Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography B, 831 (2006) 85–90
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

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

Ute Hofmann [a,*], Matthias Schwab [a], Gerd Treiber [b], Ulrich Klotz [a]

[a] *Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Auerbachstraße 112, D-70376 Stuttgart, Germany*
[b] *Department of Gastroenterology and Hepatology, University Hospital Magdeburg, Leipziger Str. 44, D-39120 Magdeburg, Germany*

Received 21 September 2005; accepted 21 November 2005
Available online 9 December 2005

Abstract

A sensitive method was developed for the simultaneous determination of omeprazole and its major metabolites 5-hydroxyomeprazole and omeprazole sulfone in human plasma by HPLC–electrospray mass spectrometry. Following liquid–liquid extraction HPLC separation was achieved on a ProntoSil AQ, C18 column using a gradient with 10 mM ammonium acetate in water (pH 7.25) and acetonitrile. The mass spectrometer was operated in the selected ion monitoring mode using the respective MH^+ ions, *m*/*z* 346 for omeprazole, *m*/*z* 362 for 5-hydroxy-omeprazole and omeprazol-sulfone and *m*/*z* 300 for the internal standard (2-{[(3,5-dimethylpyridine-2-yl)methyl]thio}-1*H*-benzimidazole-5-yl)methanol. The limit of quantification (LOQ) achieved with this method was 5 ng/ml for 5-hydroxyomeprazole and 10 ng/ml for omeprazole and omeprazole-sulfone using 0.25 ml of plasma. Intra- and inter-assay variability was below 11% over the whole concentration range from 5 to 250 ng/ml for 5-hydroxyomeprazol and from 10 to 750 ng/ml for omeprazole and omeprazole-sulfone. The method was successfully applied to the determination of pharmacokinetic parameters of esomeprazole and the two major metabolites after a single dose and under steady state conditions.

Keywords: Omeprazole; Metabolites; LC–MS

1. Introduction

Second to the statins, proton pump inhibitors (PPIs) such as omeprazole/esomeprazole, pantoprazole, lansoprazole or rabeprazole belong world-wide to the most extensively used drugs [1]. For the treatment of acid related gastrointestinal disorders omeprazole still represents the standard drugs but in equipotent doses all PPI have the same pharmacodynamic action and clinical efficacy [2,3]. All PPIs are metabolized towards two major metabolites, 5-hydroxy-PPI and PPI-sulfone (Fig. 1), reactions catalysed by the polymorphic CYP2C19 and the most abundant CYP3A4, respectively [4].

It has been suggested that omeprazole can be used as a probe drug for phenotyping of CYP2C19, and concerning omeprazole and its S-enantiomer esomeprazole it has been claimed by in vitro experiments that the relative contributions of both CYPs to the overall elimination of the two enantiomers might differ [5]. Furthermore, for both PPIs a time-dependent (nonlinear) pharmacokinetics has been observed [6]. To provide substantial data in terms of these three situations a specific analytical assay is needed to quantify simultaneously omeprazole or esomeprazole and their two metabolites following single and repeated dosing.

Only a few methods exist which are capable of quantitating all three analytes in plasma. Most HPLC methods used are applied to phenotyping [7–9], and are not sensitive enough for pharmacokinetic studies. Even if low LOQs are specified in the method description, validation data are shown for much higher concentrations. One tandem mass spectrometric method described already is also too insensitive [10]. HPLC coupled to tandem mass spectrometry (LC–MS–MS) has been used for the determination of omeprazole and 5-hydroxyomeprazole [11,12] with high sensitivity (10 ng/ml or 0.8 ng/ml, respectively), but the sulfone was not determined with this method.

In order to fill this methodological gap, we developed a sensitive and selective HPLC mass spectrometry (LC–MS) method for the simultaneous determination of omeprazole,

* Corresponding author. Tel.: +49 711 81013707; fax: +49 711 859295.
E-mail address: ute.hofmann@ikp-stuttgart.de (U. Hofmann).

