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Note

Advanced Method for Determination of Omeprazole in Plasma by HPLC

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An advanced and sensitive high-performance liquid chromatographic (HPLC) method for determination of omeprazole in human plasma has been developed. After omeprazole was extracted from plasma with diethylether, the organic phase was transferred to another tube and trapped back with 0.1 N NaOH solution. The alkaline aqueous layer was injected into a reversed-phase C_8 column. Lansoprazole was used as an internal standard. The mobile phase consisted of 30% of acetonitrile and 70% of 0.2 M KH_2PO_4, pH 7.0. Recoveries of the analytes and internal standard were >75.48%. The coefficients of variation of intra- and inter-day assay were <5.78 and 4.59% for plasma samples. The detection limit in plasma was 2 ng/ml. The clinical applicability of this assay method was evaluated by determining plasma concentration-time courses of the respective analytes in 24 healthy volunteers after oral administration 40 mg of omeprazole. The present assay is considered to be simple, accurate, economical and suitable for the study of the kinetic disposition of omeprazole in the body.

Key words : Omeprazole, HPLC, Plasma, Human volunteers

INTRODUCTION

Omeprazole, 5-methoxy-2-[{(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl}sulphoxide]-1H-benzimidazole, is a proton pump inhibitor in gastric parietal cells. The drug has greater antisecretory activity than histamine H_2-receptor antagonist and has been widely used in the treatment of reflux oesophagitis, peptic ulcer and Zollinger-Ellison syndrome (Berglindh, *et al.*, 1985; Im, *et al.*, 1985). Many HPLC methods have been reported for determination of omeprazole in plasma and urine. Excluding the column switching method, (Shim, *et al.*, 1994) all methods include the sample treatment of liquid-liquid extraction with dichloromethane (Michael, *et al.*, 1988) or toluene-isoamylalcohol (Macek, *et al.*, 1997) followed by evaporation of the organic phase and reconstitution of the residues with mobile phase.

In this paper, a back extraction method was used for selective determination of omeprazole in plasma. After liquid-liquid extraction with diethylether, the organic layer was back extracted with alkaline solution.

MATERIALS AND METHODS

Materials

All chemicals were used of HPLC analytical grade quality. Omeprazole and lansoprazole were obtained from Myung-In Pharmaceutical Co. Acetonitrile, methanol, potassium dihydrogenphosphate and sodium hydroxide were purchased from Merck (Darmstadt, Germany).

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Chromatographic system

Analyses were performed with the following HPLC system: LC-10AT pump, CTO-10A oven, SPD-10A UV/VIS detector, CBM-10A, SIL-10A auto injector (Shimadzu, Japan).

The separation was performed on a Symmetry C_8 column (150×3.9 mm I.D. 5 μn; Waters associates, MA, U.S.A.). The mobile phase consisted of 30% acetonitrile and 70% of 0.2 M potassium dihydrogenphosphate, pH 7.0 adjusted with 0.2 N NaOH. The flow rate was 1.5 ml/min and column temperature was 35°C. The spectrophotometric detector was operated at 302 nm.

Clinical study design

24 healthy volunteers were recruited for the study. They received a single dose (40 mg) of Myung-In omeprazole in the fasting state. After dosing, serial blood samples were collected at before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 7, 9 hour after the drug administration. Blood samples were immediately transferred to a cooled heparinized tube and spun down in a centrifuge. The

supernatant plasma was transferred to a serum separatory tube and stored at -20°C until analysis.

Sample preparation

Two hundred μl of 0.2 M potassium dihydrogenphosphate buffer (pH 7.0) and 200 μl of internal standard solution (10 μg/ml of lansoprazole) were added to 500 μl of plasma. After omeprazole and internal standard were extracted from plasma with 10 ml of diethylether, the organic phase was transferred to another tube and trapped back with 200 μl of 0.1 N NaOH solution. 20 μl of the alkaline aqueous layer was injected into a reversed-phase C_8 column.

