ELSEVIER

Journal of Chromatography A, 949 (2002) 1–9

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Determination of omeprazole and its metabolites in human plasma by liquid chromatography–mass spectrometry

Hideko Kanazawa[a,*], Akiko Okada[a], Yoshikazu Matsushima[a], Hiromitsu Yokota[b], Shigeo Okubo[b], Fumiko Mashige[b], Kazuhiko Nakahara[b]

[a]*Kyoritsu College of Pharmacy, Shibakoen 1-5-30, Minato-ku, Tokyo 105-8512, Japan*

[b]*Department of Laboratory Medicine, The University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-8655, Japan*

Abstract

Omeprazole is a benzimidazole compound that acts as a proton-pump inhibitor. Because the metabolism of omeprazole is mainly catalyzed by cytochrome P-450 (*CYP*) *3A4* and *CYP2C19*, the genetic polymorphism of *CYP2C19* could be of clinical concern in the treatment of acid-related diseases with omeprazole. Therefore, a reliable method for omeprazole phenotyping is desirable in clinical situations. This study has demonstrated the determination of omeprazole and its metabolites in human plasma by liquid chromatography–three-dimensional quadrupole mass spectrometry with a sonic spray ionization interface. The analytical column was YMC-Pack Pro C_{18}(50×2.0 mm I.D.) using acetonitrile–50 m*M* ammonium acetate (pH 7.25) (1:4) at a flow-rate of 0.2 ml/min. The drift voltage was 30 V. The sampling aperture was heated at 110 °C and Shield temperature was 230 °C. In the mass spectrum, the molecular ions of omeprazole, hydroxyomeprazole and omeprazole sulfone were clearly observed as base peaks. This method is sufficiently sensitive and accurate for pharmacokinetic studies of omeprazol.

Keywords: Sonic spray ionization; Interfaces, LC–MS; Omeprazole; Cytochromes; Benzimidazoles

1. Introduction

Omeprazole is a class referred to as a proton-pump inhibitor. It acts to regulate acid production in the stomach, and is used to treat various acid-related gastrointestinal disorders. Omeprazole is also used to treat infection caused by *Helicobacter pylori* (*H. pylori*). Recently, cytochrome P-450 2C19 (CYP2C19) genotype-related anti-*H. pylori* efficacy by combining omeprazole and antibiotics was reported [1,2].

The oxidative metabolism of drugs in the liver is catalyzed by substrate-specific or selective *CYP*, a superfamily of haemoproteins that catalyze the metabolism of a large number of clinically important drugs. Hepatic drug oxidation is a major source of interindividual variations in drug pharmacokinetics and therapeutic response. The polymorphic oxidative metabolism of *S*-mephenytoin 4′-hydroxylation via CYP2C19 is well known [3–9]. This genetic polymorphism shows a cosegregation with the oxidative metabolism of several clinically important drug such as diazepam, imipramine, omeprazole, and propranolol as well as selective serotonin reuptake inhibitors. Furthermore, this pharmacogenetic entity has shown a marked interethnic difference in the incidence of the poor metabolizer (PM) phenotype. The frequency

*Corresponding author. Tel.: +81-354-002-657; fax: +81-354-001-378.

E-mail address: kanazawa-hd@kyoritsu-ph.ac.jp (H. Kanazawa).

0021-9673/02/$ – see front matter
PII: S0021-9673(01)01508-4

of PM phenotype of *S*-mephenytoin oxidation is approximately 2–6% of that of Caucasians, whereas the frequency of PMs in Japanese (19–23%) is much higher [10–12]. Thus, if these pharmacogenetic determinants could have some clinical implication, a drug whose metabolism is mediated via CYP2C19 might be of more clinical concern among Oriental patients compared to among Caucasian patients. De Morais et al. have reported that the primary defect in PMs is a single base-pair mutation in exon 5 of CYP2C19, resulting in an aberrant splice site [13,14]. This defect (called *CYP2C19***2*; old nomenclature $CYP2C19_{m1}$) is common in both Asian and Caucasian populations. A second mutation, in exon 4 (*CYP2C19***3*; $CYP2C19_{m2}$), appears to be present only in Asians. According to a genotyping analysis of CYP2C19, PMs consist of three genotypes (i.e. *CYP2C19**2/*2, *3/*3 or **2*/**3*), while extensive metabolizer (EM) include two genotypes, homozygous (i.e. *2C19**1/*1) and heterozygous (i.e. *2C19**1/*2, *1/*3) EMs in Japanese subjects [15].

Because the metabolism of proton-pump inhibitors is mainly catalyzed by CYP2C19 and CYP3A4, the genetic polymorphism of CYP2C19 could be of clinical concern in the treatment of acid-related diseases with proton-pump inhibitors. We have investigated the analysis of drugs in biological fluids using liquid chromatography-mass spectrometry (LC–MS) [16–19]. This study demonstrates the analysis of omeprazole in human plasma as a probe drug of CYP2C19 phenotyping by liquid chromatography–three-dimensional quadrupole mass spectrometry (LC-3DQMS) with a sonic spray ionization (SSI) interface.

