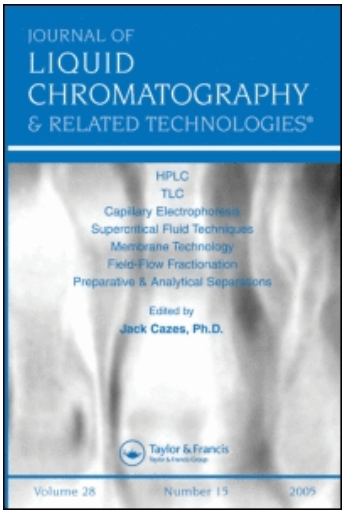

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Simultaneous Determination of Omeprazole and its Metabolites (5'-Hydroxyomeprazole and Omeprazole Sulfone) in Human Plasma by Liquid Chromatography-Tandem Mass Spectrometry

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Simultaneous Determination of Omeprazole and its Metabolites (5′-Hydroxyomeprazole and Omeprazole Sulfone) in Human Plasma by Liquid Chromatography-Tandem Mass Spectrometry

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Abstract: A method for the simultaneous determination of omeprazole (OME) and its two metabolites (5′-hydroxyomeprazole (H-OME) and omeprazole sulfone (OME-S)) in human plasma is described. OME and its metabolites were extracted from plasma with phosphate buffer (pH 7.4) by using solid phase extraction system, Oasis HLB μ Elution plates, and the eluate was directly injected into LC-MS/MS system. The analytes were chromatographed with a reversed-phase column, XTerra MS C18 column (150 × 4.6 mm) and an HPLC mobile phase which consisted of a mixture of acetonitrile/water (45/55, v/v) containing 10 mM ammonium hydroxide (pH 8.0). A Sciex API 4000 tandem mass spectrometer equipped with a heated nebulizer atmospheric pressure chemical ionization interface was used as a detector and was operated in the positive ion mode. Multiple reaction monitorings using the precursor product ion combinations of m/z 346.2 → 198.2, 362.0 → 214.0, 362.0 → 150.0, and 349.2 → 201.0 were used to detect OME, H-OME, OME-S, and internal standard (OME-d_3), respectively. The method was validated in the concentration range of 1–1000 ng/mL plasma with adequate assay precision, accuracy, and reproducibility. This sensitive and selective method with a rapid and simple sample preparation procedure was applied to a clinical pharmacokinetic study of OME.

Keywords: Omeprazole, 5′-Hydroxyomeprazole, Omeprazole sulfone

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INTRODUCTION

Omeprazole (OME, Fig. 1), which is a proton pump inhibitor and a substituted benzimidazole (5-methoxy-2-[[(4-methoxy-3, 5-dimethyl-2-pyridinyl) methyl] sulfinyl]-1H-benzimidazole), suppress acid secretion by the parietal gastric mucosa cell and is used in the treatment of gastric acid related disorders.[1,2] OME is mainly metabolized to 5′-hydroxyomeprazole (H-OME) by the genetic polymorphism enzyme, CYP2C19.[3,4] OME is also metabolized to omeprazole sulfone (OME-S) by CYP3A4, and this metabolic pathway is predominant in the poor metabolizers for CYP2C19.[5]

It is reported that OME can be used as a probe drug for pharmacogenetic studies involving the evaluation of phenotypes of CYP2C19 and CYP3A4.[6] In addition, OME is also a useful probe drug for evaluating the inhibitory effect of CYP inhibitors in different CYP2C19 genotypes.[7] Thus, a simple and sensitive assay method for the simultaneous determination of OME, H-OME, and OME-S will efficiently help to conduct both the metabolic phenotyping and drug-drug interaction studies.

Several assay methods by using high performance liquid chromatography (HPLC) for OME, H-OME, and OME-S in human plasma had already been reported. Among them, the method introduced by Lagerstrom and Persson, which uses two different analytical columns, one is a normal phase column for analysis of OME and OME-S, and the other is a reversed-phase column for H-OME analysis.[8] In another HPLC method, 1 mL aliquot of plasma