1570-0232/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2005.11.036

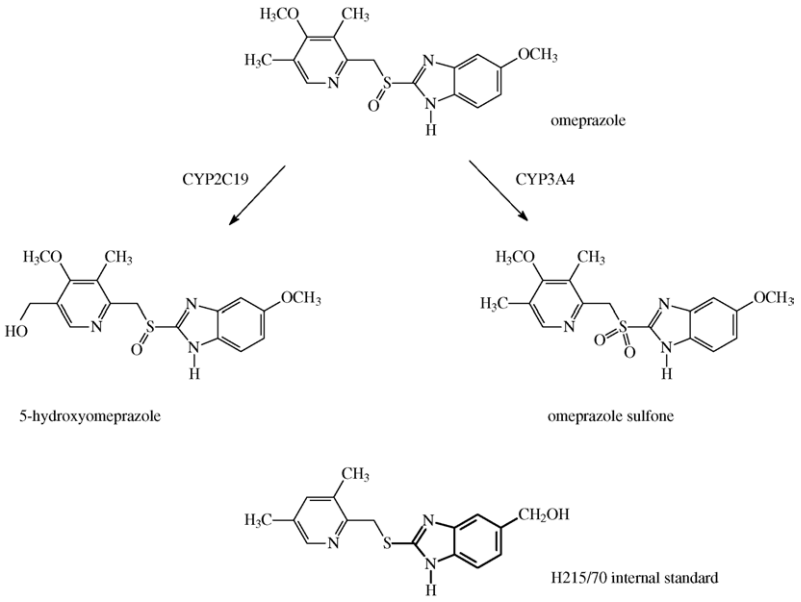

Fig. 1. Structure and metabolism of omeprazole and structure of the internal standard.

5-hydroxyomeprazole and omeprazole sulfone in human plasma. The method was applied to a pharmacokinetic study with esomeprazole in patients with gastroesophageal reflux disease (GERD).

2. Experimental

2.1. Materials

Solvents used were of HPLC quality and chemicals were of analytical grade. Omeprazole sodium, 5-hydroxyomeprazole sodium, omeprazole sulfone, and the internal standard H215/70 ((2-{[(3,5-dimethylpyridine-2-yl)methyl]thio}-1*H*-benzimidazole-5-yl)methanol) (Fig. 1) were kindly obtained from Dr. L. Weidolf (Astra Zeneca R & D, Mölndal, Sweden).

2.2. Standard solutions

The stock standard solutions of omeprazole, the metabolites and the internal standard (1 mg/ml) were prepared in methanol: sodium carbonate buffer (0.1 M, pH 9.3) 1:4 (v/v). Calibration standards were prepared by dilution of the stock solutions with methanol: sodium carbonate buffer 1:4 (v/v).

2.3. Sample preparation

Plasma samples (0.25 ml) were spiked with 10 μl of internal standard (10 ng/μl) and 100 μl of 1 M NaH_2PO_4 and extracted for 10 min with 5 ml of dichloromethane: acetonitrile (9:1, v/v). After centrifugation, the upper phase was discarded and the organic phase transferred to another vial and evaporated to dryness in a stream of nitrogen. The residue was dissolved in 100 μl of methanol/0.1 M Na_2CO_3 buffer, pH 9.3 (1:4, v/v), 10 μl were used for LC–MS analysis.

2.4. HPLC–MS analysis

A HP Series 1100 LC–MSD system (Agilent, Waldbronn, Germany) with binary pump, degasser, autosampler and mass selective detector equipped with an electrospray ion source was used. The mobile phases for HPLC were: (A) 10 mM ammonium acetate in water and (B) acetonitrile. Chromatographic separation was achieved on a ProntoSil AQ, C18 column (3 mm i.d. × 150 mm, particle size 3 μm, Bischoff, Leonberg, Germany) at a flow rate of 0.5 ml/min. Gradient runs were programmed as shown in Table 1. Equilibration time of the column was 4.5 min.