Quantitation

To examine the linearity of the assay, we prepared calibration graph for omeprazole at concentration ranging from 10 ng/ml to 2 μg/ml in plasma. Standard samples were prepared by adding the analytes to drug-free plasma and extracted and analysed as described above. Peak area ratios of each analyte to the internal standard were measured and calibration graph was obtained from the least-squares linear regression.

Pharmacokinetic parameters

Pharmacokinetic parameters were determined by a model-independent method. Area under the plasma concentration-time curve ($AUC_{0\text{-}9hr}$) of omeprazole was calculated by trapezoidal rule. The apparent oral clearance (Cl) of omeprazole was calculated as Cl= dose/AUC. The maximum plasma concentration (Cmax) and the time (Tmax) to reach Cmax for all the analytes were read from the observed data. The mean residence time (MRT) was calculated by dividing area under the first moment versus time curve (AUMC) with AUC (Dipiro, *et al.*, 1988). Elimination half-life ($t_{1/2}$) was calculated from the slope of the terminal phase.

RESULTS AND DISCUSSION

Chromatography

The chromatograms of blank plasma, spiked plasma and volunteer's plasma were shown in Fig. 1. The retention times for omeprazole and internal standard were 5.8 and 13.8 min, respectively. The peaks of these compounds were sharp, symmetrical and well resolved. The peak of blank plasma was very clear and there was no interfering peak at omeprazole and internal standard elution time. Initially, we tried liquid-liquid extraction with dichloromethane, but the extracts were less clear than those with diethylether. Omeprazole has two pKa (3.97, 8.3) as a zwitter ion and is very unstable under acidic condition. Therefore, we attempted the second liquid-liquid extraction at high pH within column resistability. The alkaline aqueous solution yields clean extracts of omeprazole and lansoprazole, an internal standard. At the second extraction, we tried liquid-liquid extraction with 200 μl of 0.01, 0.1, 1 M Na_2CO_3 and 0.01, 0.1 and 1 N NaOH solution, respectively. The maximum efficiency of extraction was obtained with 0.1 N NaOH solution.

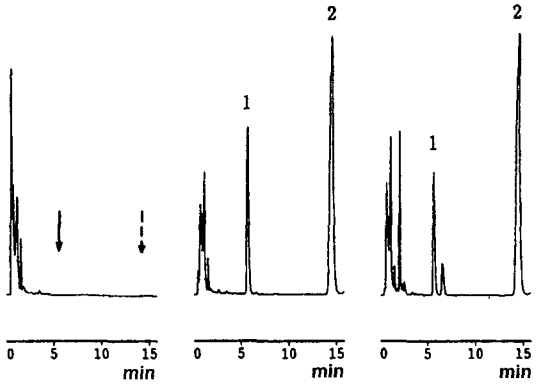

Fig. 1. Chromatograms of omeprazole in human plasma. Left: blank plasma, the arrow and the dotted one indicate omeprazole and lansoprazole elution point, respectively. Middle: spiked plasma with 1 μg/ml of omeprazole and 4 μg/ml of lansoprazole. Right: plasma sample of volunteer No. 10 at 2 hour after oral administration of omeprazole 40 mg. The peak was calculated to be 684 ng/ml of omeprazole. Peaks: 1=omeprazole; 2=lansoprazole.

Linearity and detection limit

The mean equation of calibration curve (Fig. 2) is y (the ratio of peak area of omeprazole to that of internal standard)=0.00038×(concentration of omeprazole)-0.00917 (correlation coefficient, r=0.998). The limit of quantitation in plasma was 2 ng/ml, more sensitive than that (≥10 ng/ml) of the previous method (Mecek, *et al.*, 1997; Shim, *et al.*, 1994).

