	9743 (8.8)	96
Internal standard 10	7506 (188.9)	8258 (81)	91

* Mean peak area (SD), n = 5. ****** Recovery = Mean peak area of omeprazole in human plasma divided by mean peak area in mobile phase X 100.

Table 4: Stability data for omeprazole in human plasma

Stability(%)							
Nominal	**Unprocessed**		**Processed**		**Freeze-Thaw**		
Level (µg/ml)	**24 hrs RT**	**12 wks -20°C**	**24 hrs RT**	**48 hrs -20°C**	**Cycle**		
					1	2	3
0.03	**93**	**96**	**91**	**89**	94	92	97
1.08	109	96	110	106	100	99	96

Stability (%) = mean measured concentration (n=5) at the indicated time divided by mean measured concentration (n=5) at baseline x 100. Spiked plasma samples were processed and analyzed immediately (baseline, data not shown), after 24 hours at room temperature (24 hrs RT), or after freezing at –20ºC for 12 weeks (12 wks. –20ºC), or processed and then analyzed after storing for 24 hours at room temperature (24 hrs RT) or 48 hours at –20ºC (48 hrs -20ºC).

Figure Captions

Fig. 1 Representative chromatogram of a drug-free human plasma. The arrows indicate the retention times of omeprazole (4.4 min) and the internal standard (IS), lansoprazole (7.0 min).

Fig. 2 Overlay of chromatograms of extracts of 1.0 ml human plasma blank (B), spiked with one of nine concentrations of omeprazole, 0.01, 0.02, 0.04, 0.08, 0.10, 0.24, 0.48, 0.96, and 1.20 µg/ml and with the internal standard (IS).

Fig. 3 An overlay of chromatograms of plasma samples obtained from a healthy volunteer before (A) and 2.0 hours after (B) a single oral 20 mg omeprazole dose.

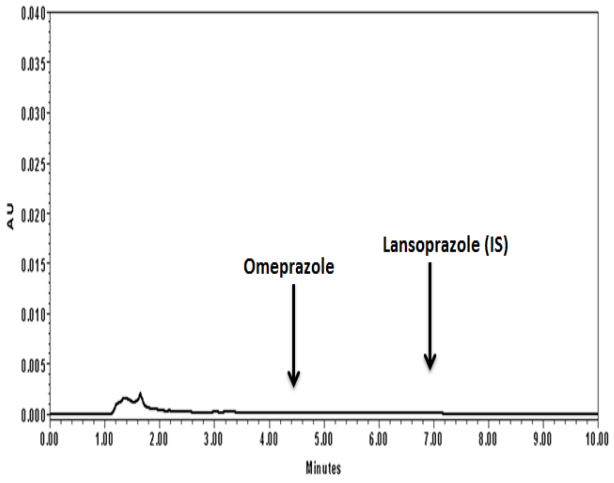

Fig. 1.

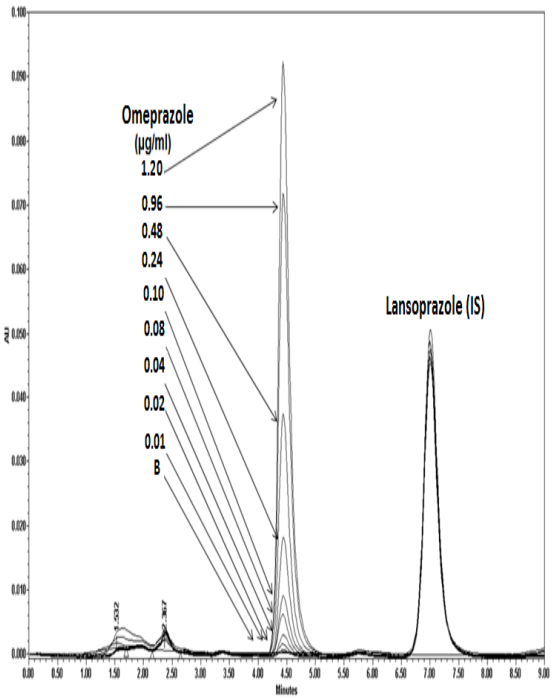

Fig. 2.

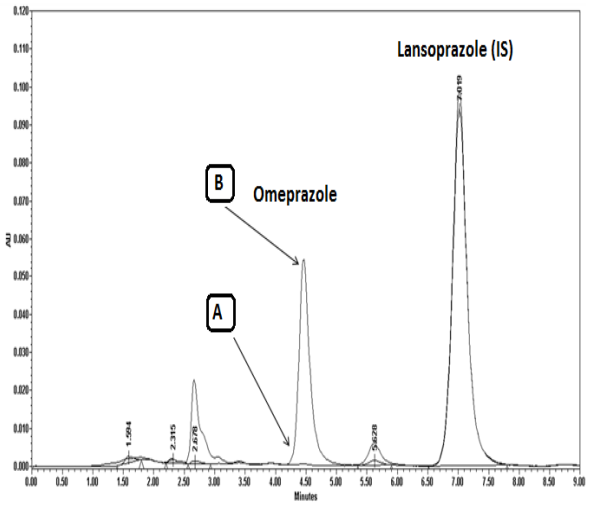

Fig. 3.