The mass spectrometer was tuned with the autotune procedure provided by the HP Chemstation software. Electrospray parameters were as follows: capillary voltage 3000 V, drying gas flow 10 l/min nitrogen, drying gas temperature 350 °C, nebulizer pressure 30 psig (207 kPa gauge) and fragmentor 80 V. The mass spectrometer was operated in the selected ion monitoring mode (SIM resolution high) using the respective MH^+ ions, *m/z* 346 for omeprazole, *m/z* 362 for 5-hydroxyomeprazole and omeprazolsulfone and *m/z* 300 for the internal standard. Peak width was set at 0.30 min.

Table 1
HPLC pump gradient program

Time (min)	%Mobile phase B
0	28
1	28
6	45
10	45
10.2	50
11	50
11.5	28
16	28

2.5. Standardisation and validation

Drug-free plasma was obtained from healthy volunteers who had not taken any medication at least 3 days before blood collection. Standard curves were prepared by adding 10 μl of standard solutions with increasing amounts of the analytes to 250 μl of drug-free plasma and extracting the samples as described above. The final concentrations obtained were 5, 10, 25, 50, 100 and 250 ng/ml for 5-hydroxyomeprazole, 10, 25, 50, 180, 500 and 750 ng/ml for omeprazole and 10, 25, 50, 175, 350 and 750 ng/ml for omeprazole-sulfone. Standard curves were evaluated by weighted ($1/x$) linear regression based on internal standard calibration and were obtained by plotting peak-area ratios against the amount of the substance. The concentration of the analytes in unknown samples was obtained from the regression line. All standardisation was performed with the HP Chemstation software (Agilent).

The reproducibility and accuracy of the method was established by analysing quality control samples, prepared by adding known amounts of the analytes to 10 ml of drug free plasma which were divided into aliquots and stored at −20°C. The final concentrations were 10, 50, and 250 ng/ml for 5-hydroxyomeprazole, 25, 180 and 750 ng/ml for omeprazole and 25, 175 and 750 ng/ml for omeprazole-sulfone. Quality control samples were always extracted and analysed together with the samples.

The intra-assay precision and accuracy was assessed by measuring the concentration of the analytes in six aliquots of the three different quality control samples extracted and analysed on a single day. Inter-assay precision and accuracy was determined from the results of the three different quality control samples which were extracted and analysed six-fold on three different days. The limit of quantification (LOQ) was determined as the lowest concentration with a coefficient of variation (CV) and a bias of <20% ($n = 6$).

Extraction recoveries were determined by comparing the peak areas from extracted standards in human plasma to the peak areas of unextracted standards at two different concentrations, 10 and 100 ng/ml for 5-hydroxyomeprazole, 25 and 500 ng/ml for omeprazole and 25 and 350 ng/ml for omeprazole-sulfone. Concentration of the internal standard in the recovery experiments was 400 ng/ml.

2.6. Pharmacokinetic study

The LC–MS assay developed was used to investigate pharmacokinetic parameters of esomeprazole and its metabolites 5-hydroxyomeprazole and omeprazole-sulfone in 10 patients with GERD. The patients received 40 mg of esomeprazole per day for 1 week. Blood samples were collected at different time points (0, 0.33, 0.5, 0.67, 1, 1.5, 2, 3, 4, 6, 8, 12 h) following the first and last dose (steady state). The study had been approved by the local ethics committee. All patients gave their written informed consent prior to participation in the study.

3. Results and discussion

3.1. Sample preparation

Liquid–liquid extraction or solid phase extraction (SPE) has been described for the determination of omeprazole and its metabolites. We have used liquid–liquid extraction with dichloromethane: acetonitrile 9:1 (v/v), the recovery (mean ± S.D.) was 84.9 ± 1.9% for omeprazole, 79.9 ± 4.1% for 5-hydroxyomeprazole, 89.3 ± 1.7% for omeprazole sulfone and 100.9 ± 2.2% for the internal standard.

3.2. LC–MS analysis

The positive ion electrospray mass spectra of all analytes and the internal standard show the protonated molecular ion $[MH]^+$ as the base peak (Fig. 2), m/z 346 for omeprazole, m/z 362 for 5-hydroxyomeprazole and omeprazole sulfone and m/z 300 for the internal standard. Fragment ions corresponding to loss of the benzimidazole moiety are present in the spectra of omeprazole and 5-hydroxy-omeprazole at m/z 198 or 214, respectively, while no fragment ions are observed for the sulfone and the internal standard.

