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Determination of omeprazole in rat plasma by high-performance liquid chromatography without solvent extraction

Huijuan Jia, Wei Li, Kang Zhao *

The College of Pharmaceuticals & Biotechnology, Tianjin University, Tianjin, PR China

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Abstract

A HPLC method without solvent extraction and using ultraviolet detection at 302 nm for the determination of omeprazole in rat plasma has been validated. Plasma samples after pretreatment with acetonitrile to effect deproteinization were dried under N_2 at 40 °C and reconstituted with mobile phase. The standard calibration curve for omeprazole was linear ($r^2 = 0.9999$) over the concentration range of 0.02–3 μg ml^{-1}. The intra- and inter-day assay variability range was 4.8–9.2% and 5.2–10.3% individually. This method has been successfully applied to a pharmacokinetic study of omeprazole in rats.

Keywords: Omeprazole; Rat plasma; High-performance liquid chromatography

1. Introduction

Omeprazole (Fig. 1) is a substituted benz-imidazole (5-methoxy-2-[[(4-methoxy-3, 5-dimethyl-2-pyridinyl) methyl] sulfinyl]-1*H*-benzimidazole), which can be applied to the treatment of gastric acid related disorders [1,2]. It works through inhibiting the (H^+, K^+)-ATPase in the gastric parietal cells to result in a diminution of gastric acid secretion. This is different from the antiacid mechanism employed by the histamine H_2-receptor antagonist (H_2RA). So this drug can be used effectively to control the gastric acidity in patients not responding satisfactorily to H_2RA such as those suffering from Zollinger–Ellison syndrome [3].

For the quantification of bulk omeprazole, several methods have been reported, which include HPLC employing electrochemical detection and coulometric detection [4,5], capillary electrophoresis [6], spectrophotometry [7], polarography [8–11], voltammetry [12] and thin-layer chromatography [13–15]. However, these methods lack the sensitivity required for quantification of omeprazole in biological fluids.

Several HPLC-MS and HPLC-UV methods for the determination of omeprazole in biological fluids have been developed [16–21]. But all of these methods involve liquid–liquid extraction under alkaline condition, which is time-consuming and not economically feasible for routine use in pharmacokinetic studies with numerous samples to be analyzed.

Here we developed and validated a sensitive method for the determination of omeprazole in rat plasma by HPLC with UV detection that can be used in pharmacokinetic studies of the drug. This method employs a micro volume of plasma (150 μl) and a simple sample preparation without organic solvent extraction which ensure the applicability of the method when only small volume of plasma is available and the reproducibility of the method. The simple sample preparation also ensures the consistent recovery, the low relative standard deviation and the good linearity which promise the elimination of the internal standard.

2. Experimental

2.1. Chemicals and reagents

Omeprazole was supplied by BBCA Pharmaceutical Co., Ltd (99.6%, China). HPLC grade acetonitrile and analytical grade triethylamine (TEA), potassium dihydrogen phosphate and concentrated phosphoric acid were obtained from Kewei Agents Company (Tianjin, China).

* Corresponding author. Tel.: +86 22 27404031; fax: +86 22 27405190.
E-mail address: combinology@yahoo.com (K. Zhao).

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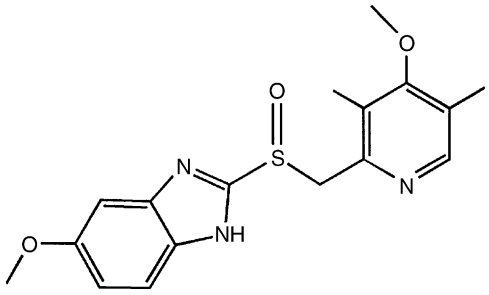

Fig. 1. The structure of omeprazole.

