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Journal of Chromatography B, 795 (2003) 227–235
JOURNAL OF CHROMATOGRAPHY B
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Simple and efficient method for enantioselective determination of omeprazole in human plasma

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Received 6 February 2003; received in revised form 12 June 2003; accepted 16 July 2003

Abstract

A practical and selective HPLC method for the separation and quantification of omeprazole enantiomers in human plasma is presented. C_{18} solid phase extraction (SPE) cartridges were used to extract the enantiomers from plasma samples and the chiral separation was carried out on a Chiralpak AD column protected with a CN guard column, using ethanol:hexane (70:30) as the mobile phase, at a flow rate of 0.5 ml/min. The detection was carried out at 302 nm. The method proved to be linear in the range of 10–1000 ng/ml for each enantiomer, with a quantification limit of 5 ng/ml. Precision and accuracy, demonstrated by within-day and between-day assays, were lower than 10%.

Keywords: Enantiomer separation; Omeprazole

1. Introduction

Omeprazole (Fig. 1) is a known and well studied substituted benzimidazole used in the treatment of gastric diseases due to its capacity to inhibit the (H^+/K^+) ATPase system in the gastric parietal cells [1]. It was demonstrated that both enantiomers have the same in vitro capacity to decrease gastric acid formation [2], but stereoselective metabolism by CYP2C19 results in different plasma concentrations [3]. The major metabolites of omeprazole found in plasma are the (+)-(*R*)- and (−)-(*S*)-hydroxyomeprazole enantiomers and the achiral omeprazolesulphone [4,5]. The CYP2C19 and CYP3A4 are responsible for hydroxylation and formation of omeprazolesulphone, respectively. Recently, this chiral drug has been marketed, by AstraZeneca, as the pure (−)-(*S*)-enantiomer (esomeprazole) under the commercial name of Nexium®. This single isomer is subjected to less first pass metabolism by CYP2C19 and lower plasma clearance than racemic omeprazole, resulting in an AUC almost two times greater than omeprazole, when equivalent doses are administered [6].

Some authors have already described the resolution of omeprazole enantiomers employing chiral columns. Balmer et al. evaluated three protein-based chiral columns (Chiral-AGP, Ultron-ES-OVM and BSA-DSC) using an aqueous mobile phase with 2-propanol as the organic modifier, and an amylose-based chiral column (Chiralpak AD) with a mixture of hexane–ethanol as the mobile phase [7]. A laboratory-made amylose *tris*-3,5-dimethylphenylcarbamate chiral column was

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doi:10.1016/S1570-0232(03)00570-1