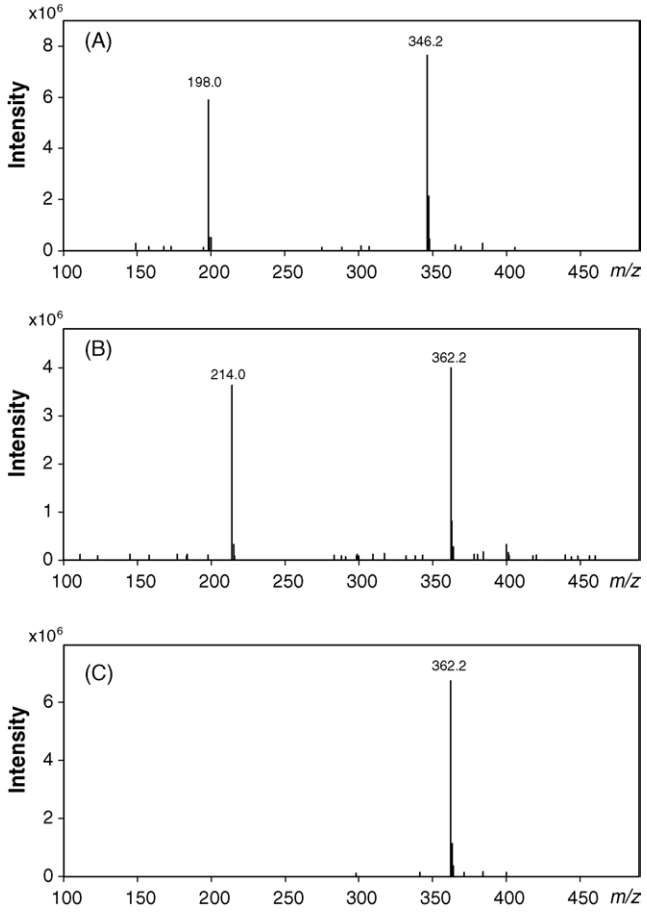

Fig. 2. Mass spectra of omeprazole (A); 5-hydroxyomeprazole (B) and omeprazole sulfone (C).