2.2. Instrumentation

The apparatus used for this work was an Agilent 1100 quaternary pump, with a variable wavelength detector, thermostatted autosampler and column thermostat. A Hypersil ODS_2 C_{18} column (250 mm × 4.6 mm i.d., 5 μm, Thermo, UK) was fitted with a Phenomenex guard column packed with octadecyl C_{18} (Phenomenex, USA). The mobile phase comprised of 50 mM potassium dihydrogen phosphate buffer (pH 7.1, contained 0.7% TEA, v/v) and acetonitrile (75:25, v/v), the detection wavelength was 302 nm.

Analyses were run at a flow rate of 1.0 ml min^{-1} at 25 °C and the samples were quantified using peak areas.

2.3. Assay standards

Stock solution of omeprazole was prepared daily by dissolving the appropriate amount of omeprazole in acetonitrile to yield a final solution (300 μg ml^{-1}). Separate solutions were prepared for the calibration standards. Further solutions were obtained by serial dilutions of stock solution with water. These solutions were added to drug free rat plasma in volumes not exceeding 8% of the plasma volume.

2.4. Sample preparation

The rat plasma sample (150 μl) was transferred into a 1.5 ml polypropylene micro-centrifuge tube. The sample was deproteinized by the addition of 450 μl acetonitrile, vortexed for 30 s and then centrifuged at 3000 rpm for 10 min. The supernatant fluid was transferred to another 1.5 ml polypropylene microcentrifuge tube and dried under a stream of N_2 at 40 °C. The residue was reconstituted with 150 μl mobile phase and 100 μl of the reconstituted solution was injected onto the HPLC column.

2.5. Assay validation

Standard calibration curve was constructed by spiking drug free plasma with a known amount of omeprazole in the concentration range of 0.02–3 μg ml^{-1}. The plasma standards were also used to determine the intra- and inter-day precision and accuracy of the method. Absolute recoveries of seven different concentrations of omeprazole in rat plasma were determined by assaying the samples as described above and comparing the peak areas of drugs with those obtained from direct injection of aqueous drug solutions of the corresponding concentrations.

2.6. Plasma collection

Male Wistar rats (250–300 g) were used in this experiment. The rats were fasted overnight before use and were given a single dose of omeprazole (10 mg kg^{-1}) through oral gavage. Heparinized samples of blood (0.4 ml) were collected at 5, 15, 30, 60, 100, 140, 180, 240 and 300 min after administration. Plasma samples were harvested after centrifugation and stored frozen at −20 °C until analysis.

2.7. Stability of omeprazole in rat plasma

The plasma sample used for the test was obtained through adding 10 μl of stock solution of omeprazole (300 μg ml^{-1}) to 3 ml drug free rat plasma. This sample was stored frozen at −20 °C and analyzed at 0, 3 and 7 days to evaluate the stability of omeprazole in rat plasma.

2.8. Pharmacokinetic data analysis

The pharmacokinetic analysis was conducted through using Practical Pharmacokinetic Program-Version 97 (3P97, published by Chinese Pharmacological Association, Beijing, China).

3. Results and discussion

3.1. Chromatography

The aim of this work, a new, simple, accurate, reproducible and sensitive HPLC method to determine omeprazole in rat plasma has been developed. A satisfactory separation of omeprazole from endogenous components in rat plasma was obtained.

Representative chromatograms of blank rat plasma, drug free rat plasma spiked with drug and rat plasma after the administration of omeprazole are shown in Fig. 2.

The plasma deproteinization by acetonitrile was employed because of its simplicity and high reproducibility. The optimum wavelength for detection was 302 nm at which omeprazole had good response. The mobile phase comprised of 50 mM potassium dihydrogen phosphate buffer (pH 7.1, contained 0.7% TEA, v/v) and acetonitrile (75:25, v/v). The composition of the mobile phase was chosen to provide the best peak resolution and retention time. As such, the added triethylamine in the mobile phase is an important factor, since it permits the peak shape of omeprazole.

3.2. Linearity and limit of quantification

The linearity of the method was evaluated with calibration curve made in rat plasma ranging from 0.02 to 3 μg ml^{-1}. A good linear relationship was found, as described by the following linear regression equations: $y = 0.0076x + 0.0021$ ($r^2 = 0.9999$), where y is the drug concentration (μg ml^{-1}) and x is the peak area. Value for the coefficient of determination is satisfactory.