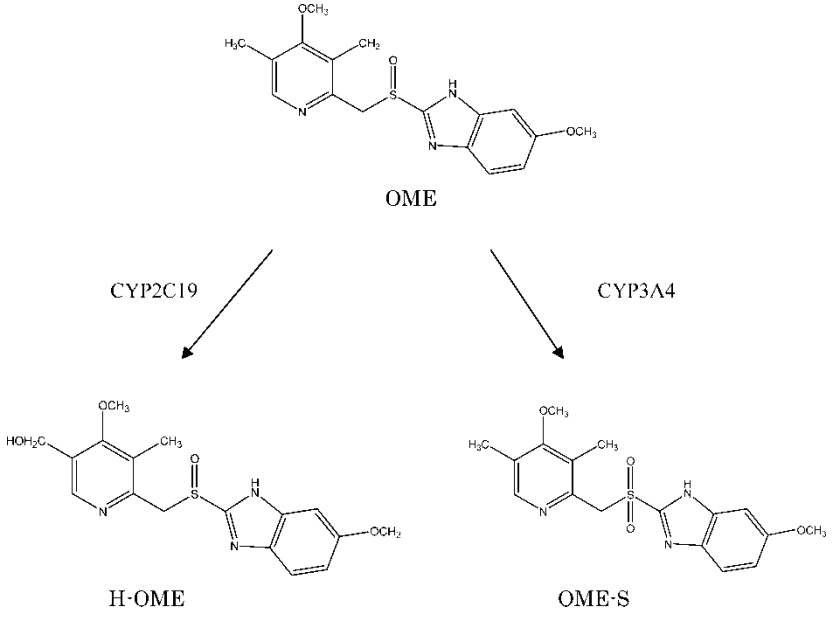

Figure 1. Chemical structures of OME, H-OME and OME-S.

was applied to a liquid/liquid extraction procedure and the minimum determinable concentration was 10 ng/mL for OME, H-OME, and OME-S.[9] Gonzalez and coworkers proposed an HPLC method for determining OME and its metabolites after dichloromethane-ether (95/5, v/v) extraction of 0.5 mL plasma, and set up the lowest limits of quantification (LLOQ) of 60 ng/mL for each analyte.[6] These HPLC assay methods using liquid/liquid extraction procedures are labor intensive, and their sensitivities are not sufficient for application to the clinical pharmacokinetic study of OME. HPLC analysis, after a solid phase extraction (SPE) using a polymeric sorbent based cartridge was also reported, and this paper only included the validation data for OME, since the purpose of this method was for the determination of OME and its metabolites, and for the quantification of OME only.[10]

Recently, a method based on micellar electrokinetic capillary chromatography with UV detection for the simultaneous determinations of OME, H-OME, and OME-S has been reported, and the calibration curves were set up for all analytes in 0.08–2.0 μg/mL concentration range. However, the high sensitive assay method is needed, since C_{max} of OME-S was reported to be approximately 90 ng/mL after an oral administration of 20 mg OME in healthy male volunteers.[9,11] In addition, applications of mass spectrometry (MS) for their detection were also reported. Woolf and Matuszewski showed an LC-MS/MS method for the simultaneous determination of OME and H-OME,[12] and the high sensitive assay method using LC-MS/MS was reported with LLOQ of 0.4 ng/mL for OME and H-OME.[13] However OME-S was not determined with both methods, since Woolf's method was only used to determine CYP2C19 phenotypes, and the other method included the analysis of midazolam and its metabolite for determining CYP3A4 phenotypes, as well as CYP2C19.

Recently, another HPLC-electrospray MS method with liquid-liquid extraction was reported with LLOQ of 5 ng/mL for H-OME and 10 ng/mL for both OME and OME-S,[14] however, liquid-liquid extraction using dichloromethane:acetonitrile (9:1, v/v) involved the use of toxic halogenated solvents. Thus, an assay method with simple sample preparation and high sensitivity will be needed in order to fill these methodological gaps.

In this paper, we describe a sensitive, selective, and reliable LC-MS/MS method with a rapid and simple sample preparation for the simultaneous determination of OME, H-OME, and OME-S in human plasma. This method was applied to the clinical pharmacokinetic studies of OME.

EXPERIMENTAL

Reagents and Materials

OME (Fig. 1) was obtained from Sigma-Aldrich (St Louis, MO, USA). OME-S (Fig. 1) and OME-d_3 were purchased from SynFine Research, Inc. (Ontario,

Canada). H-OME (Fig. 1) was kindly provided by AstraZeneca (Molndal, Sweden). Oasis HLB μ elution plate was purchased from Waters (Milford, MA, USA). Methanol and acetonitrile of HPLC grade were obtained from Junsei Chemical Co., Ltd. (Tokyo, Japan). Formic acid and triethylamine were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Phosphate buffer powder and ammonium hydroxide (4.98 N) were obtained from Sigma-Aldrich (St Louis, MO, USA). Drug free human plasma was purchased from Cosmo Bio Co., Ltd. (Memphis, Tenn, USA). All other reagents and solvents were of analytical grade.

