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Enantioselective quantification of omeprazole and its main metabolites in human serum by chiral HPLC–atmospheric pressure photoionization tandem mass spectrometry

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Abstract

Omeprazole is a proton pump inhibitor drug in widespread use for the reduction of gastric acid production. It is also proposed as a test substance for the phenotyping of cytochrome CYP3A4 and CYP2C19 enzyme activities. For this purpose, it is necessary to quantify, additionally to omeprazole, the two main metabolites 5-hydroxyomeprazole and omeprazole-sulfon in human plasma. Since omeprazole is a racemic mixture of two enantiomers and its enzymatic decomposition depends in part on its chiral configuration, full information about its metabolic breakdown can only be gained by enantioselective quantification of the drug and its metabolites. We introduce a new LC–MS/MS method that is capable to simultaneously quantify omeprazole and its two main metabolites enantioselectively in human serum. The method features solid-phase extraction, normal phase chiral HPLC separation and atmospheric pressure photoionization tandem mass spectrometry. As internal standards serve stable isotope labeled omeprazole and 5-hydroxyomeprazole. The calibration functions are linear in the range of 5–750 ng/ml for the omeprazole enantiomers and omeprazole-sulfon, and 2.5–375 ng/ml for the 5-hydroxyomeprazole enantiomers, respectively. Intra- and inter-day relative standard deviations are <7% for omeprazole and 5-hydroxyomeprazole enantiomers, and <9% for omeprazole-sulfon, respectively.

Keywords: Omeprazole; Omeprazole-sulfon; 5-Hydroxyomeprazole; Chiral separation; APPI; LC–MS/MS

1. Introduction

Omeprazole (OME) is a proton pump inhibitor drug in widespread use for the reduction of gastric acid secretion. It is effective in the treatment of gastro-esophageal reflux disease, gastric ulcer or, together with antibiotic therapy, in the eradication of *Helicobacter pylori* infections [1]. OME is extensively metabolized in the liver by the cytochrome P450 (CYP) enzyme family, particularly by the enzymes CYP2C19 and CYP3A4 (Fig. 1). OME is commonly administered as a racemic mixture of *R*(+)-omeprazole (*R*-OME) and *S*(−)-omeprazole (*S*-OME). The metabolic pathway is enantioselective in such a way that CYP3A4 acts mainly on *S*-OME to produce achiral omeprazole-sulfone (OMES), whereas CYP2C19 acts mainly on *R*-OME to produce *R*-5-hydroxyomeprazole (*R*-HOME), together with minor amounts of *S*-5-hydroxyomeprazole (*S*-HOME) [2]. The clinical efficacy of OME depends in part on the CYP2C19 phenotype of the patient. CYP2C19 fast metabolizers show relatively lower serum values of OME and have lower success rates in the treatment of various disorders than CYP2C19 poor metabolizers [3]. The application of the pure *S*-OME enantiomer (Nexium®, AstraZeneca, Wedel, Germany) avoids the polymorphism prone CYP2C19 metabolic pathway and it is reported that it may lead to overall higher success rates in the treatment of various acid secretion related diseases [3,4]. On the other hand, racemic OME can serve as a test substance to characterize the