2. Experimental

2.1. Chemicals

Omeprazole (5-methoxy-2-{[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulphinyl}-1H-benzimidazole) and β-diphosphopyridine nucleotide disodium salt reduced form (NADPH) were purchased from Wako (Osaka, Japan). Recombinant *CYP2C19* in yeast microsomes was purchased from Sumitomo (Osaka, Japan). All other reagents and solvents were of analytical grade.

2.2. LC–MS conditions

The assay was developed using a Model M-8000 LC–MS system (Hitachi, Tokyo, Japan). The absorbance was monitored at 302 nm. The analytical column was a YMC-Pack Pro C_{18} (50×2.0 mm I.D., YMC, Japan) operated at 25 °C. The mobile phase was acetonitrile–50 m*M* ammonium acetate (pH 7.25) (1:4) at a flow-rate of 0.2 ml/min. The drift voltage was 30 V. The sampling aperture was heated at 110 °C and the shield temperature was 230 °C.

2.3. Sample preparation

Venous blood samples were collected into EDTA tubes and separated by centrifugation for 10 min at 2000 *g*. To remove proteins prior to injection, the plasma sample was pretreated with a solid-phase extraction as follows: after a 200 μl volume of the plasma was loaded into a Sep-Pak C_{18} cartridge (Waters, Milford, MA, USA) after conditioning the cartridge with methanol, water and 50 m*M* ammonium acetate, a 5 ml volume of 50 m*M* ammonium acetate as a washing solution was passed through the cartridge. The sample fraction was then obtained by elution with 5 ml of methanol–50 m*M* ammonium acetate (4:1). After evaporation under reduced pressure, the residue was dissolved in 100 μl of the eluent.

Standard samples for calibration were prepared as follows: after a series of calibration standards were prepared by dissolving known amounts of omeprazole (the concentration range 0.5–10 μg) in 1 ml of drug-free human plasma, calibration curves were constructed by plotting the peak area against the concentration of the omeprazole, the data were then subjected to a linear-regression analysis.

Plasma samples were collected from healthy adults 3.5 h after a single oral dose of 20 mg omeprazole (Omepral tablets; AstraZeneca, Osaka, Japan) following an overnight fast. A 2 ml volume of the plasma was loaded into a Sep-Pak C_{18} cartridge. After the same treatments as described above, the residue was dissolved in 100 μl of the eluent.

2.4. Genotyping procedures for CYP2C19

Blood samples were obtained from three Japanese subjects (female), and genomic deoxyribonucleic acid (DNA) was isolated from peripheral lymphocytes with an extraction kit (*SepaGene*, Sanko, Tokyo, Japan). The *CYP2C19**1 (*wt*) gene and two mutated alleles associated with deficient (*S*)-mephenytoin hydroxylation, *CYP2C19**2 (*m1*) and *CYP2C19**3 (*m2*), were identified by (polymerase chain reaction (PCR) amplification using allele-specific primers according to the methods of De Morais et al. [13,14].

2.5. In vitro experiment by recombinant CYP2C19

The basic incubation medium contained 100 m*M* potassium phosphate buffer (pH 7.4), 3 m*M* NADPH and 2.9 m*M* omeprazole in a final volume of 0.5 ml. After preincubation at 37 °C for 5 min, 50 pmol of recombinant CYP2C19 was added to the mixture and incubation was carried out at 37 °C for 60 min. Adding 3 ml of cold acetonitrile stopped the reaction. After terminating the incubation, the mixture was centrifuged at 3000 rev./min for 10 min and 3 ml of the supernatant was loaded into a Sep-Pak C_{18} cartridge. After the same treatments as described above, the residue was dissolved in 50 μl of the eluent, and a 3 μl volume of the sample was injected into an LC–MS system.

3. Results and discussion

Omeprazole is metabolized to two major metabolites, 5-hydroxyomeprazole (CYP2C19) and omeprazole sulfone (CYP3A4), as shown in Fig. 1. In extensive metabolizers of mephenytoin, hydroxylation by CYP2C19 is the principal route of elimination for omeprazole [20,21]. Moreover, CYP2C19 also catalyzes the hydroxylation of omeprazole sulfone. It has been known that the rate of hydroxylation of omeprazole is well correlated with the rate of S-mephenytoin hydroxylation [15,22]. Accordingly, the results from population studies indicate that omeprazole can be used as a probe drug for phenotyping CYP2C19 [23].