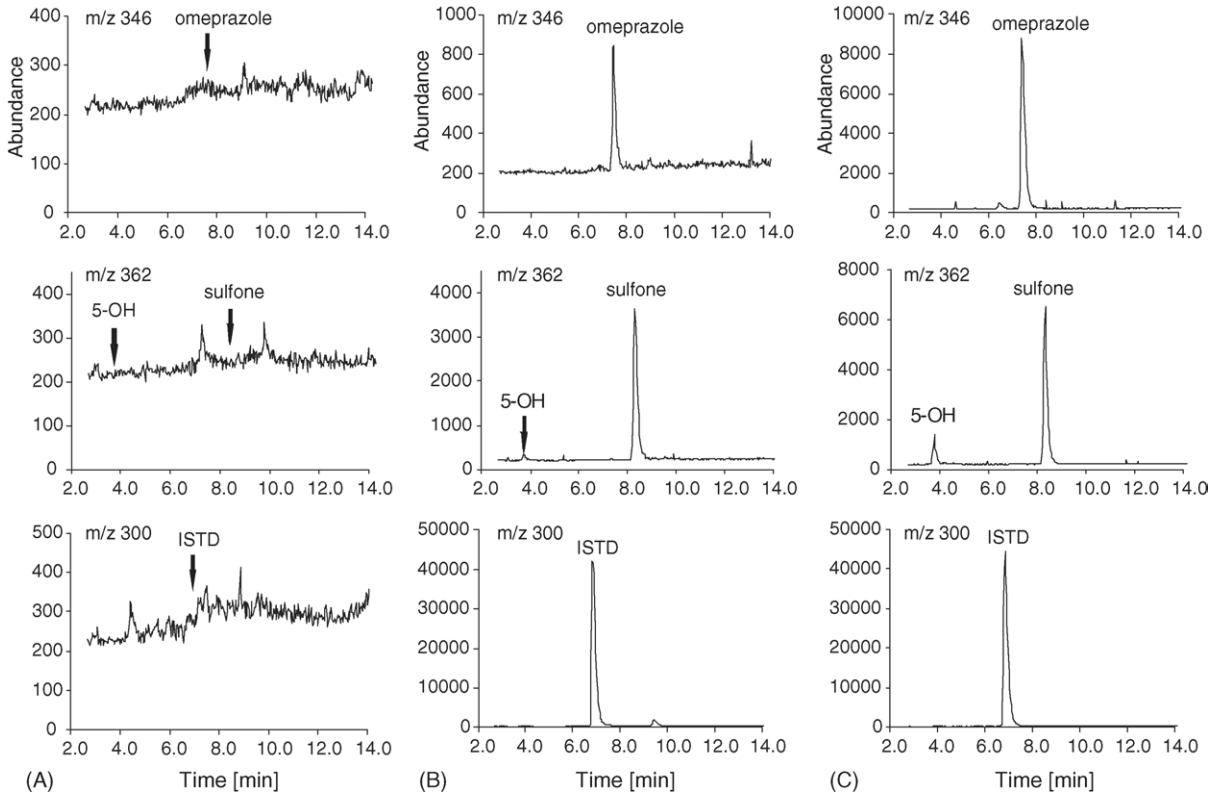

Fig. 3. Mass chromatograms of the extracts from (A) blank human plasma; (B) the lowest quality control sample and (C) plasma from a patient 40 min after a single dose of 40 mg of esomeprazole.

HPLC separation was achieved in a total runtime of 15 min on a Prontosil AQ C18 column with an ammonium acetate buffer (10 mM, pH 7.25)–acetonitrile gradient (Fig. 3C). Because of the highly selective detection method, there were no interfering peaks present in more than 10 blank samples investigated from patients or from healthy volunteers. An example of a blank plasma sample is shown in Fig. 3A, the lowest quality control sample (10 ng/ml of 5-hydroxyomeprazole and 25 ng/ml each of omeprazole and omeprazole-sulfone) in Fig. 3B.

Ion suppression due to co-eluting substances was also investigated by comparing the peak areas from pure standards to the peak areas from standards added to extracted blank plasma. No matrix effect was observed for the three analytes and the internal standard.

3.3. Assay validation

The linearity of the standard curves showed to be good over the entire concentration range measured: 5–250 ng/ml

Table 2
Intra-assay precision and accuracy for the determination of omeprazole and its metabolites 5-hydroxyomeprazole and omeprazole sulfone in human plasma

Concentration added (ng/ml)	*n*	Concentration found mean ± S.D. (ng/ml)	Bias (%)	R.S.D. (%)
Omeprazole				
10.0	6	9.98 ± 0.36	−0.2	3.7
25.0	6	25.6 ± 2	2.4	7.8
180	6	186 ± 10	3.2	5.6
750	6	688 ± 24	−8.3	3.5
5-Hydroxyomeprazole				
5.00	6	5.36 ± 0.19	7.3	3.6
10.0	6	9.86 ± 0.65	−1.5	6.6
50.0	6	48.2 ± 3.2	−3.7	6.7
250	6	233 ± 13	−6.7	5.4
Omeprazole sulfone				
10.0	6	9.98 ± 0.38	−0.2	3.8
25.0	6	26.5 ± 0.7	5.9	2.8
175	6	195 ± 5	11.5	2.6
750	6	667 ± 7	−11.1	1.1

Table 3
Inter-assay precision and accuracy for the determination of omeprazole and its metabolites 5-hydroxyomeprazole and omeprazole sulfone in human plasma