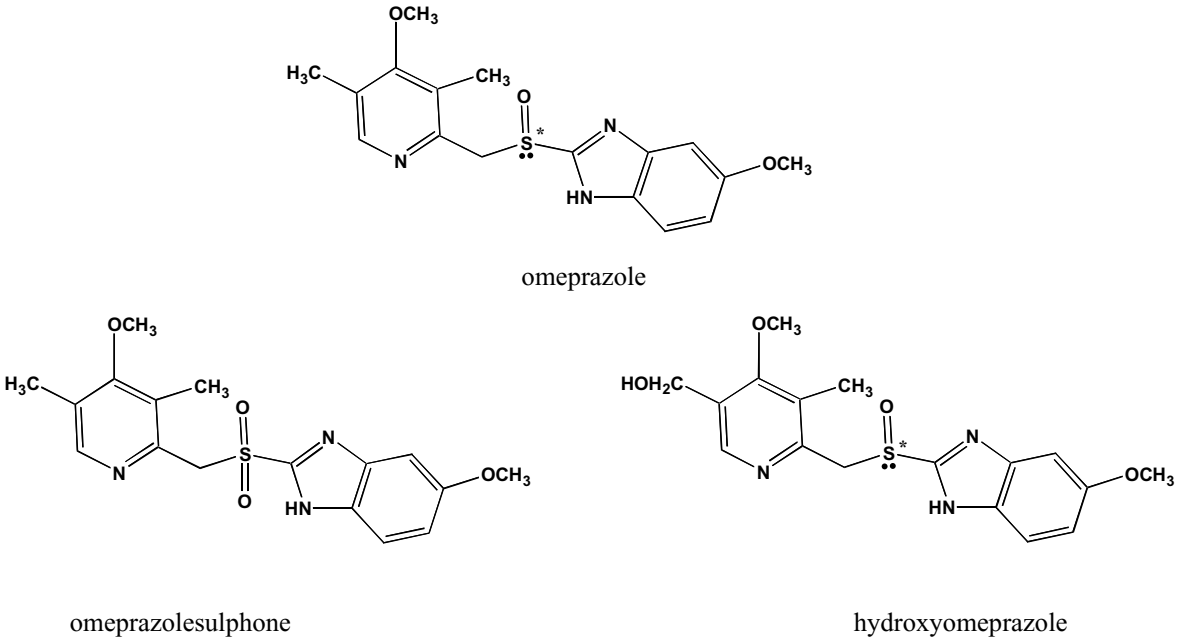

Fig. 1. Structures of omeprazole and the two metabolites hydroxyomeprazole and omeprazolesulphone.

also used by Cass et al. for the resolution of omeprazole enantiomers [8]. In a study of the effect of the omeprazole enantiomers on gastric glands, Erlandsson et al. used a triphenylcarbamoylcellulose-based stationary phase [2]. Tanaka et al. evaluated the celullose-based chiral columns Chiralcel OD-R and Chiralcel OJ-R in the reversed-phase mode [9] and Bonato et al. evaluated several chiral stationary phases based on polysaccharide derivatives and proteins [10].

In spite of this, few methods for the quantification of omeprazole enantiomers in plasma have been described. Cairns et al. used a Resolvosil BSA-7 column and solid phase extraction (SPE) process with C_2 cartridges, obtaining recoveries between 80 and 90% and quantification limit of 15 ng/ml for each enantiomer [11]. Karlsson and Hermansson evaluated and optimized the separation of omeprazole enantiomers on a Chiral-AGP column and applied the method for the analysis of plasma samples submitted to protein precipitation, but no validated data were cited [12]. Tybring et al. also used the Chiral-AGP column to quantify omeprazole and hydroxyomeprazole enantiomers after their liquid–liquid extraction from plasma samples and separation in a C_{18} column. Although the method requires two chromatography steps, a detection limit of 8.5 ng/ml for each enantiomer was obtained [3]. All methods cited above are based on UV detection. Stenhoff et al. validated a method to quantify omeprazole enantiomers in plasma using a Chiralpak AD column, liquid–liquid extraction and mass spectrometry detection with a detection limit 3.45 ng of each enantiomer/ml plasma [13]. Recently, Kanazawa et al., in a study of CYP2C19 phenotyping, determined omeprazole enantiomers in plasma using a Chiralpak AD-RH column, SPE on C_{18} cartridges and circular dichroism and UV detection [14].

The aim of the present study was to develop and validate a method for the quantification of the omeprazole enantiomers in human plasma using simple equipment and procedures suitable for pharmacokinetic and metabolism studies.

2. Experimental

2.1. Chemicals and reagents

Ethanol, methanol, hexane, 2-propanol and acetonitrile used to prepare the mobile phase and in the extraction procedure were all HPLC grade, supplied

by Merck (Darmstadt, Germany). Diethylamine, P. A. grade, was supplied by Fluka (Switzerland). Purified water was obtained from a Milli-Q plus system (Bedford, Massachusetts, USA). In the SPE procedure, Bakerbond C_{18} cartridges (500 mg, 6 ml) obtained from Baker (Phillipsburg, New Jersey, USA), were used with a Supelco Visiprep 24 system (Bellefonte, Pennsylvania, USA).

Omeprazole, hydroxyomeprazole and omeprazole-sulphone were kindly supplied by AstraZeneca (Mölndal, Sweden).