3.1. Oxidation of omeprazole by recombinant CYP2C19

Omeprazole (2.9 m*M* concentration) was incu-

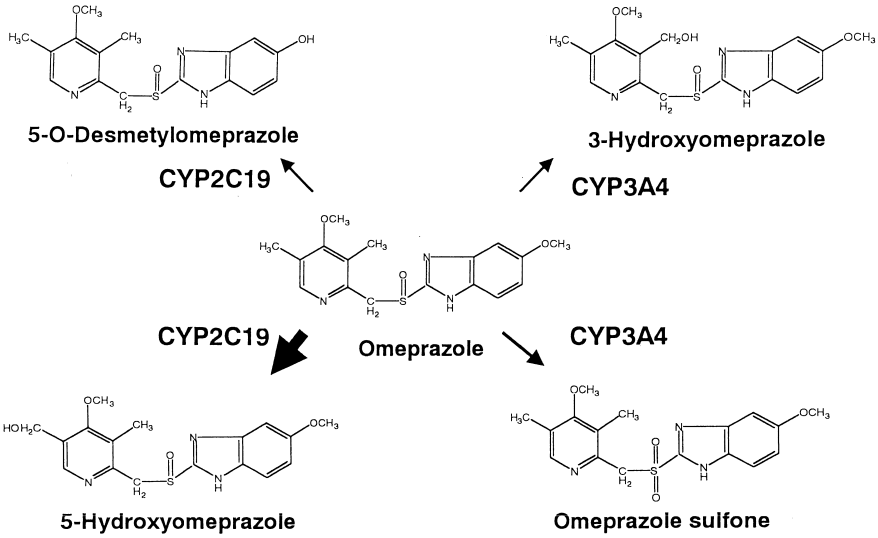

Fig. 1. Structure and metabolic pathways of omeprazole. The thickness of the arrows indicates an approximate contribution of CYP isoforms to each of the metabolic pathways.

bated in vitro with recombinant CYP2C19 isoform. The incubation mixture and other conditions are described in the Experimental section. The LC–3DQMS system with an SSI interface was used to analyze omeprazole and its metabolites in human plasma. Following recombinant CYP2C19 incubations, chromatographic peaks were detected with retention times corresponding to those of 5-*O*-desmethylomeprazole (10.80 min), 5-hydroxyomeprazole (11.64 min) and omeprazole (49.71 min). Fig. 2 shows the total ion chromatogram (TIC), mass chromatograms and mass spectra of omeprazole and its metabolites under positive ion conditions, respectively, at a drift voltage of 30 V. There was no interference from the extracted components of the incubation system. Well-resolved chromatograms were obtained with acetonitrile–50 m*M* ammonium acetate (15:85) as the eluent at a flow-rate of 0.2 ml/min. The mass spectrum of omeprazole is almost the same as that obtained by direct analysis. The protonated molecular ions, $[M+H]^+$, of 5-*O*-desmethylomeprazole, 5-hydroxyomeprazole and omeprazole were clearly observed at *m*/*z* 331, 361 and 345, respectively, as base peaks. Fragment ions of 5-*O*-desmethylomeprazole, 5-hydroxyomeprazole and omeprazole were observed at *m*/*z* 198, 214 and 198, respectively. Thus the identification of the omeprazole and its metabolite could be possible knowing their molecular ion and the fragmentation pattern.

Fig. 3 shows the TIC, mass chromatograms and mass spectra of omeprazole and its metabolites at a drift voltage of 60 V. Comparing with the mass spectra at a drift voltage of 30 V, the fragment ions