Concentration added (ng/ml)	n	Concentration found mean ± S.D. (ng/ml)	Bias (%)	R.S.D. (%)
Omeprazole				
25.0	18	25.9 ± 2.8	3.7	10.8
180	18	192 ± 19	6.4	10.0
750	18	720 ± 57	−4.0	7.9
5-Hydroxyomeprazole				
10.0	18	10.2 ± 1.1	2.3	10.7
50.0	18	50.1 ± 5.6	0.2	11.1
250	18	247 ± 21	−1.1	8.6
Omeprazole sulfone				
25.0	18	26.4 ± 1.1	5.5	4.3
175	18	201 ± 6	14.7	3.0
750	18	682 ± 24	−9.0	3.6

for are given in Table 2, inter-assay results in Table 3 5-hydroxyomeprazole and 10–750 ng/ml for omeprazole and omeprazole-sulfone. The correlation coefficients (r^2) ranged between 0.99680 and 0.99974 for 5-hydroxyomeprazole, from 0.99705 to 0.99975 for omeprazole and 0.99365 to 0.99878 for omeprazole-sulfone ($n = 14$).

Accuracy and precision of the method was determined with quality control samples as described in Section 2.5. The relative standard deviation (R.S.D.) and mean values of the deviation from the amount added (% bias) were calculated. Intra-assay results . The data show good precision and accuracy of the method with an intra-day R.S.D. of below 8% for all three analytes even at the LOQ (5 ng/ml for 5-hydroxyomeprazole and 10 ng/ml for omeprazole and omeprazole sulfone). The inter-day R.S.D.s are somewhat higher with up to 10.8% for omeprazole, 11.1% for 5-hydroxyomeprazole and 4.3% for omeprazole-sulfone. The intra-assay accuracy as expressed by the bias ranged between −8.3 and 3.2% for omeprazole, between −6.7 and 7.3% for 5-hydroxyomeprazole and between −11.1 and 11.5% for omeprazole sulfone. For inter-assay accuracy, the bias was also always below 15%.

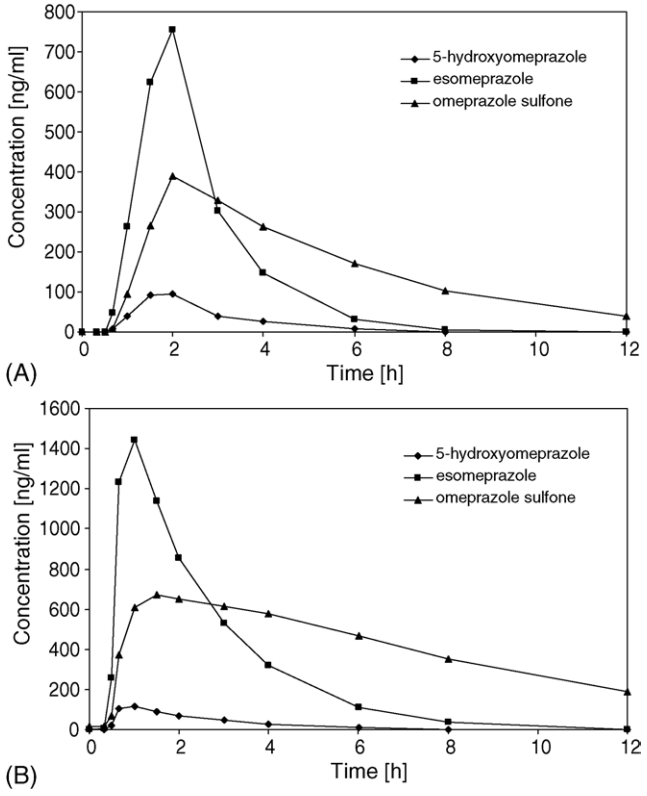

Fig. 4. Plasma concentration–time profiles of esomeprazole and the metabolites 5-hydroxyomeprazole and omeprazole sulfone in a patient after the first dose (A) and after seven days (B) of 40 mg of esomeprazole daily.

3.4. Assay application

The assay was successfully applied to a pharmacokinetic study in 10 patients with GERD. The patients received 40 mg of esomeprazole daily and pharmacokinetic parameters of esomeprazole and its metabolites were determined after the first dose and after 1 week [13]. Representative plasma concentration time curves in one patient after single dose and under steady state are shown in Fig. 4. Concentrations of esomeprazole and both metabolites – expressed as area under the plasma concentration time profiles (AUC) – increased after multiple dosing and elimination was slower if compared to single dosing as summarized in Table 4.