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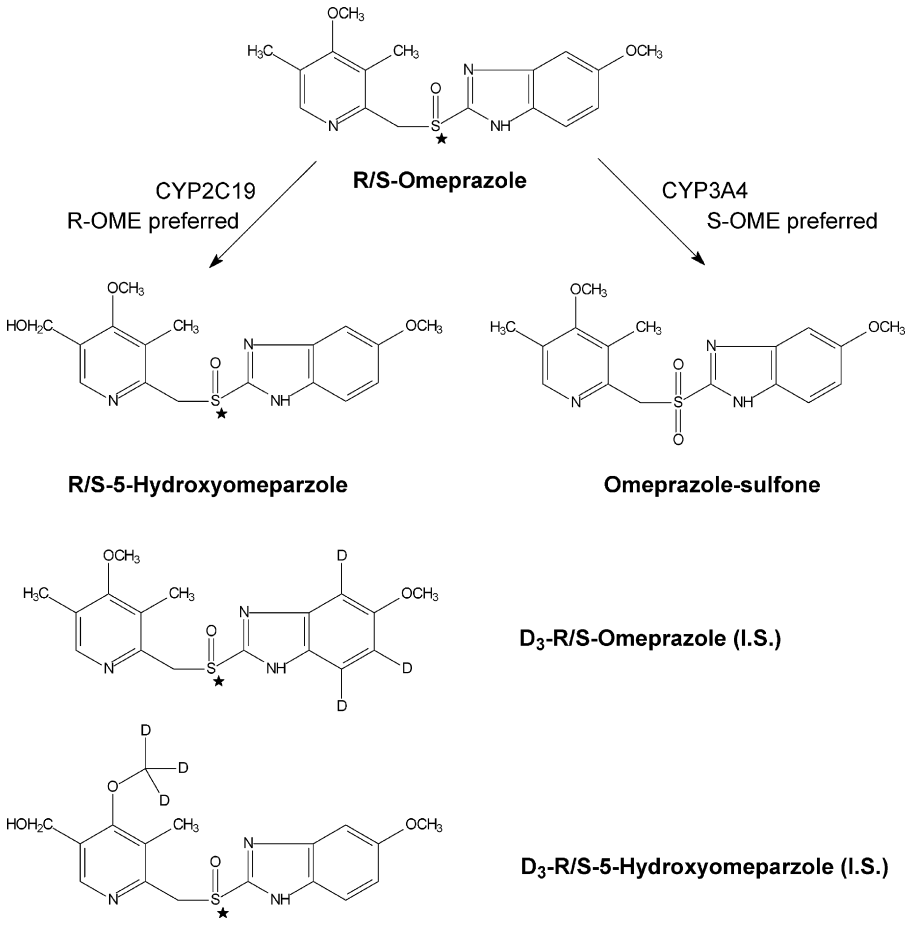

Fig. 1. Molecular structures of OME, its two main metabolites and the two I.S.s, together with the enzymes involved mainly in the biotransformation of OME. CYP2C19 acts principally on the *R*-enantiomer of OME, whereas *S*-OME is the main target of CYP3A4. The asterisks mark the chiral centers of OME and HOME and the I.S.s.

phenotype of a patient for both CYP3A4 and CYP2C19 enzymes in a single test [5–7]. To fully exploit the information in such an experiment, to gain more insight into the enantioselective metabolism and to investigate possible chiral inversion during the biotransformation processes, OME and its metabolites have to be quantified in a enantioselective manner.

A wealth of achiral methods for the detection of omeprazole in human serum with or without its main metabolites has been published. Some utilize UV absorption for detection [5,8–12], others mass spectrometric methods [13–17], whereas the mass spectrometric methods generally result in more selective determinations. However, chiral separations of OME and its metabolites provide considerably more difficulties. Chromatographic evaluations under various conditions like normal phase chromatography, reversed phase chromatography or capillary electrophoresis were performed for OME alone [18] or OME together with its main metabolites [19]. In these two papers the optimization of the chiral separation was in the main focus, whereas the determination in biological fluids like serum played a minor role and was not validated. Kanazawa et al. [7] described an enantioselective reversed phase HPLC method with UV detection and mass spectrometric identification of the substances, but performed also no complete validation of the method in biological fluids. A validated method for the enantioselective quantification of OME in human serum has been reported by Cass et al. [20]. This method featured in-line sample preparation with column switching, normal phase chiral separation and UV detection, but it suffered from quite long chromatographic run times, broad analyte peaks and interferences from endogenous substances. Another fully validated normal phase chiral separation method of OME from human serum with UV detection has been published by Orlando and Bonato [21]. A method providing high selectivity, fast chromatography and very low limits of detection was described by Stenhoff et al. [22], utilizing isotope dilution mass spectrometric detection after normal phase chiral separation of OME. To achieve proper ionization in the ion source of the mass spectrometer and to avoid the explosion hazard of the normal phase eluent, they had to add post-column make up liquid containing ethanol and aqueous buffer and split the combined liquid flow before entering the ion source.

In this work, we present for the first time a method for the enantioselective quantification of OME together with its main metabolites HOME and OMES in human serum. The method features solid-phase extraction, chiral separation on a ReproSil Chiral-CA column in normal phase mode and tandem mass spectrometric detection after atmospheric pressure photoionization (APPI). Due to the use of isotope labeled I.S.s both for OME and its metabolites, the method is very precise and accurate.

2. Experimental

2.1. Instrumentation

The HPLC part of the analytical apparatus consisted of an Agilent 1100 system (Waldbronn, Germany) comprising a binary pump, an autosampler, a thermostatted column compartment and a diode array UV–vis detector. The enantioselective chromatographic separation took place on a ReproSil Chiral-CA 5 μm 250 mm × 2 mm column (Dr. Maisch GmbH, Ammerbuch, Germany), protected by a SecurityGuard system (Phenomenex, Aschaffenburg, Germany) equipped with a 4 mm × 2 mm silica filter insert. The analytes were detected by a Thermo Scientific TSQ Discovery Max triple quadrupole mass spectrometer (San Jose, CA, USA) equipped with a APPI ion source with a krypton UV-lamp (PhotoMate®, Syagen, Tustin, CA, USA). System control and data handling was carried out by the Thermo Scientific Xcalibur software, version 1.2.

