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Assay of omeprazole and omeprazole sulfone by semi-microcolumn liquid chromatography with mixed-function precolumn

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Abstract

A column-switching system based on semi-microcolumns was used for direct analysis of omeprazole and omeprazole sulfone in human plasma samples. Plasma samples were injected into a mixed-function (MF Ph-1) column (35 mm×4.6 mm I.D.) to remove proteins and other non-specific peak producing substances from the analyte-containing time zone. The analyte-containing fraction was thereafter transferred to a C-18 semi-microcolumn (250 mm×1.5 mm I.D.) after concentration at the C-18 intermediate column. The absorbance at 302 nm in a ultraviolet (UV) detector was recorded to measure the concentration. The detection limit for omeprazole and omeprazole sulfone in the present method was 10 ng/ml. Interbatch variation (coefficient of variation) of the QC samples spanned less than 10% and intra-batch variation less than 2%. The recovery ratios of omeprazole and omeprazole sulfone were over 98%. The current method can be used as a simpler procedure with similar sensitivity and reproducibility as previously reported methods.

Keywords: Column switching; Omeprazole; Omeprazole sulfone

1. Introduction

Omeprazole, a substituted benzimidazole, is a prototype of H^+/K^+-ATPase inhibitor in gastric parietal cells [1,2]. Its therapeutic potential has been documented as a potent long-acting inhibitor of gastric acid secretion for the treatment of peptic ulcer, refractory gastroesophageal reflux disease, Zollinger-Ellison syndrome and other related hypersecretory conditions [3–5]. Omeprazole undergoes extensive hepatic metabolism [6,7] and its main metabolites are omeprazole sulfone, 5-hydroxyomeprazole and omeprazole sulfide [6,8,9]. Because omeprazole is metabolized extensively by the hepatic cytochrome P450 (CYP) 2C19 [10,11] and exhibits wide inter-individual variability in plasma concentration, its metabolic profile has been investigated in the evaluation of metabolic activity related to CYP 2C19.

We developed the current method to assay the concentrations of omeprazole and omeprazole sulfone without time-consuming extraction and concentration processes because we had to assay a number of plasma samples without sufficient laboratory manpower. Moreover, as we also had to measure the plasma concentration of moclobemide, the

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coadministered drug, the allowed amount of plasma samples per time point was not large enough. To date, most of the chromatographic methods for assaying drugs in human plasma have needed some extraction steps using more than 1 ml of plasma per sample. Under such conditions, we were inclined to establish a simpler omeprazole assay method than those already reported.

To obtain feasible sensitivity, semi-microcolumns or ''microbore''columns, having diameters smaller than 2.0 mm were used. In this article, a column-switching system was applied with semi-microcolumns. The primary separation was performed with a mixed-function column (Capcell Pak MF Ph-1). The Capcell Pak MF Ph-1 column is known to possess long polyoxyethylene chains and phenyl groups on the surface of 80-Å silica, which limits the access of large molecules such as proteins and retains drug molecules longer [12]. Omeprazole and omeprazole sulfone (Fig. 1) were sensitively detected by the column-switching method using the ''concentration'' column (intermediate column) and analysis column in the present report. Though the column-switching method is not a new concept, assaying plasma omeprazole and its sulfone metabolite in this way without an extraction step has not previously been reported.

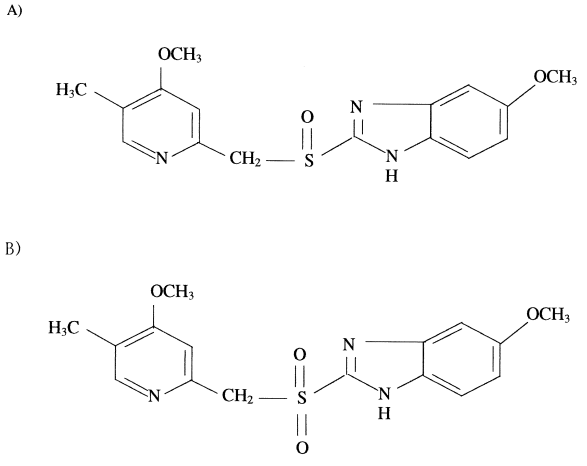

Fig. 1. Chemical structure of omeprazole (A) and omeprazole sulfone (B).

2. Experimental

2.1. HPLC system

This system consisted of a NANOSPACE® series (Shiseido, Tokyo, Japan). Two 2001 inert pumps, a 2003 autosampler, a 2005 UV–Vis detector, a column oven, a switching valve and a degassing unit (Shiseido, Tokyo, Japan) were prepared (Fig. 2). All tubings were made of ''polyetheretherketone'' (PEEK) materials. The chromatogram was recorded with dsChrom® (Donam Instrument, Seongnam, Korea) software on an IBM-compatible Pentium-grade PC.

