Pharm. Pharmacol. Commun. 1999, 5: 265–268
Received December 22, 1998
Accepted January 21, 1999

Simultaneous Determination of Omeprazole and its Metabolites in Human Plasma by HPLC using Solid-phase Extraction

M. MOTEVALIAN, G. SAEEDI*, F. KEYHANFAR, L. TAYEBI* AND M. MAHMOUDIAN

*Pharmacology Department, Iran University of Medical Sciences, PO Box 14155-6183, Tehran, and *Pars Biopharmacy Research Co., Tehran, Iran*

Abstract

A rapid, simple and sensitive HPLC assay was developed for the simultaneous determination of omeprazole and its major metabolites in human plasma using a solid-phase extraction procedure.

Eluent (50 μL) was injected on an μBondapak C_{18} reversed-phase column (4·6 mm i.d., 250 mm; 10 μm). The mobile phase consisted of 0.05 M phosphate buffer (pH 7·5) and acetonitrile (75 : 25, v/v) at a flow rate of 0·8 mL min^{-1}. UV detection was at 302 nm. Mean recovery was greater than 96% and the analytical responses were linear over the omeprazole concentration range of 50–2000 ng mL^{-1}. The minimum detection limits were 10, 10 and 15 ng mL^{-1} for omeprazole, omeprazole sulphone and hydroxyomeprazole, respectively. The method was used to determine the plasma concentration of the respective analytes in four healthy volunteers after an oral dose of 40 mg omeprazole.

The extraction procedure and HPLC method is simple, precise and quick, and suitable for the study of pharmacokinetic disposition and metabolism of omeprazole, which is extensively metabolized in man.

Omeprazole, a selective H^+/K^+ ATPase inhibitor in gastric parietal cells is widely used in various gastric acid-related disorders (Lamers et al 1984; Holt 1991; Gunasekaran & Hassall 1993). Omeprazole undergoes rapid and extensive metabolism by the liver (Cederberg et al 1989; Andersson 1996). Three major primary metabolites of omeprazole are hydroxyomeprazole, omeprazole sulphone and omeprazole sulphide. Omeprazole sulphone and hydroxyomeprazole are the major metabolites found in plasma. The concentration of omeprazole sulphide is usually too low to be determined in plasma (Lagerstrom & Persson 1984) and that of omeprazole and omeprazole sulphide is also negligible in urine (Naesdal et al 1986; Regardh et al 1990). Several HPLC methods are available for the determination of omeprazole and its metabolites in biological fluids (Mihaly et al 1983; Lagerstrom & Presson 1984; Amantea & Narang 1988; Kobayashi et al 1992). However, the extraction procedures used are tedious and time-consuming. We report the simultaneous determination of omeprazole, hydroxyomeprazole and omeprazole sulphone in plasma using a conventional extraction procedure and HPLC system. The method was applied to a pilot pharmacokinetic study of omeprazole and its metabolites in healthy volunteers.

Correspondence: M. Mahmoudian, Pharmacology Department, Iran University of Medical Sciences, PO Box 14155-6183, Tehran, Iran.
E-Mail: massoud@nrcgeb.ac.ir.

Materials and Methods

Materials

Omeprazole and omeprazole sulphone were generous gifts from Union Quimico, Farmaceutica (Mallora, Barcelona). Hydroxyomeprazole was a kind gift from Astra Hassle (Molndal, Sweden). HPLC grade acetonitrile and methanol were purchased from Merck (Germany) and Daroupakhsh (Tehran, Iran), respectively. All other reagents were of analytical grade.

Solid-phase extraction

Phenacetin (5 μg mL^{-1}), the internal standard, was added to 1-mL plasma samples. The plasma was passed through a 500 mg C_{18} cartridge connected to

a vacuum elution system. The cartridge was washed twice with 1 mL double-distilled water to remove any potential interfering compounds, and washed once with 1 mL phosphate buffer (0·125 M, pH 8). After drying, omeprazole and its metabolites were eluted by 500 μL acetonitrile and 50 μL eluent was injected onto the HPLC column.