Solid-phase extraction (SPE) of the samples was performed on OASIS HLB 1 ml extraction columns containing 30 mg sorbent (Waters, Eschborn, Germany).

2.2. Chemicals

Pure omeprazole was purchased as a racemic mixture from Sigma–Aldrich (Seelze, Germany), enantiopure *S*-omeprazole was utilized as Nexium® powder for the preparation of infusion solutions (45 mg powder contained 40 mg *S*-OME, AstraZeneca, Wedel, Germany). Racemic 5-hydroxyomeprazole, racemic D_3-5-hydroxyomeprazole and omeprazole-sulfone were purchased from SynFine Research (Ontario, Canada). The other I.S. D_3-omeprazole was obtained from CDN-Isotopes (Augsburg, Germany). All other chemicals were of analytical grade or better.

2.3. Sample collection

Blood samples of about 7.5 ml were drawn into sampling tubes (BD Vacutainer Systems, Plymouth, UK) without anticoagulant. After coagulation, blood cells were separated by centrifugation at 2440 × *g* for 5 min. The resulting blood serum was stored at −20 °C until analysis.

2.4. Stock solution, calibration and quality control samples

The I.S. stock solution was prepared by dissolving 1.0 mg D_3-omeprazole and 0.5 mg D_3-5-hydroxyomeprazole in 10 ml 2-propanol. Stock solutions of the analytes were prepared by dissolving 5.0 mg OME in 50 ml 2-propanol, 1.0 mg HOME in 5 ml 2-propanol and 1.0 mg OMES in 5 ml 2-propanol. A calibration working solution was prepared by diluting 100 μl of the OME stock solution and 25 μl each of the HOME and OMES stock solutions with water containing 0.1% triethylamine (TEA) up to a final volume of 1000 μl. This working solution contained racemic OME in the concentration of 10 μg/ml, racemic HOME in the concentration of 5 μg/ml and OMES in the concentration of 5 μg/ml, respectively. Pooled human serum was spiked by appropriate volumes of this calibration working solution to obtain calibration samples and quality control samples in the concentrations ranges summarized in Table 1.

2.5. Sample preparation

Prior to each batch of samples, a I.S. working solution was prepared by diluting 50 μl of the I.S. stock solution with 950 μl water containing 0.1% TEA. To 250 μl serum sample, 20 μl of this I.S. solution and 500 μl of 0.1 M ammonium acetate buffer pH 8.5 were added. After activating the SPE columns with 1 ml methanol and 1 ml water, the sample mixtures were drawn through the columns by applying slight vacuum. Subsequently, the columns were washed with 1 ml of the ammonium acetate buffer and with 1 ml of a mixture of methanol/ammonium acetate buffer 30/70 (v/v). The analytes were eluted from the columns with 1 ml methanol and the eluates were evaporated in vacuum. The residues were redissolved in 10 μl 2-propanol containing 1% TEA and 90 μl hexane and were transferred into brown autosampler vials with microliter inserts.

2.6. Chromatographic conditions and tandem MS detector settings

After injection of 10 μl of the prepared samples, enantioselective chromatographic separation was achieved by HPLC normal phase gradient elution. The mobile phase A consisted of 2-propanol/acetic acid/diethylamine (DEA) 100/4/1 (v/v), whereas mobile phase B was pure hexane. At a flow rate of 0.35 ml/min, the gradient started with a composition of 10:90 A:B, the fraction of A was increased to 15% in the next 10 min and was hold constant for 1 min. Subsequently, a washing step with 25% A for 1 min was performed. After this washing step the mobile phase composition was turned back to starting conditions. The column temperature was held constant at 20 °C. A divert valve directed the HPLC effluent without splitting to the mass spectrometer in the run-time window of 5–15.9 min, otherwise to the waste container. A complete chromatographic cycle including reequilibration of the column took 19 min.