Chromatographic Conditions

An HPLC system consisted of a quaternary and a binary pump, a degasser, and a well plate sampler (Hewlett-Packard Model 1100 series: Yokokawa Analytical Systems, Tokyo, Japan). The HPLC mobile phase was a mixture of acetonitrile/10 mM ammonium hydroxide (pH 8.0) (45/55, v/v), and flow rate at 0.8 mL/min. The apparent pH of the mobile phase was adjusted to 8.0 with formic acid. The analytical column, a XTerra® MS C_{18} column (5 μm, 4.6 $\times$ 150 mm, Waters, USA), was operated at room temperature. The guard column, a Capcell C_{18} column (5 μm, 4.0 $\times$ 10 mm, Shiseido, Tokyo, Japan), was also connected and it was washed by the mixture of acetonitrile/10 mM ammonium hydroxide (pH 8.0) (90/10, v/v), using switching valves after passing the analytes through it. The sample injection volume was 10 μL and the total run time was 5 min.

Mass Spectrometric Conditions

An MSD SCIEX API 4000 LC-MS/MS system equipped with a heated nebulizer atmospheric pressure chemical ionization (APCI) interface was operated in the positive ion mode (Applied Biosysyems, Toronto, Canada). Using multiple reaction monitoring (MRM), the transitions m/z 346.2 $\rightarrow$ 198.2, 362.0 $\rightarrow$ 214.0, 362.0 $\rightarrow$ 150.0, and 349.2 $\rightarrow$ 201.0 were used for quantitation of OME, H-OME, OME-S, and internal standard (IS, OME-d_3), respectively. Optimized MS parameters were as follows: curtain gas, gas 1 and gas 2 were 12, 20, and 31 psig, respectively; collision gas and nebulizer current was set to 4 and 3, respectively; heated nebulizer probe at 400°C. The declustering potential, entrance potential, collision energy, collision cell exit potential, and dwell time were set to 51, 5, 22, 6 V, and 100 msec for OME, 51, 5, 22, 14 V, and 100 msec for H-OME, 71, 10, 31, 5 V, and 100 msec for OME-S, and 71, 10, 17, 14 V, and 100 msec for IS, respectively. Applied Biosystems/MDS SCIEX Analyst software (Version 1.3.1) was used for data acquisition and processing.

Sample Preparation Procedure

Frozen plasma samples were thawed at ambient temperature and centrifuged at 1,800 × *g*, 4°C for 10 min. An aliquot of the plasma (200 μL) was placed in a disposable tube and mixed with 200 μL of 0.5 M phosphate buffer (pH 7.4), 50 μL of IS solution (400 ng/mL), and 50 μL of methanol/water (50/50, v/v) including 0.05% triethylamine. The mixture was centrifuged at 8,000 × *g*, 4°C for 5 min. Each well of Oasis HLB μ elution plate was preconditioned with 300 μL of methanol followed with 300 μL of water using a vacuum manifold (Waters, USA). The centrifuged mixture was applied to each well of the plate under vacuum aspiration, and it was washed with 500 μL of distilled water and then 300 μL of methanol/water (15/85, v/v). Finally, 300 μL of acetonitrile/10 mM ammonium hydroxide (pH 8.0) (45/55, v/v) was added to the well in order to elute the analytes. A portion of each eluent (10 μL) was directly injected into the LC-MS/MS system for analysis.

Method Validation

The selectivity between channels used for monitoring all analytes including the internal standard was evaluated by the LC-MS/MS analysis of plasma sample containing the individual analyte separately, at the concentrations of 1000 ng/mL for OME and its metabolites, and 100 ng/mL for internal standard, and monitoring the response in the other MS/MS channel used for quantification. The specificity was also evaluated using extracts from the control plasma of six individuals. The matrix effect was investigated by comparing the peak area of each analyte spiked into extracts from the control plasma with that in standard solution at concentrations of 2, 50, and 500 ng/mL. The recovery of the analytes after SPE was evaluated by comparing the peak area of each analyte spiked into extracts from control plasma with that in spiked plasma samples at concentrations of 2, 50, and 500 ng/mL.