2.2. Instrumentation and chromatographic conditions

The HPLC system used was a LC-AT Vp solvent pump, a 7725 Rheodyne injector with a 50 μl loop, na SPD-10A UV-Vis detector and a Chromatopak CR6A integrator (Shimadzy, Kyoto, Japan). The resolution of omeprazole enantiomers was carried out on the following chiral columns: Chiralpak AD (250 mm × 4.6 mm, 10 μm particle size), Chiralpak AD-RH (150 mm × 4.6 mm, 5 μm particle size) and Chiralcel OD-H (150 mm × 4.6 mm, 5 μm particle size), all purchased from Chiral Technologies (Exton, Pennsylvania, USA). A CN guard column (4 mm × 4 mm, 5 μm particle size, Merck, Darmstadt, Germany) was used to protect the chiral columns during the analyses of plasma samples. The wavelength for detection was adjusted to 302 nm. The analyses were carried out in a thermostated room with the temperature set at 23 (±2) °C.

2.3. Standard solutions

Omeprazole solutions were prepared in methanol alkalinized with 0.1% diethylamine due to its instability in acid conditions [15]. A stock solution of omeprazole was prepared at the concentration of 100 μg/ml of each enantiomer. Working solutions at concentrations of 0.4, 2, 8, 20 and 40 μg/ml of each enantiomer were obtained by appropriate dilutions. All these solutions were stored at −20 °C and protected from direct light.

2.4. Sample preparation

The drug-free plasma samples were obtained from healthy volunteers and stored frozen at −20 °C prior to use. The plasmas were allowed to thaw at room temperature and then mixed and centrifuged. Aliquots of 1 ml were spiked with 25 μl working solutions and mixed for 1 min. The spiked plasma samples were diluted with 2 ml water before being submitted to solid phase extraction onto C_{18} cartridges which had been previously conditioned by eluting 2 ml × 1 ml methanol followed by 2 ml × 1 ml water. The samples were eluted slowly (approximately 2 ml/min) under vacuum and after that, the columns were washed with 5 ml × 1 ml water:5% acetonitrile solution. The columns were dried under vacuum (−15 mmHg) for 15 min and 250 μl methanol were then added to each tube to eliminate the residual water and other interferents. The vacuum was applied for 5 min more. Omeprazole was eluted from the SPE cartridges with 1.7 ml methanol and recovered in conical glass tubes. The elution was carried out under atmospheric pressure and negative pressure was applied only to recover the remaining methanol. The eluate was evaporated under a stream of nitrogen, the residues were dissolved in 100 μl mobile phase and a 50 μl aliquot was injected into the HPLC system.

2.5. Assay specification

Calibration curves were constructed by analyzing human plasma samples (1 ml, $n = 2$ for each concentration) spiked with 25 μl omeprazole standard solutions in the range of 50–1000 ng of each enantiomer/ml of plasma. The results were plotted on a graph of peak height versus plasma concentration and the best relationship was obtained by linear least-squares regression analysis. No internal standard was used. Linearity was determined in a similar way by analyzing human plasma samples (1 ml, $n = 3$ for each concentration) spiked in the range of 10–1000 ng of each enantiomer/ml of plasma.

The quantification limit was assayed by analyzing aliquots of human plasma (1 ml, $n = 5$) spiked at concentrations of 8 and 5 ng/ml of each enantiomer.

The absolute recoveries were determined by comparing the concentration of spiked plasma samples (in the 50–1000 range, $n = 3$ for each concentration) calculated based on a calibration curve constructed by direct analysis of standard solutions of omeprazole in the mobile phase, with the nominal concentration of

these samples. Recovery was expressed as percentage of the amount extracted.

Precision was expressed as coefficient of variation (CV) and accuracy as percent of deviation between the true and the measured value. To assess within-day precision and accuracy, replicate analyses ($n = 10$) of 1 m1 of plasma spiked at concentrations of 64, 160 and 400 ng/ml of each enantiomer were performed. For between-day assays these plasma samples were analyzed for five consecutive days ($n = 2$).

The selectivity of the method was assured by analyzing 25 μl standard solutions of several types of drugs at the concentration of 1 mg/ml. The drug that was detected at similar retention times of omeprazole enantiomers was added to aliquots of 1 ml plasma at their maximum therapeutic concentrations and submitted to the SPE process before chromatographic analysis. Drug-free plasma samples were also analyzed to assess the capacity of sample pretreatment to eliminate endogenous interferents.