In the mass spectrometric detector, ions were formed by photoionization using a krypton light source radiating at two emission lines with energies of 10.0 eV and 10.6 eV. Vaporizer and capillary temperatures were set to 300 °C and 220 °C, respectively. Nitrogen served as sheath and AUX gas, with flow settings of 41 and 8 arbitrary units, respectively. Under these conditions, the analytes were ionized exclusively to $[M+H]^+$ parent ions. Prior to detection, collision induced fragmentation of the parent ions was achieved with argon serving as collision gas at a pressure of 1.0 mTorr. The parent and fragment ion masses and other mass spectrometric settings are summarized in Table 2.

3. Results and discussion

3.1. Sample preparation

OME is known to decay quite rapidly in aqueous solutions at acidic pH [23]. Therefore, it is essential for accurate quan-

Table 1
Calibration- and quality control sample concentrations and calibration results

Calibration level	Quality control level	Spike concentrations				
		R-OME (ng/ml)	*S*-OME (ng/ml)	*R*-HOME (ng/ml)	*S*-HOME (ng/ml)	OMES (ng/ml)
1	Low	5	5	2.5	2.5	5
2		10	10	5	5	10
3		25	25	12.5	12.5	25
4	Medium	50	50	25	25	50
5		100	100	50	50	100
6		250	250	125	125	250
7		500	500	250	250	500
8	High	750	750	375	375	750
Internal standards used						
I.S.		D_3-*R*-OME	D_3-*S*-OME	D_3-*R*-HOME	D_3-*S*-HOME	D_3-*S*-OME
Concentration (ng/ml)		200	200	100	100	200
Calibration results						
Slope		$5.925 \times 10^{-3} \pm 0.014 \times 10^{-3}$	$5.750 \times 10^{-3} \pm 0.014 \times 10^{-3}$	0.01350 ± 0.00003	0.01320 ± 0.00004	$2.150 \times 10^{-2} \pm 0.035 \times 10^{-2}$
r^2		0.9997	0.9999	0.9999	0.9999	0.9927

tification to avoid acidic pH values during the whole sample preparation process. This was achieved by adding small amounts of the organic base TEA to all solutions or buffering the solutions to pH 8.5 with ammonium acetate. In this basic solutions, no degradation of OME and its metabolites during the sample preparation process and the subsequent analysis was observed.

The optimization of the extraction process and the determination of the extraction yield was carried out by comparing pure solutions of the analytes resembling 100% extraction yield with the actual extracts of the analytes. Since the extraction procedure features no enantioselectivity, we were able to characterize the extraction procedure on an achiral HPLC system with UV detection at 302 nm, following the method described by Shimizu et al. [11].

In the second washing step of the SPE extraction, the ratio of the methanol/ammonium acetate buffer is critical to the extraction yield. We observed that with a methanol ratio of up to 40%, no premature elution of the analytes occurred. At 50% methanol first losses of HOME became obvious, whereas at 60% or more methanol in the washing step all analytes were prematurely eluted to a certain degree. With 30% methanol in the washing step, as it was set in the final procedure, sufficiently clean extracts were achieved, without the danger of analyte loss. Under these conditions, the extraction yield was almost quantitative, i.e. $89.0 \pm 1.2\%$ for 1000 ng/ml OME, $88.9 \pm 1.0\%$ for 500 ng/ml HOME and $86.2 \pm 4.0\%$ for 500 ng/ml OMES, respectively. At concentrations of 50 ng/ml for OME and 25 ng/ml for HOME and OMES each, the respective extraction yields were $94.7 \pm 5.2\%$, $95.5 \pm 4.6\%$ and $95.7 \pm 1.2\%$.

Table 2
Tandem mass spectrometric conditions

Analyte	Parent ion mass (*m/z*)	Collision energy (V)	Product ion mass (*m/z*)	Time window[a] (min)
OME	346	14	198	5–16
D_3-OME	349	14	198	5–16
HOME	362	14	213	8.75–16
D_3-HOME	365	14	216	8.75–16
OMES	362	18	297	5–8.75

[a] Chromatographic run-time window at which the ion trace of the corresponding substance is detected by the mass spectrometer.

Stability with respect to chiral inversion during the extraction procedure was tested by analysis of a serum sample spiked with 200 ng/ml of the pure *S*-enantiomer of OME with the enantioselective assay. No chiral inversion of *S*-OME to *R*-OME was observed, thus the measured enantiomeric ratios in unknown samples could be regarded as uninfluenced by the assay (Fig. 2a).