2.2. Columns and reagents

A precolumn (35 mm length×4.6 mm I.D., particle size 5 μm, Shiseido) packed with MF Ph-1 packing material was used. This material is designed so that the polar protein molecules are quickly eluted from the system and relatively fewer polar molecules are retained. A Capcell Pak C-18 UG 120 (250 mm×1.5 mm I.D., particle size 5 μm, Shiseido) column was used as a main separation column after the concentration process in the intermediate column (Capcell Pak C-18 UG 120, 35 mm×2.0 mm I.D., particle size 5 μm, Shiseido). Omeprazole and omeprazole sulfone were a kind gift from Astra (Mölndal, Sweden). Acetonitrile of HPLC grade was purchased from Fisher Scientific (Seoul, Korea) and potassium phosphate and phosphoric acid to mix 50 m*M* buffer (pH 7.0) were purchased from Kanto (Tokyo, Japan). The composition of mobile phase for the precolumn (MP1) was buffer:acetonitrile 90:10, v/v, and that for the analytic column (MP2) was buffer:acetonitrile 60:40, v/v. All three columns were kept under 40°C throughout the analytic process.

2.3. Sample collection

The current method was developed to measure samples from a pharmacokinetic study performed at the Clinical Trial Center, Gachon Medical School. The protocol was approved by the IRB of Gachon Medical School Ghil Hospital. Participants came to

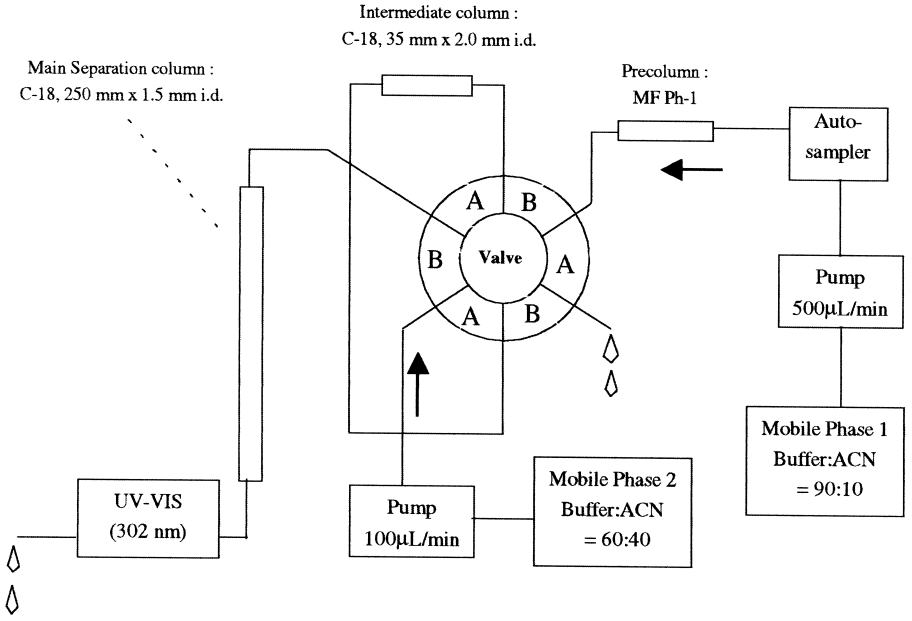

Fig. 2. Schematic diagram of the column-switching HPLC system. Valve status A: mobile phase flows around the A section. Valve status B: mobile phase flows around the B section. The intermediate column's direction of flow and its mobile phase's composition alternate with the valve status (A: mobile phase 1, left to right; B: mobile phase 2, right to left).

the study site after overnight fasting. Each participant received a p.o. dose of 2×20 mg omeprazole capsule (Losec®, Yuhan, Seoul, Korea) together with 200 ml of water. Venous blood samples (10 ml each) were collected into heparinized tubes at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12 and 24 h post dose. All samples were spun immediately after collection, and the plasma aliquots were stored at −80°C until assay.

2.4. Sample preparation.

Stock solutions of omeprazole and omeprazole sulfone were prepared at a concentration of 1 mg/ml in methanol and stored in the dark at −80°C. Solutions of various concentrations were prepared by serial dilution.

Plasma samples were thawed just before use and 200 μl of each sample was diluted with the same volume of 50 m*M* potassium phosphate buffer (pH 7.0). After centrifugation at 3000 rpm for 1 min, the supernatant was filtered by a 0.22 nm-sieved syringe filter (Millipore, Bedford, USA) to protect the primary separation column (MF Ph-1 column). Filtrates were transferred into the vials for the autosampler and 160 μl of each filtrate was injected.