Table 4
Pharmacokinetic parameters of esomeprazole and its two major metabolites in patients with GERD [13]

Parameter	Mean ± S.D. ($n = 10$)		p-value
	Single dose (sd)	Steady state (ss)	sd vs. ss
Esomeprazole			
AUC (μmol h/l)	8.03 ± 9.51	13.8 ± 9.8	0.0009
$t_{1/2}$ (h)	1.30 ± 1.03	1.70 ± 1.00	<0.0001
5-Hydroxyomeprazole			
AUC (μmol h/l)	0.72 ± 0.23	0.91 ± 0.41	0.15
$t_{1/2}$ (h)	1.61 ± 0.84	2.31 ± 1.36	0.0028
Omeprazole sulfone			
AUC (μmol h/l)	5.88 ± 4.48	15.2 ± 7.63	0.0003
$t_{1/2}$ (h)	3.87 ± 4.28	7.48 ± 5.50	<0.0001

4. Conclusions

A method has been developed for the simultaneous determination of omeprazole and its two major metabolites 5-hydroxyomeprazole and omeprazole sulfone in human plasma using liquid–liquid extraction and HPLC–mass spectrometry. Sensitivity, selectivity and reproducibility allow for the application in pharmacokinetic studies. In 10 patients with GERD the most relevant pharmacokinetic parameters of the three compounds have been evaluated following single and multiple therapeutic dosing.

Acknowledgments

Omeprazole sodium, 5-hydroxyomeprazole sodium, omeprazole sulfone, and the internal standard H215/70 ((2-{[(3,5-dimethylpyridine-2-yl)methyl]thio}-1*H*-benzimidazole-5-yl) methanol) were a kind gift from Dr. L. Weidolf (Astra Zeneca R & D, Mölndal, Sweden).

The technical assistance of Monika Seiler is greatfully acknowledged.

The work was supported by the Robert Bosch Foundation (Stuttgart, Germany).

References

[1] A.S. Raghunath, C. O'Morain, R.C. McLoughlin, Aliment. Pharmacol. Ther. 22 (Suppl. 1) (2005) 55.
[2] H.D. Langtry, M.I. Wilde, Drugs 56 (1998) 447.
[3] L.J. Scott, C.J. Dunn, G. Mallarkey, M. Sharpe, Drugs 62 (2002) 1091.
[4] U. Klotz, M. Schwab, G. Treiber, Basic Clin. Pharmacol. Toxicol. 95 (2004) 2.
[5] A. Äbelö, T.B. Andersson, M. Antonsson, A.K. Naudot, I. Skanberg, L. Weidolf, Drug Metabol. Dispos. 28 (2000) 966.
[6] U. Klotz, Clin. Pharmacokinet. 38 (2000) 243.
[7] P. Lagerstrom, B. Persson, J. Chromatogr. 309 (1984) 347.
[8] K. Kobayashi, K. Chiba, D. Sohn, Y. Kato, T. Ishizaki, J. Chromatogr. 579 (1992) 299.
[9] H.M. Gonzalez, E.M. Romero, T. de, J. Chavez, A.A. Peregrina, V. Quezada, C. Hoyo-Vadillo, J. Chromatogr. B 780 (2002) 459.
[10] H. Kanazawa, A. Okada, Y. Matsushima, H. Yokota, S. Okubo, F. Mashige, K. Nakahara, J. Chromatogr. A 949 (2002) 1.
[11] E.J. Woolf, B.K. Matuszewski, J. Chromatogr. A 828 (1998) 229.
[12] V.A. Frerichs, C. Zaranek, C.E. Haas, J. Chromatogr. B 824 (2005) 71.
[13] M. Schwab, U. Klotz, U. Hofmann, E. Schaeffeler, A. Leodolter, P. Malfertheiner, G. Treiber, Clin. Pharmacol. Ther., in press.