3.2. Enantioselective chromatographic separation

The choice of a specific chiral stationary phase for a given chiral separation problem is by no means an easy task owing to the fact that achieving enantioresolution is often purely empirical [24]. Prior work on the enantioresolution of OME and HOME suggest that these enantiomers are best separated on chiral stationary phases that are designed for carboxylic acids and are operated in the normal phase mode [18,19,24]. We chose the ReproSil Chiral-CA (CA stands for carboxylic acids) stationary phase because of its factory-demonstrated capability of separating the enantiomers of OME, its robustness against different mobile phase compositions and its comparable low price. Optimization of the enantioresolution was performed with respect to the type and quantity of the alcohol in the hexane/alcohol mobile phase and the acidic and basic modifiers. It turned out that 2-propanol was superior over ethanol or methanol, which both lead to very short retention times and poor enantioresolution even at very low concentration ratios in the mobile phase. The choice of the basic modifier had a great influence on the peak shape. TEA and 1-methylpiperidine caused only minor improvements in the peak shape, whereas the addition of DEA leads to nearly tailing free peaks for all analytes. The choice and concentration

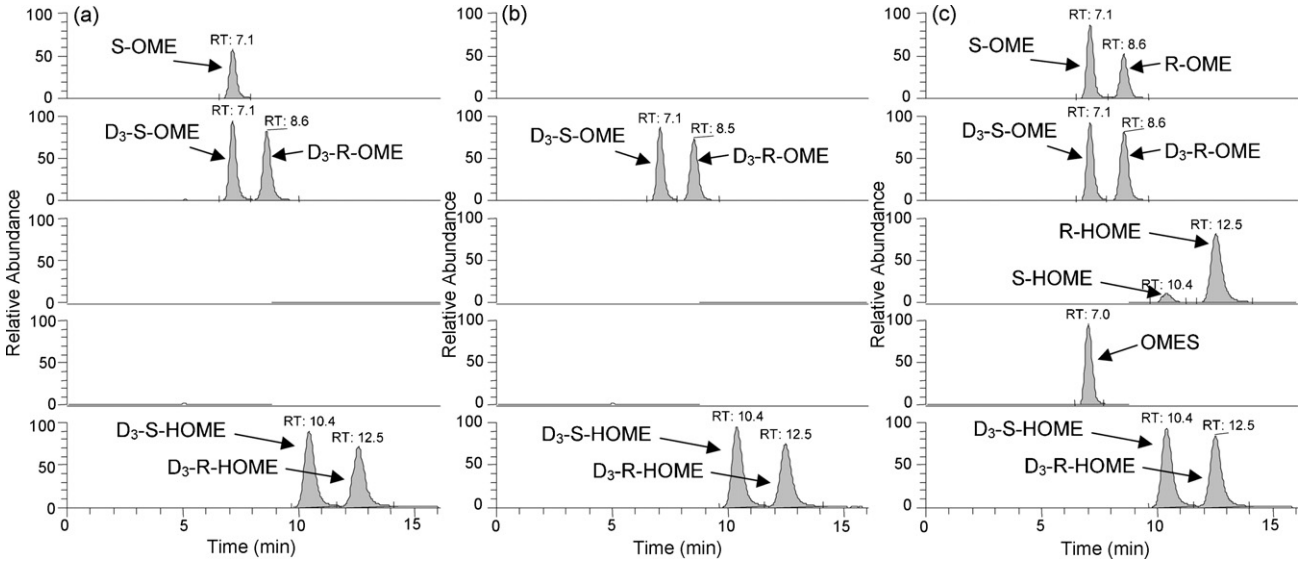

Fig. 2. Typical chromatograms obtained from human serum samples: (a) blank serum spiked with 200 ng/ml *S*-OME, (b) blank serum, and (c) patient serum 2 h after the application of 20 mg OME oral with concentrations of 274.8 ng/ml *S*-OME, 186.5 ng/ml *R*-OME, 95.1 ng/ml OMES, 27.6 ng/ml *S*-HOME and 238.8 ng/ml *R*-HOME, respectively. Depicted are the selected reaction monitoring chromatograms of the fragment ions *m/z* 346 → 198 for OME, *m/z* 349 → 198 for D_3-OME, *m/z* 362 → 213 for HOME, *m/z* 362 → 297 for OMES and *m/z* 365 → 216 for D_3-HOME.

of the acid modifier had only minor effects on peak shape and resolution, so we chose acetic acid in a concentration to obtain an apparent pH of about 4, which is in the middle of the allowed range for the stationary phase. Under these conditions, the stability of the analytical column was satisfying. After more than 800 injections, no degradation in terms of enantiomeric separation, peak shape or back pressure was observed.