Inversion of the configuration of chiral compounds may occur during the extraction procedure, mainly when the chiral center is formed by a sulphoxide group with a free electron pair. In order to verify the occurrence of racemization, omeprazole enantiomers were separated and collected in the chromatographic system. After the mobile phase evaporation, the residues were dissolved in methanol containing 0.1% diethylamine. Then, 1 ml plasma samples ($n = 2$) spike with 25 μl of each enantiomer solution were submitted to the SPE process and subsequent chromatographic analysis.

After validation, the method was used to quantify omeprazole enantiomers in plasma samples collected from a volunteer after oral administration of 20 mg omeprazole. The blood samples were collected into heparinized tubes at 0, 0.5, 1, 2, 3, 5, and 8 h after drug administration and the plasma samples were separated by centrifugation for 10 min at 1800 × *g*. This assay was approved by the Ethics Committee of Escola de Enfermagem de Ribeirão Preto-USP (0081/2000).

3. Results and discussion

The chiral resolution of omeprazole and its metabolites was evaluated using *tris*-3,5 dimethylphenylcarbamate derivative-based chiral columns, i.e Chiralpak AD, Chiralpak AD-RH, and Chiralcel OD-H. Although the method described in this paper was not optimized for the analysis of omeprazole metabolites, the evaluation of their chromatographic behavior was important to avoid their interference. Under the optimized conditions reported on Table 1, the three columns used were suitable for the separation of omeprazole enantiomers and avoided the interference of the metabolites (Fig. 2). The Chiralpak AD column was chosen because it demonstrated the best resolution with a relatively short time of analysis (Fig. 2). The chromatographic behavior of this column did not change significantly when a CN guard column was used. The flow rate employed assured low solvent consumption. The Chiralcel OD-H column was also suitable for the separation of omeprazole enantiomers and avoided the interference of metabolites.

Table 2
Mean recoveries of omeprazole nantiomers

Plasma concentration (ng/ml, $n = 3$)	(+)-(*R*)-Omeprazole		(−)-(*S*)-Omeprazole	
	Recovery (%)	CV (%)	Recovery (%)	CV (%)
50	100.3	1.8	101.4	1.5
200	90.7	2.5	90.8	4.3
500	89.2	1.6	90.2	1.6
1000	96.2	1.4	96.9	0.6
Range (50–1000)	93.8	5.0	94.6	5.2

n: number of samples.

Table 1
Columns evaluated for the resolution of omeprazole enantiomers

Columns[a]	Chromatographic conditions	R_s	k	α
Chiralpak AD-RH	Mobile phase: water:acetonitrile (50:50, v/v), flow rate of 0.5 ml/min	1.18	4.11	1.34
Chiralpak AD	Mobile phase: ethanol:hexane (70:30, v/v), flow rate of 0.5 ml/min	4.30	2.74	1.31
Chiralpak OD-H	Mobile phase: ethanol:hexane:2-propanol (6:91:3, v/v/v), flow rate of 1.5 ml/min	1.80	8.86	1.50

[a] Columns protected by a CN (4 mm × 4 mm, 5 μm particle size) guard column.