2.5. Total column-switching procedure

The switching procedure was as follows.

(1) Determination of switching time zone. First, we applied concentrated stocks and then standard plasma samples into the precolumn, which was connected to a UV–Vis detector. To attain an appropriate time zone, it was necessary to change the composition and pH of MP1 through trial and error.

(2) Once the time zone was determined, the valve system was conditioned to alternate the flow direction (status B during the switching interval and then back to A) and UV–Vis detector was connected to the tube from the analytic column.

(3) Determination of mobile phase (MP2) condition for analysis. The process of finding the

optimal mobile phase condition for the analytic column was performed in the same way as the conventional HPLC conditioning process.

(4) Summary of established work flow:

- A sample is injected by the autosampler and the elute from a precolumn is discarded until the switching time zone begins (valve status A).
- During the switching time zone, the elute is directed to an intermediate column, where target substances are concentrated (valve status B).
- After the end of the switching time zone, the substances which were concentrated at the intermediate column are transported to an analytic column by MP2 (valve status A), and then to UV–Vis detector. The flow direction and mobile phase composition (MP1 or MP2) for the intermediate column is alternated by the valve status.
- When a cycle of analysis is complete, the above procedure repeats itself for the next sample.

3. Results

3.1. Assay condition

The results of a conditioning experiment to determine the switching time zone for omeprazole and omeprazole sulfone are shown in Fig. 3. Peaks of omeprazole (1 μg/ml) and omeprazole sulfone (1 μg/ml) are shown separated from the protein peaks on the MF Ph-1 precolumn. The mobile phase (MP1, 50 m*M* potassium phosphate buffer (pH 7.0):acetonitrile 90:10, v/v) was eluted with a flow-rate of 500 μl/min in this primary separation. The chromatogram in Fig. 3 was obtained by connecting the end of the MF Ph-1 column directly to the UV–Vis detector. Non-specific peaks from plasma proteins and/or other large polar molecules were recorded within 2 min, while those of omeprazole and omeprazole sulfone were in the 8–10-min time zone. But their retention times gradually shortened and shapes got blunter with the ''senescence'' of the precolumn. So the switching time zone (the valve is in B status during this interval, otherwise A) was initially set as 7–10 min after injection to transfer the analyte-containing elute to the intermediate column for concentration. This time zone has periodically been changed according to the RT change in the primary separation process. Mobile phases tested with 0 or 5% acetonitrile were found inappropriate due to retention times longer than 20 min. Final chromatograms were obtained from the main separation column (C-18 UG 120, 250×1.5 mm, Shiseido) by its mobile phase (MP2, 50 m*M* potassium phosphate buffer (pH 7.0):acetonitrile 60:40, v/v) at a flow-rate of 100 μl/min (Fig. 4). The peak of omeprazole (RT: 22.3 min, 12.3 min after the end

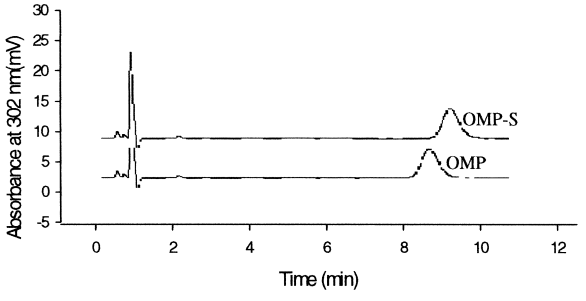

Fig. 3. Chromatogram of omeprazole (OMP: RT 8.5 min) and omeprazole sulfone (OMP-S: RT 9.2 min) from an MF Ph-1 column. Concentration of the samples injected was 1 μg/ml. Non-specific protein peaks separated from the peaks of the analytes are seen within 2 min after injection. As the MF Ph-1 column was used for primary separation, the peaks are not as sharp as those in Fig. 4.