The absolute retention order of the *R*- and *S*-enantiomers was determined by analyzing pure *S*-OME. It turned out that the *S*-enantiomers are those to be first eluted under the described conditions. By using an isocratic mobile phase, reasonable retention times for both *R*/*S*-OME and *R*/*S*-HOME in one run could not be achieved, so we diverted to gradient elution. Using this technique, additional time had to be invested for reequilibration of the column after the run, but the time needed for separation could be shortened and the peaks of the late eluting *R*/*S*-HOME become sharper and higher, thus improving detection limits. Under the final conditions, the peaks were sharp and symmetrical and the enantiomers of OME and HOME, respectively, were baseline separated. The retention time of the achiral OMES was under all conditions very close to the retention time of *S*-OME, with considerable peak overlapping, but the tandem mass spectrometric detection provided full selectivity between the two substances (typical chromatograms see Fig. 2).

3.3. Mass spectrometric detection

Mixtures of alcohols with hexane, as they are used in normal phase chiral chromatography, are normally considered as not compatible with ESI or APCI ionization techniques in mass spectrometry due to concerns of potential explosion hazards and poor ionizability [22,25]. A common remedy of these shortcomings is to add post-column make-up liquids containing alcohols and aqueous buffers. However, care has to be taken that such a make-up liquid is completely miscible with the HPLC mobile phase. Furthermore, it unavoidably dilutes the mobile phase and thus worsens the sensitivity of the assay. On the other hand, with APPI a technique is available that is capable of overcoming these problems [25]. Usually a "dopant", i.e. a substance which is easily ionized by the light source and transfers the charged state to the analytes, for example toluene, has to be added to the mobile phase in APPI for effective ionization. However, the 2-propanol-hexane mobile phase used in the assay described here has self-doping properties, making the addition of dopants unnecessary [25]. Under the described conditions, OME and its metabolites were efficiently ionized to the $[M+H]^+$ parent ions by the krypton light source, with signal intensities found to be about a factor of 1.5 increased in comparison to APCI. The selectivity of the assay could be enhanced by detecting the product ions after collision-induced fragmentation of the corresponding parent ions. Applying this tandem MS technique, no interferences from endogenous substances were observed. Also, both metabolites of OME were clearly distinguishable from OME, as well as the isotopic labeled I.S.s from their corresponding natural compounds. On the other hand, OMES and HOME share identical molecular masses and their collision induced fragmentation mass spectra are quite similar, thus making their mass spectrometric distinction impossible. Nevertheless, OMES was chromatographically separated from both enantiomers of HOME, so the quantification of all analytes was not impaired.

Matrix effects on the ionization efficiency of the analytes were investigated by flow injection analysis. In short, a mixture of the analytes were injected into the column effluent constantly by a syringe pump via a tee-union, producing a constant mass spectrometric signal of each analyte. During the chromatographic run of a matrix sample not containing the analytes, any ionization suppression by matrix components

Table 3
Intra- and inter-day precision and accuracy

Analyte	Concentration (ng/ml)	Intra-day precision and accuracy				Inter-day precision and accuracy			
		n	Mean (ng/ml)	R.S.D. (%)	Accuracy (%)	*n*	Mean (ng/ml)	R.S.D. (%)	Accuracy (%)
R-OME	5	10	4.79	2.17	−4.22	5	4.80	2.92	−3.98
	50	10	49.61	2.10	−0.79	5	50.62	2.52	1.24
	750	10	736.43	1.60	−1.81	5	720.02	1.66	−4.00
S-OME	5	10	5.06	1.47	1.17	5	5.05	2.99	0.92
	50	10	50.69	1.23	1.37	5	51.38	1.91	2.76
	750	10	750.60	1.41	0.08	5	729.61	2.07	−2.72
R-HOME	2.5	10	2.436	2.72	−2.57	5	2.49	7.00	−0.20
	25	10	24.84	1.19	−0.65	5	25.51	2.07	2.05
	375	10	362.99	1.52	−3.20	5	357.27	2.07	−4.73
S-HOME	2.5	10	2.49	4.21	−0.54	5	2.58	6.46	3.28
	25	10	24.97	1.00	−0.13	5	25.71	2.13	2.83
	375	10	362.48	1.33	−3.34	5	359.29	1.15	−4.19
OMES	5	10	5.12	4.18	2.36	5	5.45	5.09	9.00
	50	10	53.69	7.77	7.38	5	53.15	8.90	6.30
	750	10	811.78	5.79	8.24	5	769.54	4.32	2.60

would result in an attenuation of the otherwise constant analyte signal. With this experimental set up, we could not detect any obvious matrix effects with three different human serum samples.