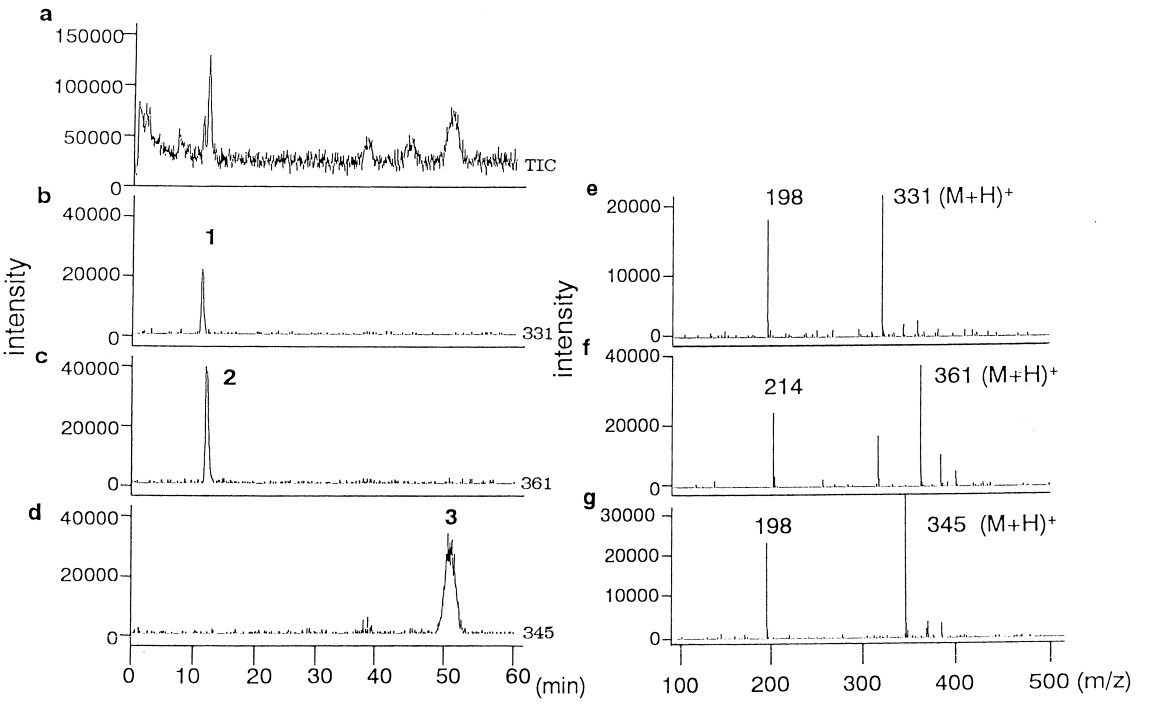

Fig. 2. Total ion chromatogram (TIC), mass chromatograms and mass spectra of omeprazole and its metabolites under positive-ion conditions at a drift voltage of 30 V. TIC (a), mass chromatogram at *m*/*z* 331 (b), at *m*/*z* 361 (c), at *m*/*z* 345 (d), and mass spectrum of 5-*O*-desmethylomeprazole (e), 5-hydroxyomeprazole (f), omeprazole (g). Mobile phase is acetonitrile–50 m*M* ammonium acetate (15:85) at a flow-rate of 0.2 ml/min. Other conditions are described in the Experimental section. Peaks: (1) 5-*O*-desmethylomeprazole; (2) 5-hydroxyomeprazole; (3) omeprazole.

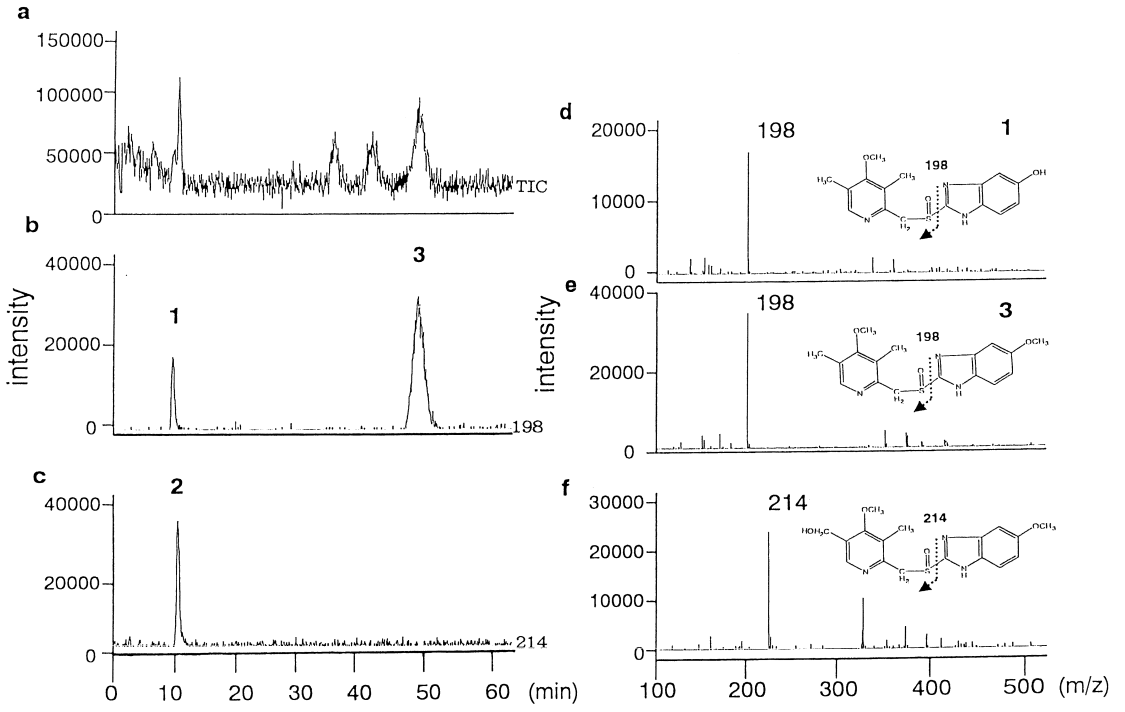

Fig. 3. TIC, mass chromatograms and the mass spectra of omeprazole and its metabolites at a drift voltage of 60 V. TIC (a), mass chromatogram at m/z 198 (b), at m/z 214 (c), a mass spectrum of a peak of 5-*O*-desmethylomeprazole (d), of omeprazole (e) on the mass chromatogram at m/z 198, a mass spectrum of 5-hydroxyomeprazole (f) on mass chromatogram at m/z 214. The chromatographic conditions and peak numbers as in Fig. 2.

of 5-*O*-desmethylomeprazole, 5-hydroxyomeprazole and omeprazole were strongly observed at m/z 198, 214 and 198, respectively, as base peaks.