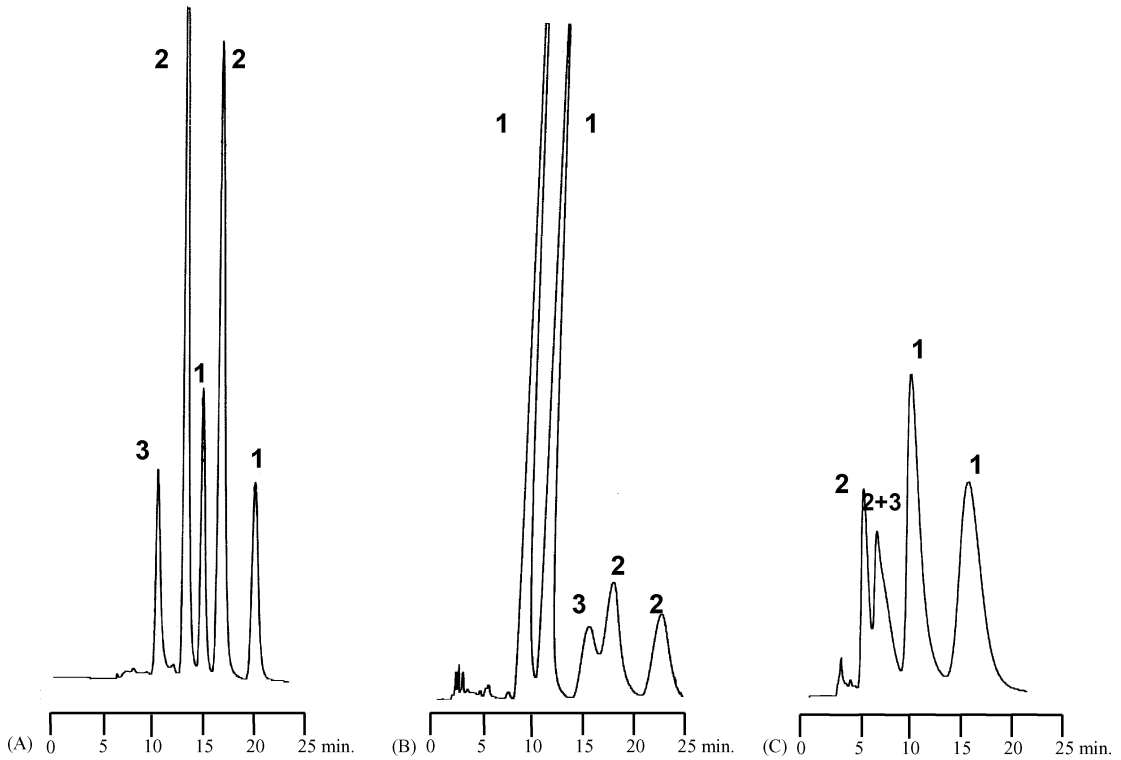

Fig. 2. Chromatograms referring to the separation of omeprazole enantiomers (1) from hydroxyomeprazole (2) and omeprazolesulphone (3) on Chiralpak AD (A), Chiralcel OD-H (B), and Chiralpak AD-RH (C) columns. Chromatographic conditions: (A) ethanol:hexane (70:30, v/v) at a flow rate of 0.5 ml/min; (B) hexane:ethanol:2-propanol (91:6:3, v/v/v) at a flow rate of 1 ml/min and (C) acetonitrile:water (50:50, v/v) at a flow rate of 0.5 ml/min.

Table 3
Precision and accuracy data

Nominal standard concentration (ng/ml)	Analyzed concentration (ng/ml)		Accuracy[a]		Precision[b]	
	(−)-(*S*)-	(+)-(*R*)-	(−)-(*S*)-	(+)-(*R*)-	(−)-(*S*)-	(+)-(*R*)-
Within-day assay of omeprazole enantiomers in plasma ($n = 10$)[c]						
64	62.5	61.8	−2.3	−3.4	4.1	4.1
160	159.6	162.1	−0.2	1.3	2.0	3.3
400	407.3	406.9	1.8	1.7	1.8	1.9
Between-day assay of omeprazole enantiomers in plasma ($n = 5$)[d]						
64	66.3	66.7	3.6	4.2	5.1	5.5
160	158.4	158.4	−1.0	−0.9	2.1	2.9
400	408.3	404.6	2.1	1.2	3.0	3.5

[a] Expressed as percentage of systematic error.
[b] Expressed as coefficient of variation.
[c] Number of samples.
[d] Number of days.