3.4. Calibration and limits of detection

Calibration samples were made from pooled blank human serum, which were spiked in the concentration range of 5–750 ng/ml in the case of *R*-OME, *S*-OME and OMES, respectively, whereas *R*-HOME and *S*-HOME were calibrated in the range of 2.5–375 ng/ml. The calibration functions were linear and crossed the origin with no significant deviation. The slopes with their corresponding standard deviations and the correlation coefficients of the calibration functions are summarized in Table 1. The lower limits of quantification were defined as the lower ends of the calibration ranges. The limits of detection were defined as three times the noise in blank chromatograms from pooled human plasma. The values were 0.2 ng/ml for *R*-OME, *S*-OME and OMES, and 1 ng/ml for *R*-HOME and *S*-HOME. The higher limits of detection for the HOME enantiomers resulted from small impurities of unlabeled *R*/*S*-HOME in the I.S. D_3-5-hydroxy-omeprazole, which led to small peaks in the blank chromatograms.

3.5. Precision and accuracy

The application of isotope labeled I.S.s for each enantiomer of both OME and HOME made their quantification very reliable and accurate. The data for inter- and intra-day precision and accuracy are summarized in Table 3. All R.S.D.s and accuracy deviations for these four substances were less than 5%, except the lowest levels of the HOME-enantiomers, where the values were less than 7%. In the case of OMES, we used D_3-*S*-OME as I.S. due to the nearly identical retention times of the two substances. However, the precision and accuracy performance was slightly inferior compared to the other analytes with values of <8% intra-day and <9% inter-day, respectively.

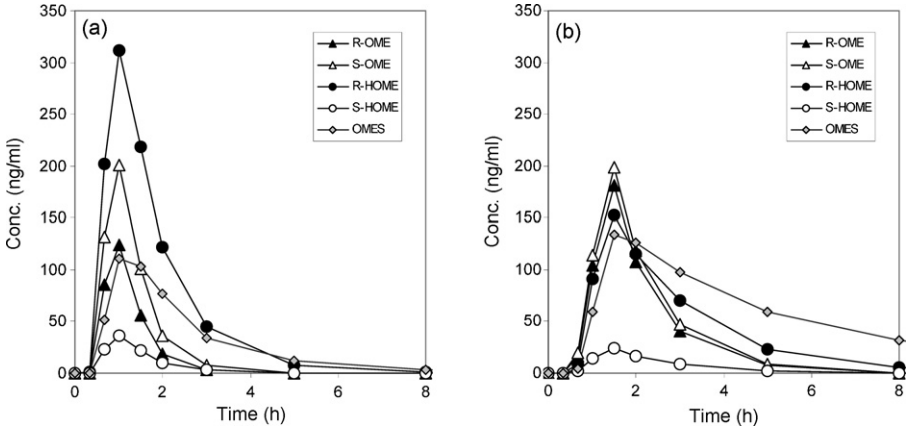

Fig. 3. Concentration–time curves of *R*-OME, *S*-OME, *R*-HOME, *S*-HOME and OMES from (a) a CYP2C19 extensive metabolizer and (b) a CYP2C19 slow metabolizer after a single oral 20 mg OME application.

4. Application of the method

The method described here for the enantioselective quantification of OME and its two main metabolites HOME and OMES is currently used in the course of a study to predict OME therapy efficacy in gastro-esophageal reflux disease with respect to CYP3A4 and CYP2C19 geno- and phenotyping. The complete results of this study will be published elsewhere. As an example, in Fig. 3 the concentration–time courses of OME and its two metabolites are depicted from two subjects after the administration of a single 20 mg OME dose. One of the subjects was genotyped as homozygote CYP2C19 wt/wt extensive metabolizer (Fig. 3a), the other subject as heterozygote extensive (i.e. slow) CYP2C19 mt/wt metabolizer (Fig. 3b). As can be seen in Fig. 3, the slow metabolizer shows levels of *R*/*S*-HOME that are only half the size when compared to the extensive metabolizer. On the other hand, in the slow metabolizer case the concentration–time curves for *R*-OME and *S*-OME are nearly parallel, whereas in the case of the extensive metabolizer *R*-OME concentrations are about 30% lower than *S*-OME concentrations. This comparison reflects the diminished CYP2C19 activity in the slow metabolizer, leaving the *R*-OME levels higher than normal and the *R*/*S*-HOME levels lower than normal.