Apparatus and chromatographic conditions

A Waters liquid chromatograph system comprising a pump model 600, UV detector model 486, integrator model 746, a 4·6 × 250 mm μBondapak C_{18} column (10 μm) and a μBondapak C_{18} precolumn were used. The mobile phase consisted of acetonitrile and 0.05 M phosphate buffer (pH 7·5) (25 : 75, v/v) at a flow rate of 0·8 mL min^{-1} and the injection volume was 50 μL. UV detection was set at 302 nm, chromatograms were traced on an integrator and peak heights were determined. The procedure was performed at room temperature. Quantification was based on peak height ratio using phenacetin as internal standard.

Standard solutions and calibration

Standard solutions for plasma determination of omeprazole and metabolites were prepared by dissolving omeprazole in acetonitrile (1 mg mL^{-1}), omeprazole sulphone and hydroxyomeprazole in methanol (1 mg mL^{-1}), and then serial dilutions were made in 0·05 M phosphate buffer (pH 7·5). Different amounts of standard solutions were added to blank plasma to prepare plasma standards. Calibration samples were prepared in the same way and assayed.

Subjects and sample preparation

The method was used in a preliminary study of the concentration–time profile of omeprazole and its metabolites in the plasma of four healthy male volunteers (aged 28–33 years). Informed consent was obtained from each volunteer and the experiment was approved by an institutional ethical committee. After overnight fasting, each subject was given two 20-mg omeprazole capsules (Losec). Blood samples (5–10 mL) were collected at 0, 0·5, 1, 1·25, 1·5, 1·75, 2, 2·25, 2·5, 4, 6 and 8 h after drug administration, centrifuged and plasma collected and stored at −70°C until assayed. All volunteers were non-smokers and had not taken any drugs the week before or during experiment. A light breakfast was given 2 h, and standard lunch 4 h, after drug administration.