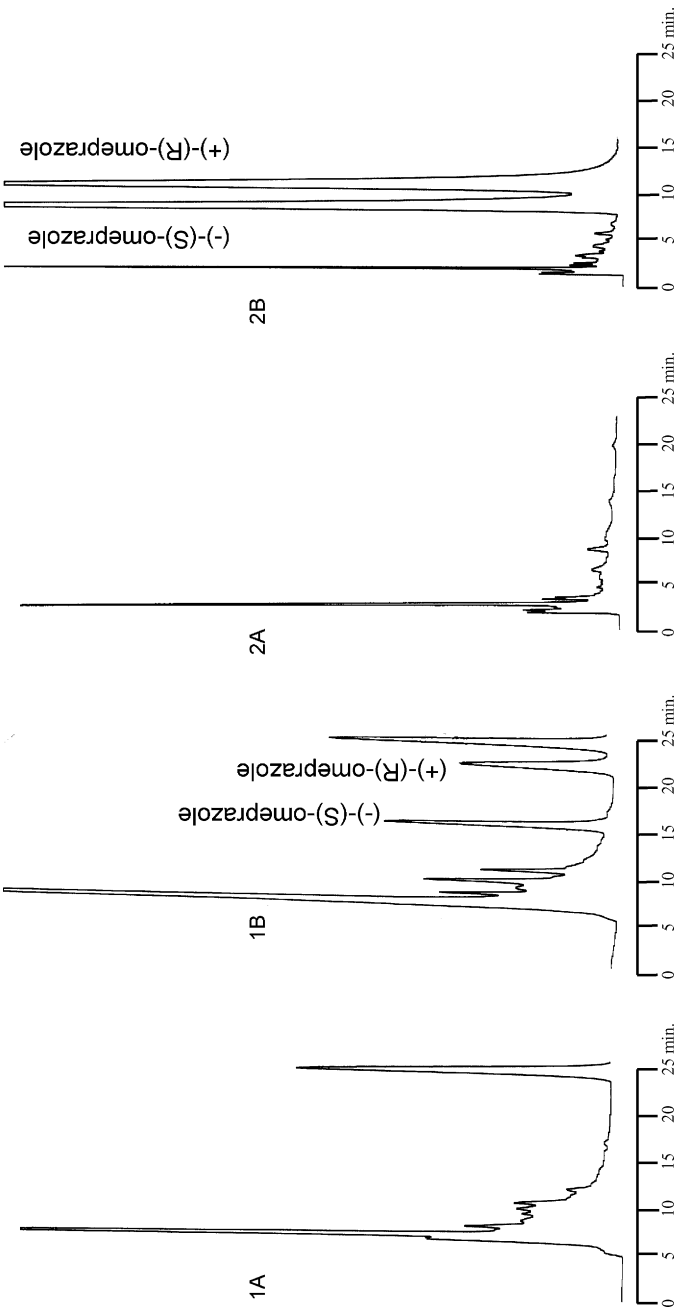

Fig. 3. Drug-free and spiked plasma samples analyzed on Chiralpak AD (chromatograms 1A and 1B, respectively) and Chiralcel OD-H columns (chromatograms 2A and 2B, respectively). Chromatographic conditions are described in Fig. 2.

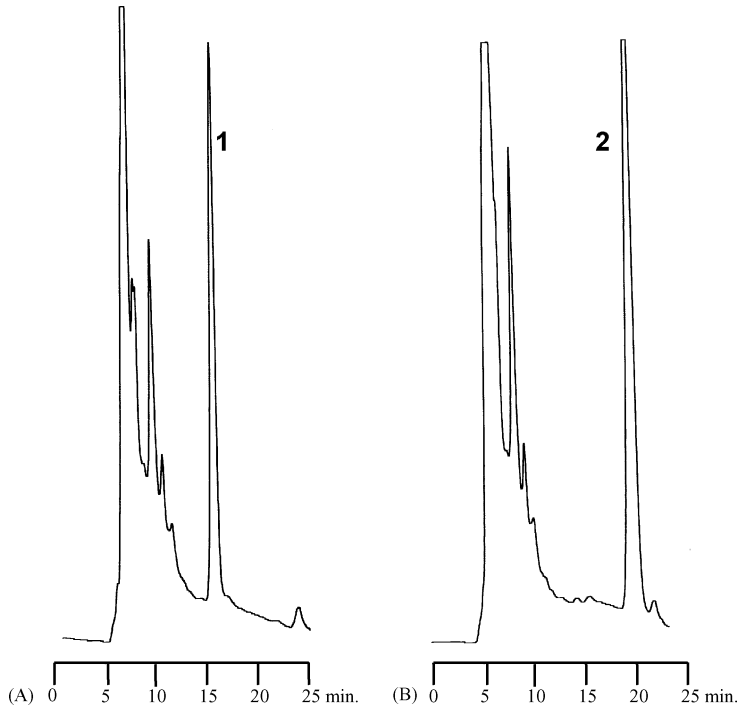

Fig. 4. Racemization during SPE test of omeprazole enantiomers in plasma. Chromatogram A represents (−)-(*S*)-omeprazole (1) and chromatogram B represents (+)-(*R*)-omeprazole (2). Chromatographic conditions are described in Fig. 2.