5. Conclusion

In this work, we present for the first time a method for the simultaneous enantioselective quantification of OME together with its main metabolites HOME and OMES in human serum. The solid-phase extraction sample preparation results in high recoveries and clean extracts. The chromatographic analysis on a ReproSil Chiral-CA column in normal phase mode resulted in sharp and symmetric peaks and separated the enantiomers of OME and HOME with baseline resolution. The APPI ion source was capable to cope efficiently with the normal phase HPLC effluent and the tandem mass spectrometric detection was selective and sensitive enough to follow the concentration–time course of OME and its metabolites for up to 24 h after the application of a single 20 mg OME-dose, uncompromised by any endogenous substances. The use of isotope labeled I.S.s both for OME and its metabolites led to precise and accurate results. With this method it is possible to simultaneously characterize a subject for its CYP3A4 and CYP2C19 phenotype, avoiding the difficulties of genotyping. We applied the method successfully in a clinical study investigating the efficacy of OME treatment with respect to the CYP3A4 and CYP2C19 geno- and phenotype of the patients.

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References

[1] F. Gomollon, X. Calvet, Drugs 65 (Suppl. 1) (2005) 25.
[2] A. Abelo, T.B. Andersson, M. Antonsson, A.K. Naudot, I. Skanberg, L. Weidolf, Drug Metab. Dispos. 28 (2000) 966.
[3] U. Klotz, Int. J. Clin. Pharmacol. Ther. 44 (2006) 297.
[4] B.S. Sheu, A.W. Kao, H.C. Cheng, S.F. Hunag, T.W. Chen, C.C. Lu, J.J. Wu, Aliment Pharmacol. Ther. 21 (2005) 283.
[5] H.M. Gonzalez, E.M. Romero, T.J. Chavez, A.A. Peregrina, V. Quezada, C. Hoyo-Vadillo, J. Chromatogr. B 780 (2002) 459.
[6] Y. Böttiger, Eur. J. Clin. Pharmacol. 62 (2006) 621.
[7] H. Kanazawa, A. Okada, M. Higaki, H. Yokota, F. Mashige, K. Nakahara, J. Pharm. Biomed. Anal. 30 (2003) 1817.
[8] M.C. Dubuc, C. Hamel, M.S. Caubet, J.L. Brazier, J. Liquid Chromatogr. Relat. Technol. 24 (2001) 1161.
[9] K. Kobayashi, K. Chiba, D.R. Sohn, Y. Kato, T. Ishizaki, J. Chromatogr. 579 (1992) 299.
[10] N.L. Rezk, K.C. Brown, A.D.M. Kashuba, J. Chromatogr. B 844 (2006) 314.
[11] M. Shimizu, T. Uno, T. Niioka, N. Yaui-Furukori, T. Takahata, K. Sugawara, T. Tateishi, J. Chromatogr. B 832 (2006) 241.
[12] K.H. Yuen, W.P. Choy, H.Y. Tan, J.W. Wong, S.P. Yap, J. Pharm. Biomed. Anal. 24 (2001) 715.
[13] V.A. Frerichs, C. Zaranek, C.E. Haas, J. Chromatogr. B 824 (2005) 71.
[14] U. Hofmann, M. Schwab, G. Treiber, U. Klotz, J. Chromatogr. B 831 (2006) 85.
[15] H. Kanazawa, A. Okada, Y. Matsushima, H. Yokota, S. Okubo, F. Mashige, K. Nakahara, J. Chromatogr. A 949 (2002) 1.
[16] J. Wang, Y. Wang, J.P. Fawcett, Y. Wang, J. Gu, J. Pharm. Biomed. Anal. 39 (2005) 631.
[17] E.J. Woolf, B.K. Matuszewski, J. Chromatogr. A 828 (1998) 229.
[18] P.S. Bonato, F.O. Paias, J. Braz. Chem. Soc. 15 (2004) 318.
[19] A. Karlsson, S. Hermansson, Chromatographia 44 (1997) 10.
[20] Q.B. Cass, V.V. Lima, R.V. Oliveira, N.M. Cassiano, A.L. Degani, J. Pedrazzoli, J. Chromatogr. B 798 (2003) 275.
[21] R.M. Orlando, P.S. Bonato, J. Chromatogr. B 795 (2003) 227.
[22] H. Stenhoff, A. Blomqvist, P.O. Lagerström, J. Chromatogr. B 734 (1999) 191.
[23] A. Riedel, C.S. Leopold, Drug Dev. Ind. Pharm. 31 (2005) 151.
[24] M.E. Andersson, D. Aslan, A. Clarke, J. Roeraade, G. Hagman, J. Chromatogr. A 1005 (2003) 83.
[25] S.S. Cai, K.A. Hanold, J.A. Syage, Anal. Chem. 79 (2007) 2491.