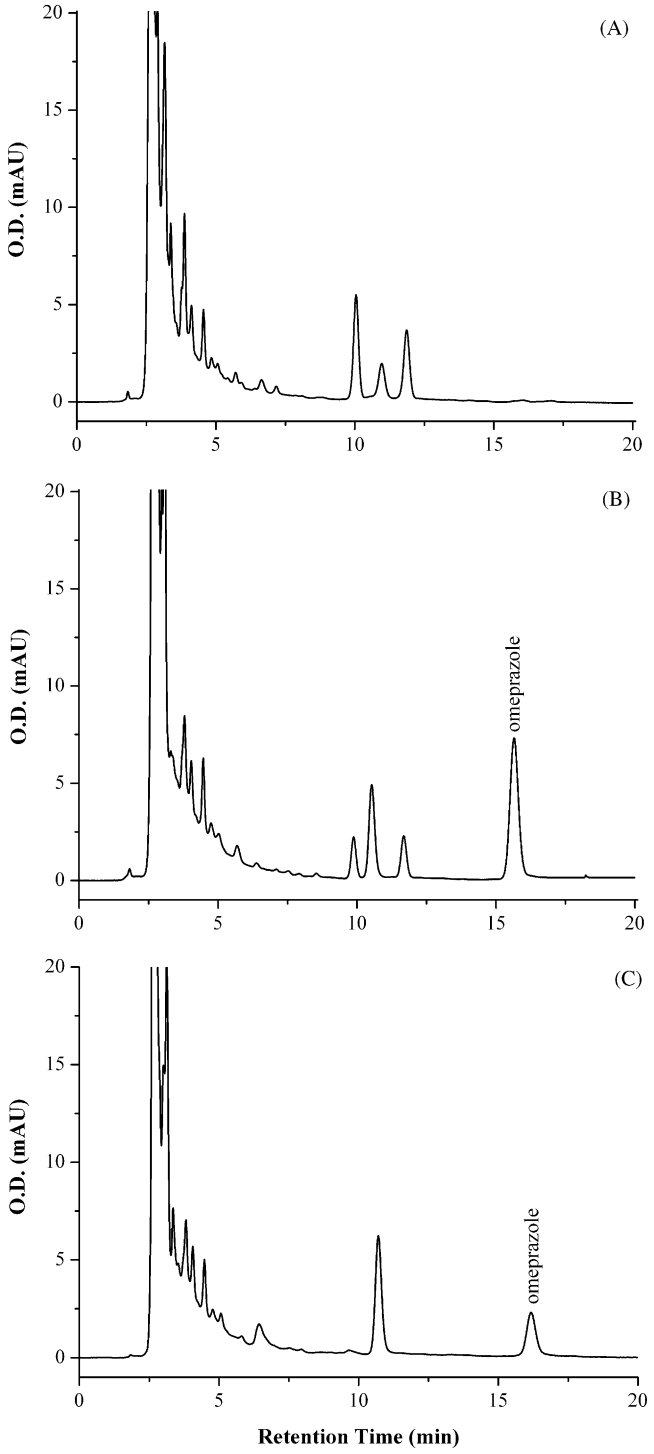

Fig. 2. (A) HPLC chromatogram of drug free rat plasma. (B) HPLC chromatogram of rat plasma containing omeprazole (μg ml^{-1}). (C) Rat plasma after oral administration of omeprazole (10 mg kg^{-1}).

Detection limits were determined as the concentration of components giving a ratio of signal-to-noise = 4:1. The limit of detection for omeprazole in rat plasma was found to be 0.01 μg ml^{-1}. The limits of quantitation (LOQ) for omeprazole in rat plasma was chosen as the concentrations used for the lowest concentration level on the calibration curves and for which the R.S.D. was <15% (i.e. 0.02 μg ml^{-1}).