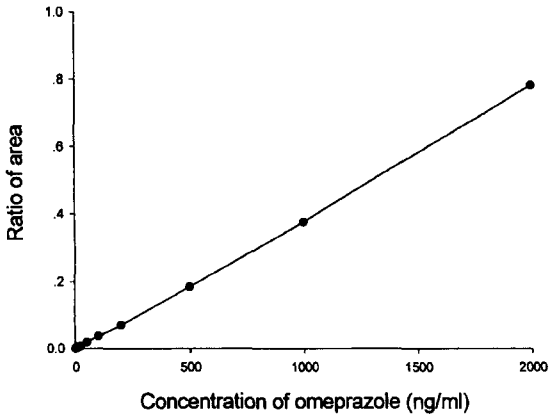

Fig. 2. Calibration of omeprazole in plasma concentration (r=0.998).

Table I. Precision and accuracy of intra-day assay of omeprazole

n	Concentration (ng/ml) Added (ng/ml)	Measured (mean±S.D.)	C. V. (%)	Mean recovery (%)
5	100	97.58±18.40	5.78	75.48±0.28
5	200	189.45±25.35	4.35	79.21±0.26
5	500	512.13±15.14	2.31	81.56±0.19
5	1000	1016.24±10.34	1.05	79.38±0.11
5	2000	2035.32± 5.38	0.86	81.23±0.08

Table II. Precision and accuracy of inter-day assay of omeprazole

n	Concentration (ng/ml) Added (ng/ml)	Measured (mean±S.D.)	C. V. (%)	Mean recovery (%)
6	100	114.56±24.40	4.59	77.79±0.08
6	200	203.83±24.85	2.61	79.11±0.06
6	500	490.94±27.69	1.62	80.56±0.18
6	1000	1065.96±30.54	1.00	75.83±0.06
6	2000	2061.21±51.39	1.53	80.31±0.06

Table III. Pharmacokinetic parameters of omeprazole in 24 human volunteers after oral administration of 2 capsules (40 mg as omeprazole)

Parameters	Omeprazole (mean±S.E.M.)
$AUC_{0\text{-}9hr}$ (μg · hr/ml)	5.09±0.70
Cmax (μg/ml)	1.61±0.17
Tmax (hr)	2.38±0.16
Cl (L/hr)	13.26±2.30
MRT_{9hr}	3.58±0.13
T1/2 (hr)	1.72±0.24

Intra-day and inter-day precision and accuracy

Intra-day and inter-day precision and accuracy of this method is presented in Table I and II, respectively.

Five sets of quality control samples were analysed with calibration samples on one day for the intra-day assay validation. The inter-day assay validations were evaluated by processing a set of calibration and quality control samples on six separate days.

Pharmacokinetics of omeprazole

The major pharmacokinetic parameters ($AUC_{0\text{-}9hr}$, Cmax, Tmax) were calculated from the plasma concentration-time data of each volunteer (Table III). The $AUC_{0\text{-}9hr}$, Cmax and Tmax of omeprazole were 5.09±0.70 μg·hr/ml, 1.61±0.17 μg/ml and 2.38±0.16 hr, respectively. The MRT and $t_{1/2}$ were 3.58±0.13 hr and 1.72±0.24 hr, respectively (Fig. 3).

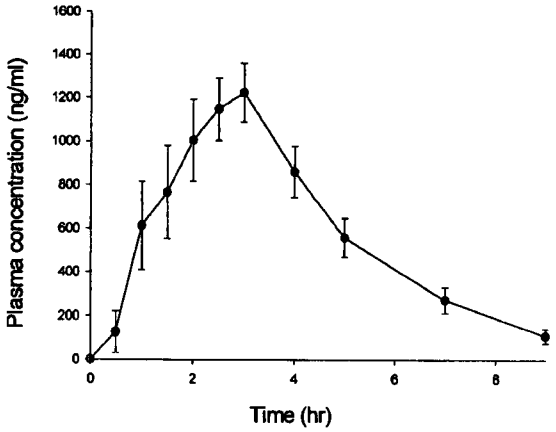

Fig. 3. Time course of plasma omeprazole concentrations after oral administration of 2 capsules (20 mg/capsule) of omeprazole in human volunteers (mean±S.E.M., n=24).

CONCLUSION

This advanced method for determination of omeprazole in plasma is sensitive, simple and economical when compared to earlier methods.

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