The samples of the calibration curve were prepared by adding 50 μL of a mixture of OME and its metabolites solution, 200 μL of 0.5 M phosphate buffer (pH 7.4), and 50 μL of the internal standard solution (400 ng/mL) to 200 μL of plasma in a range of final concentrations from 1 to 1000 ng/mL plasma. The calibration curve was constructed by weighted ($1/x^2$) least squares liner regression analysis of the peak area ratios of each analyte to internal standard at the concentrations. The intra-day precision and accuracy were evaluated by analyzing the spiked samples in six replicates at 1, 2, 5, 10, 50, 100, 500, and 1000 ng/mL. The inter-day precision and accuracy were evaluated by analyzing the spiked samples in a replicate on 3 days, at 2, 50, and 500 ng/mL. The precision was evaluated by the coefficient of

variation (CV), while the accuracy was estimated by comparing the nominal concentration with the calculated concentration.

Stability

The stabilities of each analyte in eluate from the extraction plate stored in the autosampler at 4°C were examined in six replicates at 2, 50, and 500 ng/mL. The stabilities of OME, H-OME, and OME-S in human plasma at room temperature were investigated in six replicates at 2.5, 75, and 750 ng/mL. The solution stabilities of each analyte containing internal standard in a mixture of methanol/water (50/50, v/v) with 0.05% triethylamine were tested at room temperature and at 4°C in the concentrations of 8, 200, and 2000 ng/mL for OME, H-OME, and OME-S, and in the concentration of 400 ng/mL for omeprazole-d_3, respectively.

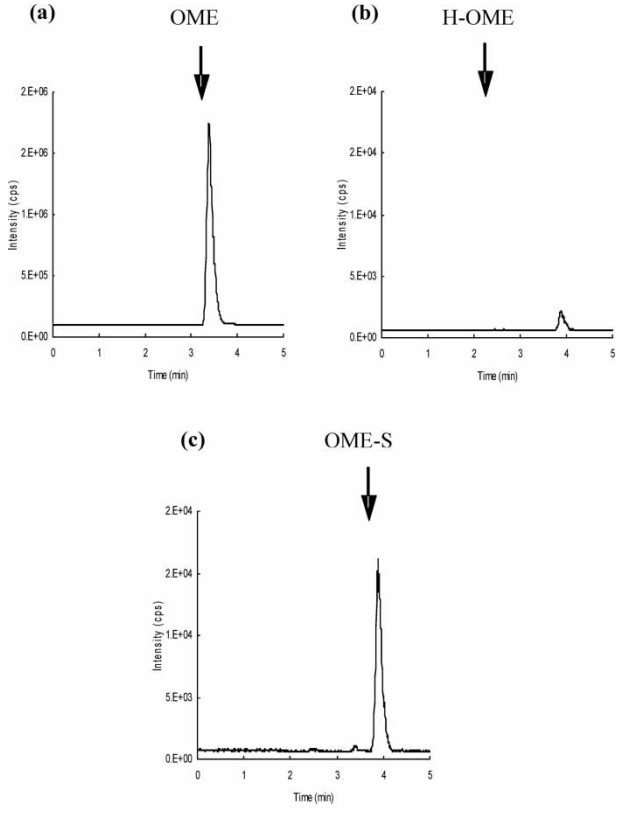

Figure 2. MRM chromatograms of human plasma spiked with 1000 ng/mL of OME monitored at 3 channels after evaporation. (a): m/z for OME, (b): m/z for H-OME, (c): m/z for OME-S.

Pharmacokinetic Study

The method was applied to evaluate the pharmacokinetic of OME and its metabolites. Twelve Japanese healthy male volunteers received a single oral dose of 20 mg OME. Blood samples were collected pre-dose and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, and 12 hours post-dose. Plasma was separated immediately and kept at $-80^{\circ}C$ until analysis. The pharmacokinetic analysis was carried out by WinNonlin (Pharsight Co., CA, version 4.1). The elimination rate constant β was calculated by least square regression of the log plasma concentration time curve of the terminal concentration time points. The elimination half-life ($t_{1/2}$) was determined by $0.693/\beta$. The area under the plasma concentration versus time curve ($AUC_{(0\text{-}12hr)}$) was calculated by the trapezoidal rule from zero to 12 hr after dose administration. The peak concentration (C_{max}) and the time to peak concentration (T_{max}) were obtained directly from the plasma concentration-time curves.