3.2. Calibration curves and precision

The peak areas were calculated on a selected-ion chromatogram of omeprazole at m/z 345 under positive conditions. The linearlities between the amount of omeprazole and the peak area in the mass chromatogram were obtained between 0.5 and 10 μg. The linear relationship calculated between the peak area (R) and the concentration (x μg/ml) of omeprazole up to 10 μg/ml and the correlation coefficient (r) were as follows:

Omeprazole: $R = 6.91x - 2.37$ ($r^2 = 0.989$).

The lower limit for the quantification of omeprazole was 0.5 ng at a signal-to-noise ratio of 3. The present method is sufficiently sensitive and accurate to measure the pharmacokinetic parameters.

3.3. Determination of plasma levels of omeprazole and its metabolites

The allele-specific PCR based method allows genetic determinations, thus predicting their phenotype. Therefore, we examined the pharmacokinetic profile of omeprazole as a probe drug in relation to genotyping for two known mutations, *CYP2C19*★2 and *CYP2C19*★3.

Three different human samples which were genotyped for the CYP2C19 gene were used for the analysis. Fig. 4 shows the TIC, mass chromatograms and mass spectra of an extract of plasma sample after

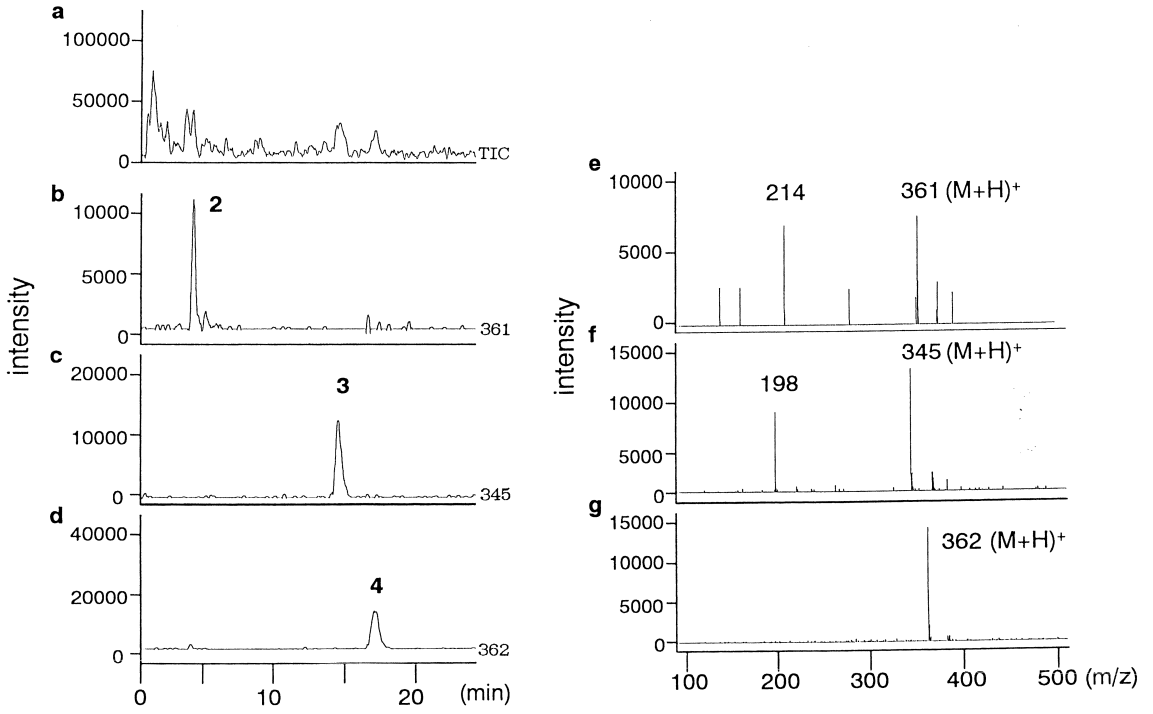

Fig. 4. TIC, mass chromatograms and the mass spectra of an extract of a plasma sample obtained after a single oral dose of 20 mg omeprazole in a healthy subject with heterozygous EM. TIC (a), mass chromatogram at m/z 361 (b), at m/z 345 (c), at m/z 362 (d), and mass spectrum of 5-hydroxyomeprazole (e), omeprazole (f), omeprazole sulfone (g). Mobile phase is acetonitrile–50 m*M* ammonium acetate (1:4) at a flow-rate of 0.2 ml/min. Other conditions are described in the Experimental section. Peaks: (2) 5-hydroxyomeprazole; (3) omeprazole; (4) omeprazole sulfone.