CONCLUSION

The described HPLC assay is precise and rapid. It requires only 1.0 ml plasma and utilizes a simple and convenient method for sample preparation. The assay was applied to monitor stability of omeprazole under various conditions generally encountered in the clinical laboratories. Further, it was successfully used to measure the levels of omeprazole in samples obtained from a healthy volunteer.

ACKNOWLEDGEMENT

This work was funded by a grant to Dr. Muhammad M Hammami, from the King Abdul-Aziz City for Science and Technology, Riyadh, Saudi Arabia (National Comprehensive Plan for Science and Technology # 10-BIO 961).

REFERENCES

1. Fellenius E. Berglindh T. Sachs G, Substitute benzimidazoles inhibit gastric acid secretion by blocking (H+ K+)-ATPase. Nature., 1981; (290): 159-161.
2. Indian Pharmacopia, Govt. of India, Ministry of Health and Family Welfare, Ghaziabad, New Delhi. The Indian pharmacopeia commission publisher., 2010; 1813-1814.
3. Andersson T. Andren K. Cederberg C. Lagerstrom PO. Lundborg P. Skanberg I, Pharmacokinetics and bioavailaibility of omeprazole after single and repeated oral administration in healthy subjects. Br. J. Clin. Pharmac., 1990; (29): 557-563.
4. Sajal KS. Jayanta KC. Sreedam CD. Tasfina I. Sitesh CB, Comparative pharmacokinetic analysis with two omeprazole formulations “Proception” and “Losec” in healthy subjects. Arch Med Biomed Res., 2014; (1): 147-155.
5. Asif HC. Tanveer AM, Kazi MA. Muhammad S. Muhammad A. Muhammad SA, A simple HPLCnethod for the detrermination of omeprazole in vitro. Int. J. Pharm. Chem., 2012; 2(4): 126-128.
6. Pujeri1 SS. Khader MA. 1 and Seetharamappa. J. Development and validation of LC method for the assay of omeprazole enantiomers in pharmaceutical formulations. Scholars Research Library Der Pharmacia Lettre., 2012; 4(1): 76-86.
7. Cristina I. Mirela M. Adina P. Leucuta SE, Validation of Hplc-UV method for analysis of omeprazole in presence of its metabolites in human plasma. Farmacia., 2008; 56(3): 254-260.
8. Gopalakrishnan S. Jothy K. Dhanalakshmi K, Analytical method development and validation of HPLC method for the determination of omeprazole in capsule dosage form. Elixir Appl. Chem., 2012; (52): 11283-11286.
9. Motevalian M. Saeedi G. Keyhanfar F. Tayebi L. Mahmoudian M, Simultanous determination of omeprazole and its metabolites in human plasma by HPLC using solid-phase extraction. Pharm. Pharmacol. Commun., 1999; (5): 265-268.
10. Noubarani M. Keyhanfar F. Motevalian M. Mahmoudian M. Improved HPLC method for determination of four PPIs, omeprazole, pantoprazole, lansoprazole and rabeprazole in human plasma., J Pharm Pharm Sci., 2010; 13(1): 1-10.
11. Yeung PK. Little R. Jiang Y. Buckley SJ. Pollak PT, Kapoor H. Veldhuyzen van Z SJ, A simple high performance liquid chromatography assay for simultaneous determination of omeprazole and metronidazole in human plasma and gastric fluid. J Pharm Biomed Anal., 1998; 17(8): 1393-1398.
12. Won KK. Dong SK. Kwang IIK, Advance method for determination of omeprazole in plasma by HPLC. Archives Pharm. Res., 1999; 22(1): 86-88.
13. Ray S, De. PK. HPTLC and TLC method for rapid quantification and identification of omeprazole. Indian drugs., 1994; 31(11): 543-547.
14. Ozaltin N. Temizer A, Differential pulse polarographic determination of omeprazole in pharmaceutical preparations. Electroanalysis., 1994; 6(9): 799–803.
15. Bonato PS, Paias O. Enantioselective analysis of omeprazole in pharmaceutical formulations by chiral high-performance liquid chromatography and capillary electrophoresis. Journal of the Brazilian Chemical Society., 2004; 15(2): 318-323.
16. Pinzauti S, Gratteri P, Furlanetto S, Mura P, Dreassi E, Phan-Tan-Luu R. Experimental design in the development of voltammetric method for the assay of omeprazole. Journal of pharmaceutical and biomedical analysis., 1996; 4: 881-889.
17. Qinying Z, Anna P, Grace EB, Cynthia E, Jason G, Pamela J, Bernadette DR, Rita GA, Bharat D. Bioequivalence of omeprazole delayed-release capsules in healthy Filipino subjects. J. Bioequiv Availab., 2014; 6(5): 144-149.
18. Bioanalytical Method Validation, Food and Drug Administration, Centre for Drug Evaluation and Research (CDER), May 2001.