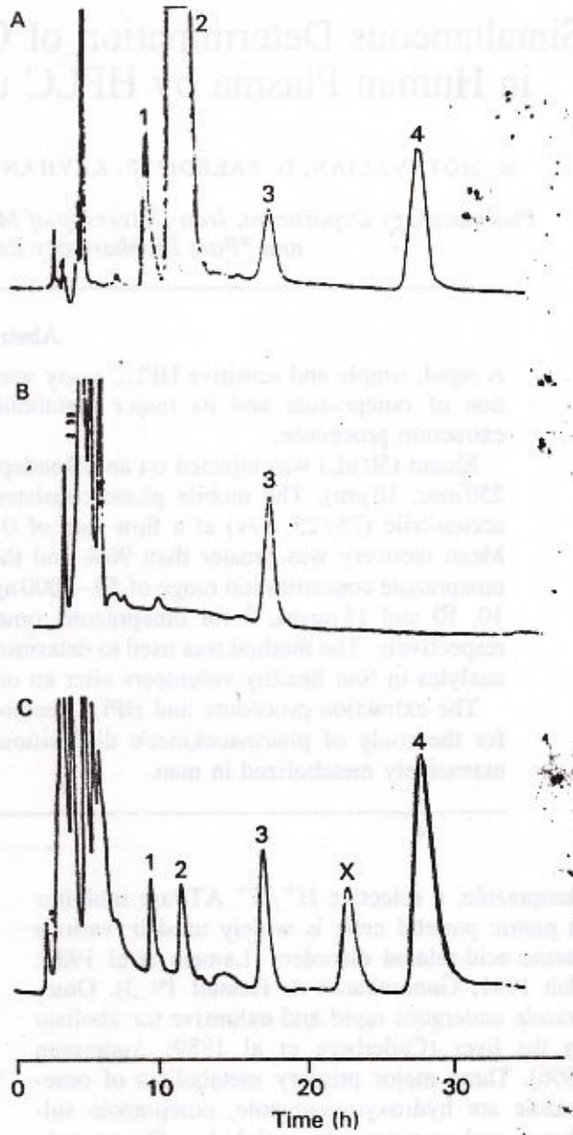

Figure 1. Representative chromatograms of blank plasma with omeprazole, metabolites and phenacetin (A), blank plasma with phenacetin (B) and human plasma 6 h after oral administration of 40 mg omeprazole (C). Peaks: 1. hydroxyomeprazole; 2. omeprazole sulphone; 3. phenacetin (internal standard); 4. omeprazole. An unknown metabolite is indicated by X.

Pharmacokinetic parameters were determined by a model-independent method using a computer program developed in our laboratory. The slope of the terminal portion of the plasma concentration–time curve was determined by least-squares regression analysis, the apparent elimination half-life ($t_{½}$) of each of the analytes was calculated as $t_{½} = 0·693/K_e$. Areas under the plasma concentration–time curve (AUC) of omeprazole, omeprazole sulphone and hydroxyomeprazole were calculated by the trapezoidal rule with time extra-

Table 1. Extraction yields of omeprazole and its metabolites from plasma.

Analyte	Concn added ($ng\,mL^{-1}$)	Recovery (%)	CV (%)
Omeprazole	50	100·0 ± 0·03	0·03
	500	98·5 ± 0·2	0·20
	1000	89·3 ± 0·02	0·02
Omeprazole sulphone	100	97·3 ± 4·1	4·20
	250	102·8 ± 7·2	6·97
Hydroxyomeprazole	100	92·8 ± 4·9	5·27
	250	93·4 ± 3·2	3·40

Results are mean ± s.e of 3–6 samples. CV = coefficient of variation.

polated to infinity. The apparent oral clearance of omeprazole and its metabolites were calculated as dose/$AUC_{0-\infty}$. The maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) for all the analytes were read from the obtained data.

Results and Discussion

The proposed method is more convenient and less time-consuming with respect to a liquid–liquid extraction procedure. Typical chromatograms of omeprazole and its metabolites are shown in Figure 1. No interfering peaks were observed in chromatograms of blank plasma samples. The detection limit was 10, 10 and 15 $ng\,mL^{-1}$ for omeprazole, omeprazole sulphone and hydroxyomeprazole, respectively, which is below the drug concentration usually expected in samples from patients taking therapeutic doses of omeprazole. An extra peak (Figure 1C) was also observed. This may be due to an unidentified metabolite of omeprazole and needs to be identified and characterized. The standard curves were linear over the concentration range 50–2000 $ng\,mL^{-1}$ for omeprazole, omeprazole sulphone and hydroxyomeprazole with regression coefficients of 0·981, 0·995 and 0·998, respectively.

The mean recovery of omeprazole and omeprazole sulphone and hydroxyomeprazole from plasma ranged from 89·3 to 102·8% (in Table 1), indicating that the proposed method can achieve complete recovery of the compounds studied from plasma. The coefficient of variation was less than 0·2% for omeprazole (Table 1). The analytical precision and accuracy is given in in Table 2.

The method was used to determine omeprazole and its metabolites in plasma samples from four healthy volunteers who had received an oral dose of 40 mg omeprazole. A sample concentration–time curve of omeprazole and the metabolites is shown in Figure 2 and the individual pharmaco-