omeprazole administration obtained from a healthy subject with a heterozygous-extensive metabolizer (hetero EM). Well-resolved chromatograms were obtained without any influence of endogenous compounds in plasma with acetonitrile–50 m*M* ammonium acetate (pH 7.25) (1:4) as an eluent at a flow-rate of 0.2 ml/min. The peaks of 5-hydroxyomeprazole, omeprazole and omeprazole sulfone were identified with retention times of 4.02, 14.87 and 17.45 min, respectively. The molecular ions, $[M+H]^+$, of 5-hydroxyomeprazole were observed at m/z 361 as the base peak, and the fragment ion was observed at m/z 214. The molecular ions, $[M+H]^+$, of omeprazole were observed at m/z 345 as the base peak, and the fragment ion was observed at m/z 198. The molecular ions, $[M+H]^+$, of omeprazole sulfone were clearly observed at m/z 362 as base peaks.

Fig. 5 shows TIC, the mass chromatograms and mass spectra of an extract of a plasma sample obtained from a healthy subject with a homozygous extensive metabolizer (homo EM). Although the molecular ions, $[M+H]^+$, of 5-hydroxyomeprazole, omeprazole and omeprazole sulfone were observed as base peaks, the peaks of free omeprazole and hydroxyomeprazole were smaller than that in subjects with hetero EM. A greater sensitivity of the present method for omeprazole was obtained than that of the conventional HPLC method using UV detection. Omeprazole was not detected by comparing the UV chromatogram under the same condition.

Fig. 6 shows the TIC, mass chromatograms and mass spectra of an extract of plasma sample obtained from a healthy subject with a heterozygous poor metabolizer (hetero PM). The peaks of free omeprazole and omeprazole sulfone were greater than that in subjects with hetero, homo EMs. Although

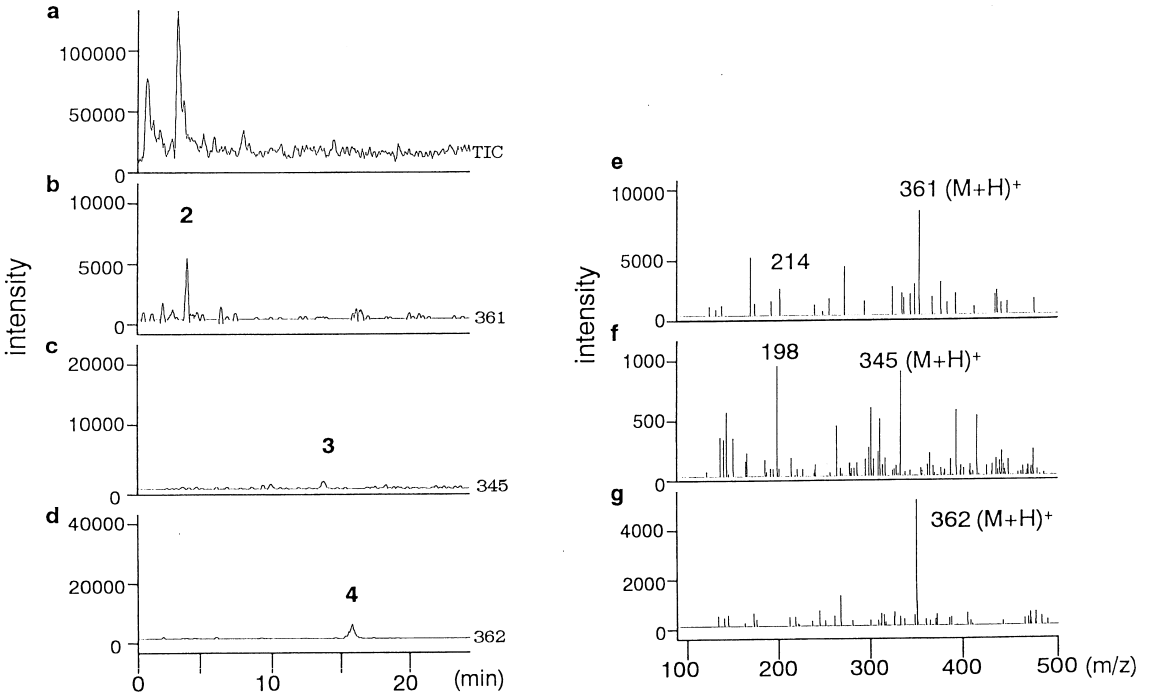

Fig. 5. TIC, mass chromatograms and mass spectra of an extract of a plasma sample obtained after a single oral dose of 20 mg omeprazole in a healthy subject with homozygous EM. TIC (a), mass chromatogram at m/z 361 (b), at m/z 345 (c), at m/z 362 (d), and mass spectrum of 5-hydroxyomeprazole (e), omeprazole (f), omeprazole sulfone (g). Chromatographic conditions and peak numbers as in Fig. 4.

the molecular ion, $[M+H]^+$, of hydroxyomeprazole was observed, the peak was smaller than that in subjects with EMs. The molecular ions, $[M+H]^+$, of omeprazole were clearly observed at m/z 345 as the base peak, and the fragment ion was observed at m/z 198. The molecular ions, $[M+H]^+$, of omeprazole sulfone was strongly observed at m/z 362 as the base peaks.