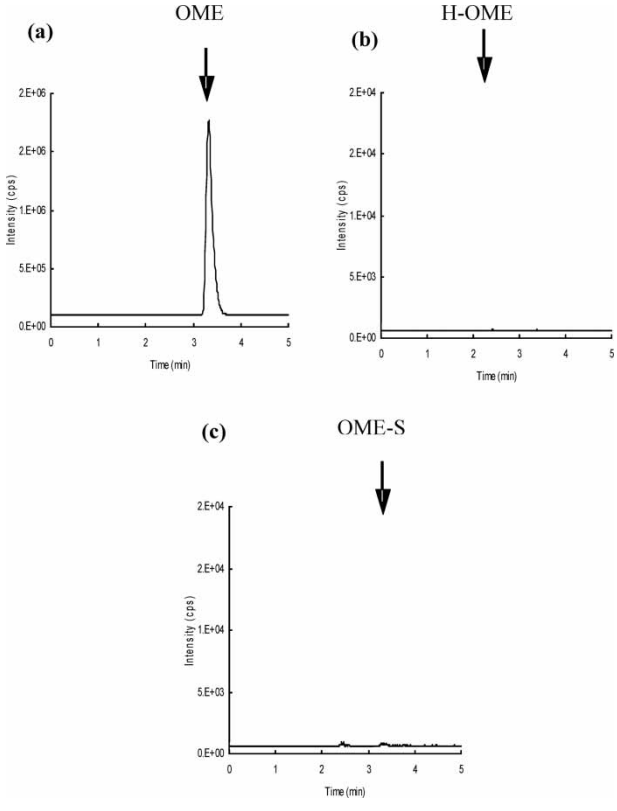

Figure 3. MRM chromatograms of human plasma spiked with 1000 ng/mL of OME monitored at 3 channels after direct injection. (a): m/z for OME, (b): m/z for H-OME, (c): m/z for OME-S.

RESULTS AND DISCUSSION

Selection of Direct Injection

In this paper, we have applied a solid phase extraction (SPE) for the sample preparation, since SPE was generally simpler and faster than liquid-liquid extractions. However, an artificial formation of OME-S was found during the evaporation of the eluate from SPE and the peak area of OME-S accounted for about 1% of that of OME, indicating interference with its quantitation for low concentration levels (Fig. 2 (c)). As shown in Figs. 3–5, after using the HLB μ elution 96 well plate with direct injection to LS-MS/MS for sample preparation, the artificial generation of OME-S disappeared, and there were no significant interfering peaks detected at the mass transition and the retention time of each analyte and IS in MRM chromatograms of human

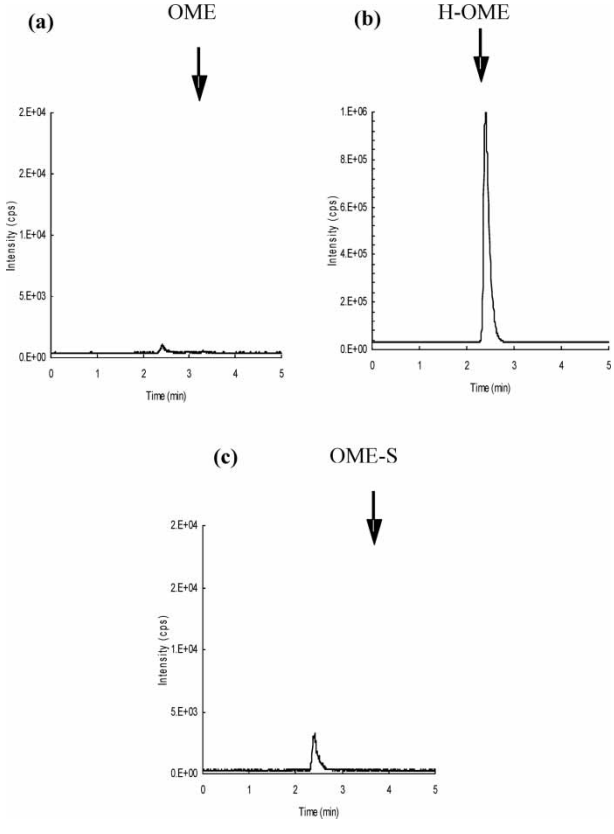

Figure 4. MRM chromatograms of human plasma spiked with 1000 ng/mL of H-OME monitored at 3 channels after direct injection. (a): m/z for OME, (b): m/z for H-OME, (c): m/z for OME-S.

plasma spiked with OME (1000 ng/mL) (Fig. 3), H-OME (1000 ng/mL) (Fig. 4), or OME-S (1000 ng/mL) (Fig. 5), separately. Therefore, direct injection was chosen to prevent this phenomenon.