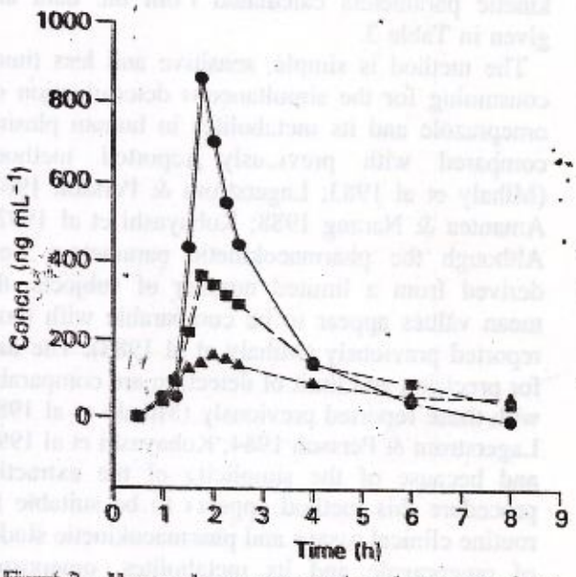

Figure 2. Human plasma concentration–time curves of omeprazole (●), omeprazole sulphone (■) and hydroxyomeprazole (▲) after an oral dose of 40 mg omeprazole.

Table 2. Precision and accuracy of the intra- and inter-day assay of omeprazole and its metabolites in plasma.

Analyte	Concn added ($ng\,mL^{-1}$)	n	Calculated (mean ± s.e.)	CV (%)	Accuracy (%)
Intra-day					
Omeprazole	250	6	242·2 ± 11·2	4·6	−3·1
	500	6	467·2 ± 24·2	5·1	−6·6
Omeprazole sulphone	500	3	481·5 ± 11·9	2·5	−3·7
Hydroxyomeprazole	500	3	464·1 ± 7·2	1·5	−7·2
Inter-day					
Omeprazole	100	3	101·8 ± 5·9	5·8	1·8
	500	3	526·9 ± 25·0	4·7	5·4
Omeprazole sulphone	500	3	522·7 ± 5·2	0·9	4·5
Hydroxyomeprazole	500	3	475·2 ± 7·1	1·6	−4·9

CV = coefficient of variation.

Table 3. Individual pharmacokinetic parameters after oral administration of 40 mg omeprazole to four healthy volunteers.

Analyte	K_e (h^{-1})	$t_{1/2}$ (h)	C_{max} (ng mL^{-1})	T_{max} (h)	$AUC_{0-\infty}$ (ng h mL^{-1})
Omeprazole	1·41	0·49	310·75	1·00	247·97
Omeprazole sulphone	0·13	5·10	119·40	1·75	553·60
Hydroxyomeprazole	0·22	3·07	348·10	1·75	1027·20
Omeprazole	1·29	0·53	443·00	2·00	539·80
Omeprazole sulphone	0·59	1·16	35·10	1·75	90·16
Hydroxyomeprazole	0·30	2·36	399·40	2·00	651·60
Omeprazole	1·19	0·57	351·50	1·75	410·96
Omeprazole sulphone	0·43	1·61	570·50	2·00	941·90
Hydroxyomeprazole	0·17	4·00	251·30	2·00	571·80
Omeprazole	0·66	1·05	860·95	1·75	1433·33
Omeprazole sulphone	0·30	2·30	362·40	1·75	1228·20
Hydroxyomeprazole	0·17	4·00	158·90	2·00	987·30

kinetic parameters calculated from the data are given in Table 3.

The method is simple, sensitive and less time-consuming for the simultaneous determination of omeprazole and its metabolites in human plasma compared with previously reported methods (Mihaly et al 1983; Lagerstrom & Persson 1984; Amantea & Narang 1988; Kobayashi et al 1992). Although the pharmacokinetic parameters were derived from a limited number of subjects, the mean values appear to be comparable with those reported previously (Mihaly et al 1983). The data for precision and limit of detection are comparable with those reported previously (Mihaly et al 1983; Lagerstrom & Persson 1984; Kobayashi et al 1992) and because of the simplicity of the extraction procedure this method appears to be suitable for routine clinical assays and pharmacokinetic studies of omeprazole and its metabolites, omeprazole sulphone and hydroxyomeprazole.

Acknowledgement

This study was supported by Dr Abidi Pharmaceutical Company.

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