The concentrations of omeprazole, as determined by the present method, were 6.26, 274.17 and 552.43 ng/ml in subjects with homo EM, hetero EM and hetero PM, respectively. Phenotyping of CYP2C19 could be determined by measuring the omeprazole hydroxylation index (OPZ-HI; the relative concentration ratios of omeprazole to 5-hydroxyomeprazole). In this study, OPZ-HI was 0.47, 2.18 and 19.28 in subjects with homo EM, hetero EM and hetero PM, respectively. The kinetics of omeprazole sulfone in relation to the various genotype patterns showed a behavior similar to that of the parent drug. The concentration of free omeprazole was greater in subjects with 2C19*2/*3 than in those with the 2C19*1/*1, *1/*2 genotype. The OPZ-HI was approximately 10-times greater in subjects with 2C19*2/*3 than those with 2C19*1/*2. These results indicate that a correlation exists between the rate of metabolism of omeprazol and the genotype, and that significant differences exist in the disposition kinetics of omeprazol among subjects with different genotype patterns.

4. Conclusion

In this study, three different human samples which were genotyped for the CYP2C19 gene were used for the analysis. Reliable methods for phenotyping are extremely desirable under clinical situations. However the overall selectivity towards metabolites and endogenous compounds in plasma samples using

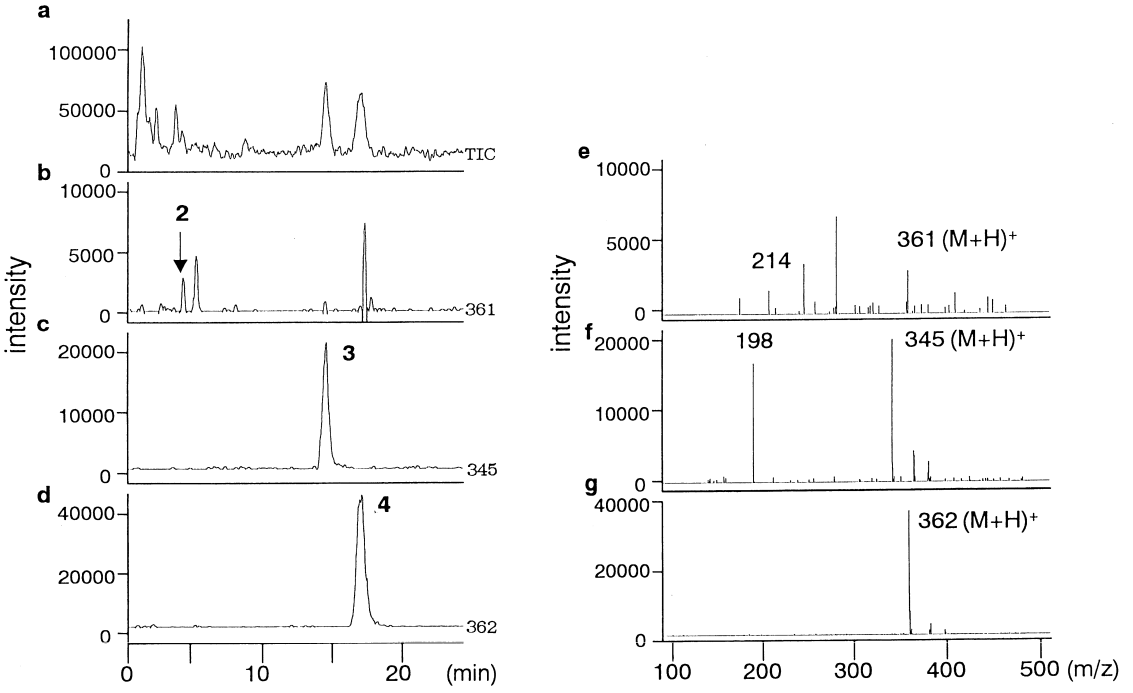

Fig. 6. TIC, mass chromatograms and the mass spectra of an extract of a plasma sample obtained after a single oral dose of 20 mg omeprazole in a healthy subject with heterozygous PM. TIC (a), mass chromatogram at m/z 361 (b), at m/z 345 (c), at m/z 362 (d), and mass spectrum of 5-hydroxyomeprazole (e), omeprazole (f), omeprazole sulfone (g). Chromatographic conditions and peak numbers as in Fig. 4.