Method Validation

Figure 6 shows chromatograms of the extracted plasma samples spiked with OME, H-OME, OME-S, or IS. The concentrations of each analyte and IS were 1 and 100 ng/mL, respectively. No endogenous interfering peaks were observed in the chromatograms of six individual control plasmas. The retention times for OME, H-OME, OME-S, and IS was 3.4, 2.4, 3.8, and 3.3 min, respectively, and the total run time was 5 min. This short total run time is considered to be desirable for increasing sample throughput. On the

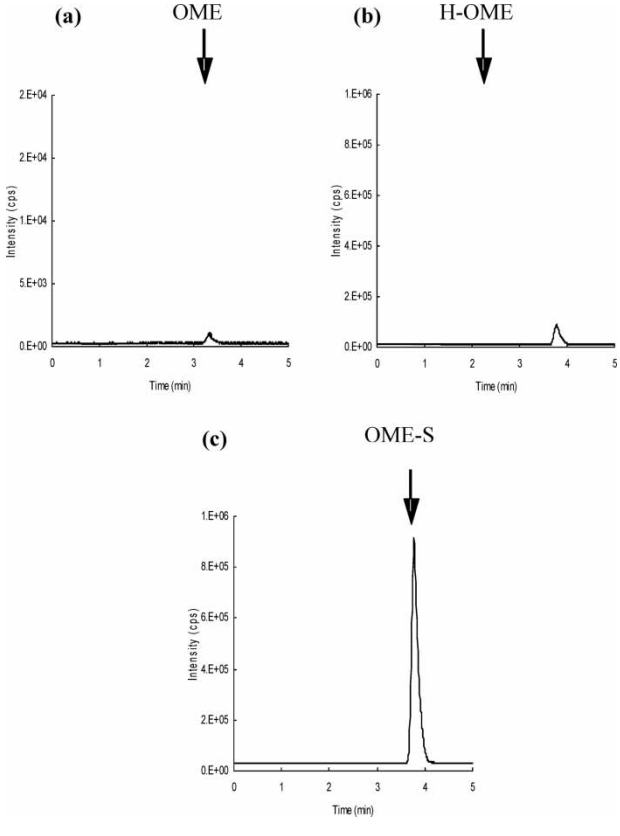

Figure 5. MRM chromatograms of human plasma spiked with 1000 ng/mL of OME-S monitored at 3 channels after direct injection. (a): m/z for OME, (b): m/z for H-OME, (c): m/z for OME-S.