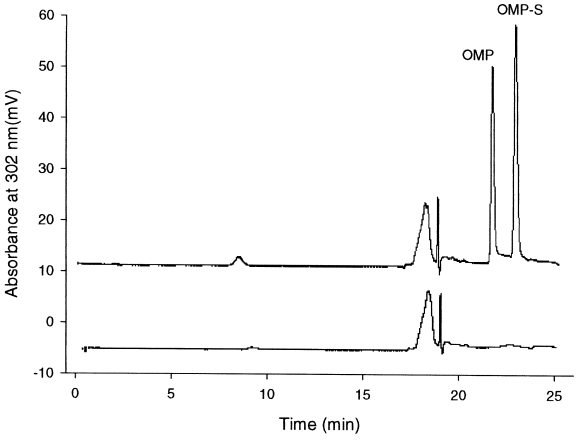

Fig. 4. Chromatogram of control plasma (lower) and 6-h sample (upper) in a volunteer after ingestion of 40 mg omeprazole. The peak of omeprazole (148 ng/ml) is followed by that of omeprazole sulfone (199 ng/ml) 20–25 min after injection, but the retention times as the switching time interval was adjusted.

of switching) was followed by its sulfone metabolite (RT: 23.6 min, 13.6 min after the end of switching). The revised RTs starting at the end of the switching interval were constant throughout the assay.

3.2. Calibration curve and detection limit

Linear calibration curves were checked for both anlytes with their standard plasmas of 0, 10, 50, 100, 200, 1000 and 2000 ng/ml (correlation coefficient $r>0.999$). The detection limits of omeprazole and omeprazole sulfone were 10 ng/ml with signal-to-noise ratio of 5. From the five calibration lines without weighting, the mean and coefficient of variation (C.V.) of slope and intersection were y (peak area)$=2.099\times10^4$ (C.V.$=9.2\%)\times x$ (ng/ml)-7.482×10^2 (C.V.$=65.7\%$) for omeprazole. For omeprazole sulfone, they were y (peak area)$=2.105\times10^4$ (C.V.$=8.5\%)\times x$ (ng/ml)-0.317×10^3 (C.V.$=78.4\%$).

3.3. Reproducibility

The variations in the estimated concentration of standard plasma samples of omeprazole used for quality assurance are tabulated as inter-batch variation (Table 1). We considered the samples measured using one bottleful of each mobile phase (~1 l) as one batch. The number of samples measured per batch was ~50–100.

As the time needed for the completion of assay of one batch was 20–40 h, and as the laboratory work was done on a batch basis, and not a day basis, we used the terms intra-batch and inter-batch instead of intra-day and inter-day.

Intrabatch variations were evaluated by a consecutive assay of 20 standard plasma samples per concentration level in the middle of the assay period (Table 1). The inter-batch variations were obtained from the QC samples which had been inserted every 13 actual samples. The mean QC peak areas of each of five batches were compared to estimate the inter-batch variation.

Table 1
Reproducibility (% coefficient of variation) of omeprazole concentration assay

Concentration, ng/ml	Omeprazole	
	Inter-batch ($n=5$[a])	Intra-batch ($n=20$)
100	6.2	1.5
20	9.1	1.7

[a] A total of five mean QC samples' peak areas obtained from five batches.

Table 2
The recovery of omeprazole and omeprazole sulfone in standard plasma samples[a]

	Omeprazole	Omeprazole sulfone
100 ng/ml	99.8±5.9	99.2±1.5
20 ng/ml	99.1±2.1	98.0±0.5

[a] A total of 20 standard plasma samples were used to test the recovery for each concentration.

3.4. Recovery

Aqueous buffer solutions containing 20- and 100-ng/ml concentrations of omeprazole and omeprazole sulfone (20 samples each) were compared with spiked plasma standard samples of corresponding concentrations (20 samples each) to estimate the recovery ratios. The ratio (%) of standard plasma peak areas divided by that of aqueous buffer was considered to be the recovery ratio. Greater than 98% recovery means that the analytes bound to plasma proteins were almost completely separated passing through the precolumn (Table 2).

4. Discussion

Typical plasma concentration curves in a volunteer are shown in Fig. 5. Though the lowest level of standard samples used for calibration was 10 ng/ml, the detection of which was our first objective, we could detect concentrations as low as 3–4 ng/ml with peak-to-noise ratio greater than 5 in some samples.

As plasma samples are directly injected, denatured plasma protein may be precipitated if the mobile phase pH is extremely acidic or basic. Additionally, as omeprazole is known to be chemically unstable in acidic conditions [13], the final pH of both mobile phases was chosen to be not less than 7.0.