Table 1
Absolute recovery of omeprazole ($n = 6$)

Concentration (μg ml^{-1})	Recovery (mean, %)	R.S.D. (%)
0.02	92.5	3.7
0.05	97.4	2.1
0.1	93.1	6.8
0.2	92.9	7.2
0.5	91.0	5.6
1.0	91.6	2.0
3.0	91.4	4.6

3.3. Analytical recovery

The percentage recoveries ($n = 6$) of omeprazole over the entire concentration range is given in Table 1. These results indicate that the simple protein precipitation procedure with acetonitrile is sufficient to ensure stable and high recovery from rat plasma.

3.4. Intra- and inter-day variations and accuracy

The intra- and inter-day variations and accuracy of the method are summarized in Table 2. All results are within acceptable ranges for bio-analytical purposes.

3.5. Stability study

The results of storage stability in rat plasma are shown in Table 3. The omeprazole was stable in rat plasma at −20 °C for at least 1 week.

3.6. Application

The developed method has been successfully applied to an analysis of plasma samples collected from rats administered with omeprazole (10 mg kg^{-1}). The concentration–time profile of omeprazole (mean ± S.D.) after oral administration is shown in Fig. 3.

A two-compartment model was fitted to the concentration–time profiles of the administered animals by the 3P97 program. The pharmacokinetic parameters were estimated as parts of the model which were summarized in Table 4.

Table 2
The intra- and inter-day variations and accuracy of omeprazole ($n = 6$)

Concentration (μg ml^{-1})	Intra-day		Inter-day	
	Precision (R.S.D., %)	Accuracy (%)	Precision (R.S.D., %)	Accuracy (%)
0.02	8.4	102.8	10.3	103.5
0.05	9.2	104.5	8.6	107.3
0.1	6.5	97.0	7.5	98.4
0.2	6.8	99.0	8.1	100.4
0.5	6.9	99.9	7.3	101.4
1.0	7.1	100.4	7.9	99.9
3.0	4.8	99.9	5.2	99.4

Table 3
Stability of omeprazole in rat plasma

n[a]	Nominal concentration ($\mu g\,ml^{-1}$)	Concentration measured ($\mu g\,ml^{-1}$, mean ± S.D.)		
		0 day	3 days	7 days
6	0.997	0.998 ± 0.021	0.993 ± 0.037	0.995 ± 0.033

[a] n = repeated times of analysis.

Table 4
Pharmacokinetic parameters of omeprazole in rat ($n = 4$)

Parameter[a]	Value (mean ± S.D.)	Parameter[a]	Value (mean ± S.D.)
A (ng/ml)	2320.6 ± 922.5	k_{10} (1/min)	0.0063 ± 0.0034
α (1/min)	0.036 ± 0.015	k_{12} (1/min)	0.027 ± 0.012
B (ng/ml)	45.3 ± 13.8	k_{21} (1/min)	0.0077 ± 0.0038
β (1/min)	0.032 ± 0.010	AUC (min ng/ml)	35532.2 ± 8310.2
k_a (1/min)	0.23 ± 0.10	$CL_{(s)}$ ((mg/kg/min)/(ng/ml))	0.00062 ± 0.00020
$t_{1/2\alpha}$ (min)	50.7 ± 23.8	T_{max} (min)	21.8 ± 13.2
$t_{1/2\beta}$ (min)	249.1 ± 100.1	C_{max} (ng/ml)	439.6 ± 116.2
$t_{1/2\,Ka}$ (min)	10.7 ± 4.3		

[a] A: distribution phase; B: elimination phase; α: distribution rate constant;, β: elimination rate constant; k_a: absorption rate constant; $t_{1/2\alpha}$: distribution half-life time; $t_{1/2\beta}$: elimination half-life time; $t_{1/2\,Ka}$: absorption half-life time; k_{12} and k_{21}: transportation constants; k_{10}: elimination constant; AUC: area under curve; $CL_{(s)}$: clearance; C_{max}: maximum concentration; T_{max}: time of C_{max}.