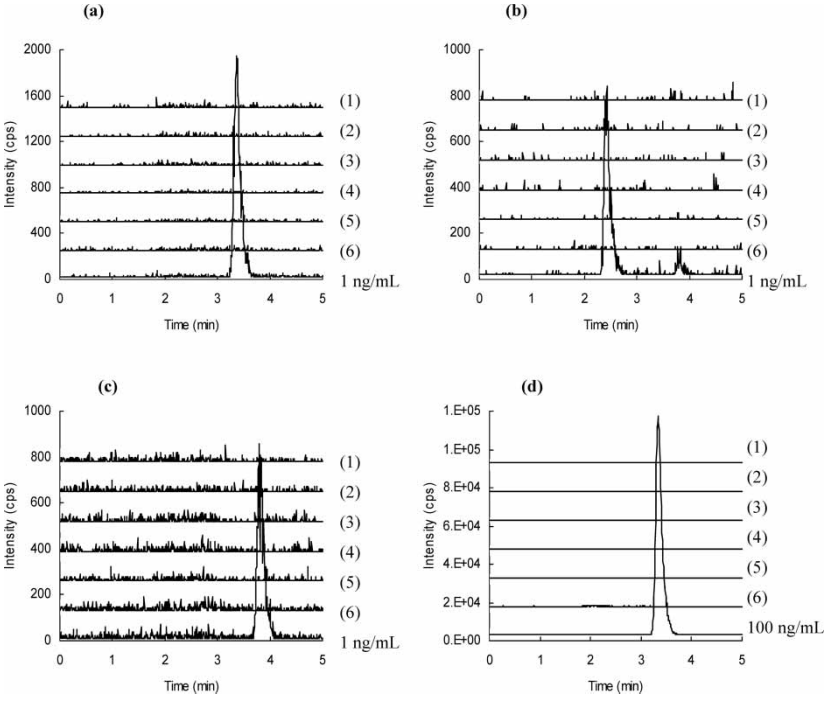

Figure 6. MRM chromatograms of OME (a), H-OME (b) and OME-S (c) and IS (d) in control plasma spiked with 1 ng/mL of each analyte or 100 ng/mL of IS and those of six individual control plasma (1–6).

other hand, formation of a mono-hydroxylated metabolite, 3-hydroxyomeprazole (3-H-OME), was reported to be a minor metabolite mediated by CYP3A4 in vitro.[15] Thus, it is possible that H-OME is not completely separated from 3-H-OME and determination of H-OME is affected in this method. However, there is no published paper regarding any assay method for separation of H-OME and 3-H-OME and also plasma concentrations of 3-H-OME in human in vivo. Therefore, it is suggested that 3-H-OME in human plasma might be negligible after administration of OME, and determined concentrations of H-OME by this method would be practically appropriate. Further studies on the assay method and pharmacokinetics in humans for 3-H-OME would be needed. The ions of each analyte and IS extracted from the plasma samples spiked with OME, H-OME, OME-S, or IS were not suppressed, indicating that no considerable matrix effects in the LC-MS/MS analysis were observed for all analytes and IS. The recoveries of each analyte from plasma following SPE procedures at 2, 50, and 500 ng/mL were found to be 80.2% − 86.3%, 84.2% − 92.2%, and 56.8% − 67.3% for OME, H-OME, and OME-S, respectively.

The calibration curves for OME, H-OME, and OME-S in plasma were linear in the concentration range of 1 to 1000 ng/mL, and the correlation coefficient was greater than 0.998 for each calibration curve. The lower limit of quantification, defined as the lowest concentration at which both precision and accuracy were less than or equal to 15%, was 1 ng/mL for all analytes. The mean accuracy and precision for the intra- and inter-day assay of the analyses are shown in Tables 1 and 2. The values of intra-day precision over the range of the standard curve were below 2.5%, 5.3%, and 6.5% for OME, H-OME, and OME-S, respectively. The accuracy ranged from −5.8% to 4.4%, −6.4% to 4.0%, and −3.4% to 4.8% for OME, H-OME, and OME-S, respectively. The inter-day precision values for the

Table 1. Intra-day precision and accuracy for the determination of OME and its metabolites (H-OME and OME-S) in human plasma

Nominal concentration (ng/mL)	Calculated concentration (ng/mL)	Accuracy (%)	Precision (%)
OME			
1	0.987 ± 0.026	−1.3	2.5
2	2.03 ± 0.04	1.5	2.2
5	5.11 ± 0.09	2.2	1.8
10	10.1 ± 0.1	1.0	0.6
50	52.2 ± 0.4	4.4	0.9
100	101 ± 2	1.0	1.3
500	485 ± 9	−3.0	1.3
1000	942 ± 9	−5.8	1.4
H-OME			
1	0.990 ± 0.057	−1.0	5.2
2	2.00 ± 0.11	0.0	5.3
5	5.20 ± 0.15	4.0	3.0
10	10.2 ± 0.3	2.0	3.1
50	51.6 ± 0.6	3.2	0.9
100	101 ± 3	1.0	3.0
500	485 ± 8	−3.0	1.6
1000	936 ± 16	−6.4	1.9
OME-S			
1	0.983 ± 0.054	−1.7	5.6
2	2.08 ± 0.14	4.0	6.5
5	4.92 ± 0.26	−1.6	5.3
10	9.97 ± 0.38	−0.3	4.0
50	52.4 ± 1.7	4.8	3.2
100	101 ± 6	1.0	5.5
500	483 ± 5	−3.4	1.3
1000	971 ± 18	−2.9	1.9

Each calculated concentration represents the mean ± s.d. (n = 6).