UV detection is not sufficient for pharmacokinetic studies. LC–3DQMS with the SSI interface is very useful for polar analytes, such as the omeprazole metabolite. The present method is sufficiently sensitive and accurate for a studying of the kinetics of the formation of omeprazole metabolites in human plasma after administrations.

References

[1] Y. Tanigawara, N. Aoyama, T. Kita, K. Shirakawa, F. Komada, M. Kasuga, K. Okumura, Clin. Pharmacol. Ther. 66 (1999) 528.
[2] T. Furuta, M. Takashima, N. Shirai, F. Xiao, H. Hanai, K. Ohashi, T. Ishizaki, Clin. Pharmacol. Ther. 67 (2000) 684.
[3] M. Chang, G. Tybring, M.-L. Dahl, E. Götharson, M. Sagar, R. Seensalu, L. Bertilsson, Br. J. Clin. Pharmacol. 39 (1995) 511.
[4] I. Ieiri, T. Kubota, A. Urae, M. Kimura, Y. Wada, K. Mamiya, S. Yoshioka, S. Irie, T. Amamoto, K. Nakamura, S. Nakano, S. Higuchi, Clin. Pharmacol. Ther. 59 (1996) 647.
[5] M. Tanaka, T. Ohkubo, K. Otani, A. Suzuki, S. Kaneko, K. Sugawara, Y. Ryokawa, H. Hakusui, S. Yamamori, T. Ishizaki, Clin. Pharmacol. Ther. 62 (1997) 619.
[6] K. Laine, G. Tybring, L. Bertilsson, Clin. Pharmacol. Ther. 68 (2000) 151.
[7] A. Äbelö, T.B. Andersson, M. Antonsson, A.K. Naudot, I. Skånberg, L. Weidolf, Drug Metab. Disos. 28 (2000) 966.
[8] T. Sarich, T. Kalhorn, S. Magee, F.A. Sayegh, S. Adams, J. Slattery, J. Goldstein, S. Nelson, J. Wright, Clin. Pharmacol. Ther. 62 (1997) 21.
[9] Y. Böttiger, G. Tybring, E. Götharson, L. Bertilsson, Clin. Pharmacol. Ther. 62 (1997) 384.
[10] T. Kubota, K. Chiba, T. Ishizaki, Clin. Pharmacol. Ther. 60 (1996) 661.
[11] H.-G. Xie, C.M. Stein, R.B. Kim, G.R. Wilkinson, D.A. Flockhart, A.J.J. Wood, Pharmacogenetics 9 (1999) 539.
[12] S. Sviri, S. Shpizen, E. Leitersdorf, M. Levy, Y. Caraco, Clin. Pharmacol. Ther. 65 (1999) 275.
[13] S.M.F. de Morais, G.R. Wilkinson, J. Blaisdel, U.A. Meyer, K. Nakamura, J.A. Goldstein, Mol. Pharmacol. 46 (1994) 594.
[14] S.M.F. de Morais, G.R. Wilkinson, J. Blaisdell, K. Nakamura, J.A. Goldstein, J. Biol. Chem. 269 (1994) 15419.
[15] T. Ishizaki, Y. Horai, Aliment. Pharmacol. Ther. 13 (1999) 27.

[16] H. Kanazawa, Y. Nagata, Y. Matsushima, N. Takai, H. Uchiyama, R. Nishimura, A. Takeuchi, J. Chromatogr. 631 (1993) 215.
[17] H. Kanazawa, R. Nishimura, N. Sasaki, A. Takeuchi, N. Takai, Y. Nagata, Y. Matsushima, Biomed. Chromatogr. 9 (1995) 188.
[18] H. Kanazawa, Y. Konishi, Y. Matsushima, T. Takahashi, J. Chromatogr. A 797 (1998) 227.
[19] H. Kanazawa, R. Atsumi, Y. Matsushima, J. Kizu, J. Chromatogr. A 870 (2000) 87.
[20] T. Andersson, C.G. Regårdh, M.L. Dahl-Puustinen, L. Bertilsson, Ther. Drug Monit. 12 (1990) 415.
[21] T. Andersson, C.G. Regårdh, Y.C. Lou, Y. Zang, M.L. Dahl-Puustinen, L. Bertilsson, Pharmacogenetics 5 (1995) 358.
[22] S. Yasuda, Y. Horai, Y. Tomono, H. Nakai, C. Yamato, K. Manabe, K. Kobayashi, K. Chiba, T. Ishizaki, Clin. Pharmacol. Ther. 58 (1995) 143.
[23] K. Kobayashi, K. Chiba, D.R. Sohn, Y. Kato, T. Ishizaki, J. Chromatogr. 579 (1992) 299.