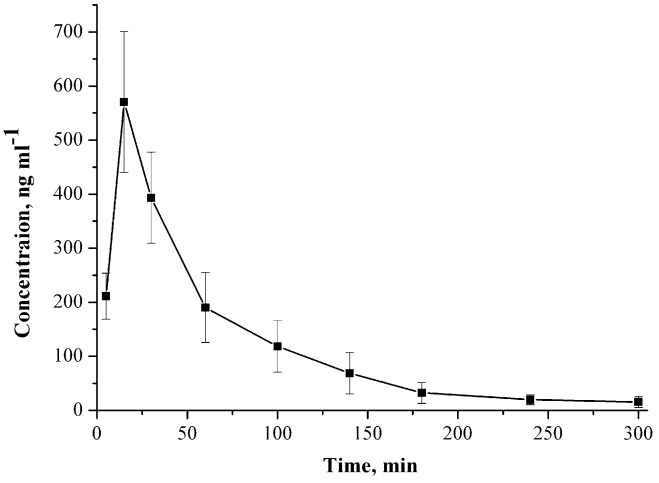

Fig. 3. The concentration–time profile (mean ± S.D.) of omeprazole after administration to rats ($n = 4$).

4. Conclusions

The method described in this paper is a new, specific and sensitive HPLC-UV method without solvent extraction to quantify omeprazole in rat plasma. According to our knowledge, no similar method has been reported. The method has been successfully applied to quantify omeprazole in rat plasma after oral administration.

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References

[1] A. Walan, Gastroenterol. Hepatol. 4 (1989) 27.
[2] E. Fellenius, T. Berglind, O. Sachs, L. Olbe, B. Elander, S.E. Sjostrand, B. Wollmark, Nature 290 (1981) 159.
[3] K. Oberg, K. Lindstrom, Lancet 1 (1983) 66.
[4] B. Persson, S. Wendsjo, J. Chromatogr. 321 (1985) 375.
[5] G.W. Sluggett, J.D. Stong, J.H. Adams, J. Pharm. Biomed. Anal. 25 (2001) 357.
[6] A. Tivesten, S. Folestad, V. Schonbacher, K. Svensson, Chromatographia 49 (1999) S7.
[7] C. Sastry, Y. Naidu, S. Murty, Talanta 44 (1997) 1211.
[8] S. McClean, E. O'Kane, V.N. Ramachandran, W.F. Smyth, Anal. Chim. Acta 292 (1994) 81.
[9] N. Ozaltin, A. Temizer, Electroanalysis 6 (1994) 799.
[10] D. Dogrukol-Ak, M. Tuncel, Pharmazie 50 (1995) 701.
[11] H. Oelschlaeger, H. Knoth, Pharmazie 53 (1998) 242.
[12] S. Pinzauti, P. Gratteri, S. Furlanetto, P. Mura, E. Dreassi, R. Phan-Tan-Luu, J. Pharm. Biomed. Anal. 14 (1996) 881.
[13] S. Mangalan, R.B. Patel, B.K. Chakravarthy, J. Planar Chromatogr. Mod. TLC 4 (1991) 492.
[14] S. Ray, P. Kumar De, Indian Drugs 31 (1994) 543.
[15] D. Dogrukol-Ak, M. Tuncel, Pharmazie 53 (1998) 272.
[16] H.M. González, E.M. Romero, T. de J. Chavez, J. Chromatogr. B 780 (2002) 459.
[17] F.C. Cheng, Y.F. Ho, L.C. Hung, J. Chromatogr. A 949 (2002) 35.
[18] K.H. Yuen, W.P. Choy, H.Y. Tan, J. Pharm. Biomed. Anal. 24 (2001) 715.
[19] E.J. Woolf, B.K. Matuszewski, J. Chromatogr. A 828 (1998) 229.
[20] J. Macek, P. Ptáček, J. Klíma, J. Chromatogr. B 689 (1997) 239.
[21] H. Kanazawa, A. Okada, Y. Matsushima, J. Chromatogr. A 949 (2002) 1.