The proportion of acetonitrile in MP2 was also

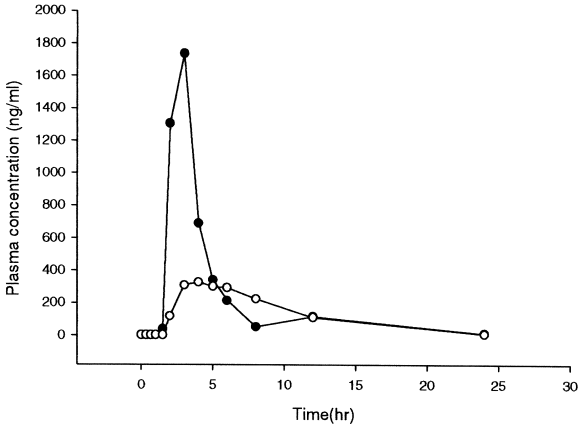

Fig. 5. Pharmacokinetic pattern of a volunteer orally administered 40 mg omeprazole. Closed circle: omeprazole; open circle: omeprazole sulfone.

chosen after some trial and error to allow the omeprazole and omeprazole sulfone peaks to be eluted behind the non-specific blunt peak appearing within 10 min after the end of the switching period. As their RTs were inversely correlated with the acetonitrile proportion in MP2, acetonitrile lower than 40% in MP2 would result in a total analysis time greater than 30 min.

The intermediate column we used for the current research consisted of the same packing material (C-18, particle size 5 μm) as that of the analytic column. The ''concentration'' occurring at the intermediate column (valve status B) relies upon the condition of MP1. Its relatively lower proportion of acetonitrile keeps the omeprazole and its sulfone metabolite retained as a layer near the proximal end (right end in Fig. 2) of the intermediate column by minimizing their elution to the opposite end of the column. Thereafter the concentrated substances are transferred to the analytic column (valve status returns to A again) by MP2 of higher acetonitrile composition. Separation of the two substances occurs in the analytic column as in other HPLC methods and finally we obtain the separated peaks sharpened by the intermediated column.

The main merits of a column-switching method are saving of time and labor, dispensability of internal standard and possibility of using a smaller sample volume. It is also of note that this method allows satisfactory quality without expert experience with HPLC. A drawback we experienced was the comparatively short life-span of precolumns, the MF Ph-1 columns. We had to use three MF Ph-1 columns to analyze ~600 human plasma samples obtained from a pharmacokinetic study in normal volunteers. Along with the waning of the precolumn's separation function, the RTs of the primarily separated peaks decreased accordingly. After injection of more than 80 samples into a precolumn, we had to check the RTs carefully approximately every 30 samples to decide whether to adjust the switching time zone. The RT of hydroxy omeprazole, another important metabolite, on a MF Ph-1 column was too short to be eluted together with omeprazole and its sulfone metabolite. We are planning to develop a new method using a double valve system and gradient elution to detect these three substances simultaneously.

There are several reports on HPLC methods assaying omeprazole and its metabolites. Lagersröm et al. [14] measured omeprazole, omeprazole sulfone and hydroxy omeprazole (LOD 6.9 ng/ml). They used an extraction method using methylene chloride with internal standard. The repeatability (C.V.) for 1 μg/ml and 100 ng/ml omeprazole was 1.1 and 3.5%, respectively. For 1 μg/ml omeprazole sulfone, it was 1.5% and the absolute recovery was ~99%. Kobayashi et al. reported an assay method for omeprazole and its metabolites with LOD of 10 ng/ml, intra-assay and inter-assay variation (C.V.) of 9 and 6%, respectively and with recovery of ~93%. Macek et al. [15] showed the precision at 9.7 ng/ml (at LOD) to be 12.1% (C.V.). and inaccuracy to be −3.1% for six calibration curves for omeprazole alone. Their intra-day and inter-day precisions (C.V.) were 3.0–7.6 and 3.0–9.2% in a 20–1000-ng/ml range for six samples per stock. The inaccuracies of intra-day and inter-day were −3.3 to +5.6 and −1.2 to +0.4%, respectively, for the same samples. Amantea et al. [16] showed that the inter-day variability/accuracy was 5.4–14.1% for 7.5–125 ng/ml. The reproducibility estimated from the calibration standard was C.V. 3.0–6.8% in the 5.0–200-ng/ml range. The LODs were 5 ng/ml for omeprazole and 10 ng/ml for omeprazole sulfone. All the reports cited above relied upon sample extraction steps using organic solvents such as dichloromethane or toluene-isoamyl alcohol. As an extraction step was involved, the use of internal standards, commercially available or not, was also indispensable.

Considering the results above, the reported method can be used as a simpler procedure with similar sensitivity and reproducibility. Though we did not report the inaccuracy, it is expected to be feasible when we look at the recovery ratios over 98% in our data.

5. Conclusion

We developed a method for simple analysis of omeprazole and omeprazole sulfone in human plasma based on a semi-microcolumn HPLC and an MF Ph-1 precolumn. Detection limits for the two substances were ~10 ng/ml. Both the system's durability and reproducibility were found acceptable for its application to mass assay necessary for various pharmacokinetic studies.

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