Table 2. Inter-day precision and accuracy for the determination of OME and its metabolites (H-OME and OME-S) in human plasma

Nominal concentration (ng/mL)	Calculated concentration (ng/mL)	Accuracy (%)	Precision (%)
OME			
2	2.05 ± 0.03	2.5	1.5
50	51.4 ± 0.5	2.8	1.0
500	481 ± 12	−3.8	2.5
H-OME			
2	2.08 ± 0.11	4.0	5.3
50	50.4 ± 1.2	0.8	2.4
500	470 ± 19	−6.0	4.0
OME-S			
2	2.19 ± 0.13	9.5	5.9
50	55.6 ± 2.5	11.2	4.5
500	517 ± 22	3.4	4.3

Each calculated concentration represents the mean ± s.d. (3 days, n = 1 per day).

determinations of OME, H-OME, and OME-S were all less than 5.9%. The accuracy for inter-day ranged from −6.0% to 11.2% for all analytes.

Stability

OME, H-OME, and OME-S in eluate from SPE plates were stable in the autosampler at 4°C for at least 8 hr, and they were also stable in human plasma when left at room temperature for at least 4.5 hr. On the other hand, OME, H-OME, OME-S, and IS in the standard solution (the mixture of methanol/water (50/50, v/v) with 0.05% triethylamine) were stable at room temperature for at least 21 hr and at 4°C for at least 1 week.

Pharmacokinetic Study

This analytical method has been applied to the assay of clinical plasma samples collected from a pharmacokinetic study in twelve Japanese healthy male volunteers receiving 20 mg OME. The mean plasma concentration time profiles for OME, H-OME, and OME-S are shown in Fig. 7. The plasma concentrations of OME, H-OME, and OME-S were higher than the lower limit of quantification (1 ng/mL) up to 8, 10, and 12 hr after dosing, respectively. Mean pharmacokinetic parameters calculated from the data were as follows: OME; $C_{max} = 295 \pm 125$ ng/mL, $AUC_{(0\text{-}12hr)} = 429 \pm 196$ ng · hr/mL,

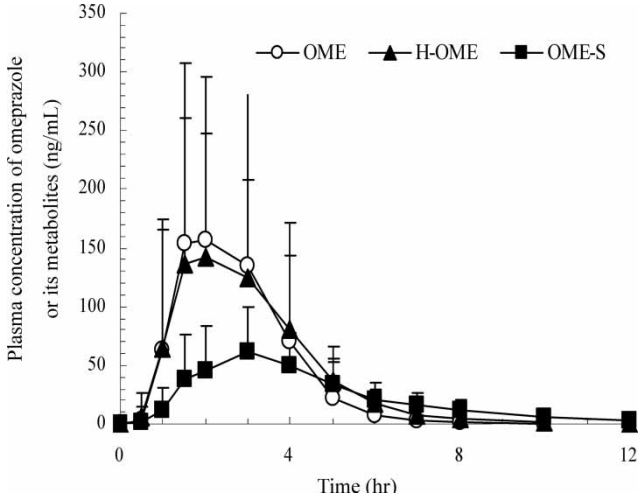

Figure 7. Mean plasma concentration-time profiles of OME, H-OME and OME-S after an oral administration of 20 mg OME to 12 Japanese healthy male volunteers. The data represents the arithmetic mean + s.d. of 12 subjects.

$T_{max} = 2.1 \pm 0.9$ hr, $t_{1/2} = 0.74 \pm 0.16$ hr, H-OME; $C_{max} = 257 \pm 48$ ng/mL, $AUC_{(0\text{-}12hr)} = 478 \pm 84$ ng · hr/mL, $T_{max} = 2.2 \pm 0.9$ hr, $t_{1/2} = 1.30 \pm 0.27$ hr, OME-S; $C_{max} = 83 \pm 22$ ng/mL, $AUC_{(0\text{-}12hr)} = 253 \pm 99$ ng · hr/mL, $T_{max} = 2.3 \pm 0.9$ hr, $t_{1/2} = 1.75 \pm 0.36$ hr, respectively.

CONCLUSION

This method is a sensitive, selective, and reliable LC-MS/MS method with a rapid and simple sample preparation procedure for the simultaneous determination of OME, H-OME, and OME-S in human plasma. Therefore, the method can be applicable to the simultaneous determination of OME, H-OME, and OME-S in human plasma, in the various clinical pharmacokinetic studies of OME including drug-drug interaction studies. Furthermore, this method will be applied to determine the CYP2C19 metabolic phenotype of subjects participating in the clinical studies.

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