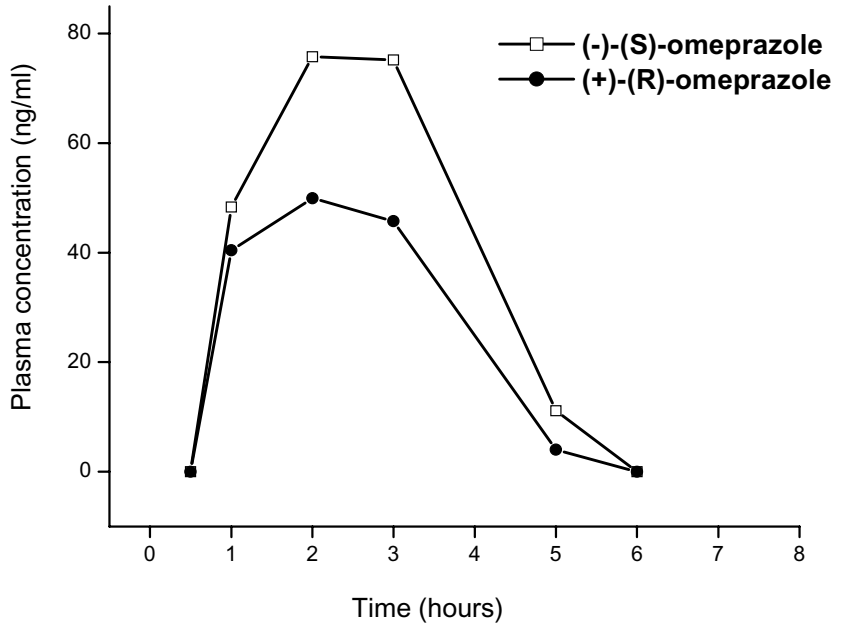

Fig. 5. Time–concentration profile of omeprazole enantiomers after oral administration of the racemic drug.

Table 4
Limit of quantification for the analysis of omeprazole enantiomers in plasma

Nominal standard concentration (ng/ml, $n = 5$)	Analyzed concentration (ng/ml)		Accuracy[a]		Precision[b]	
	(−)-(*S*)-	(+)-(*R*)-	(−)-(*S*)-	(+)-(*R*)-	(−)-(*S*)-	(+)-(*R*)-
5	5.1	5.0	1.2	0.4	4.4	3.4
8	8.3	8.1	1.9	1.8	5.6	5.3

n: number of samples.
[a] Expressed as percentage of systematic error.
[b] Expressed as coefficient of variation.

In addition, the analysis of drug-free plasma samples demonstrated chromatograms without interferents for both columns (Fig. 3). Although the Chiralpak AD-RH column produced a reasonable separation of omeprazole enantiomers from their metabolites, the peaks showed large band width and poor resolution (Fig. 2 and Table 1).

Analysis of pure (−)-(*S*)-omeprazole (obtained from a tablet of Nexium®, AstraZeneca) demonstrated that, under the chromatographic conditions described on Table 1, the first peak analyzed corresponds to the (−)-(*S*)-omeprazole and the second to the (+)-(*R*)-omeprazole in all three columns evaluated.

Table 5
Evaluation of the interference of some drugs with the analysis of omeprazole enantiomers

Drug	t_R	Drug	t_R
(−)-(*S*)-Omeprazole	15.7	Phenytoin	ND
(+)-(*R*)-Omeprazole	22.4	Phenobarbital	ND
Omeprazolesulphone	10.5	Flunitrazepam	15.8
Hydroxyomeprazole	13.0/16.5	Fluoxetine	ND
Salicylic acid	9.6	Haloperidol	10.2
Valproic acid	ND	Imipramine	7.5
(*R*,*S*)-Atenolol	7.5	Levomepromazine	9.6
Alprazolam	12.9	Lidocaine	ND
Amiodarone	8.8	Lorazepam	10.5
Amitriptyline	ND	Mexiletine	ND
Bromazepam	13.1	Pindolol	7.5
Carbamazepine	10.0	Piracetam	ND
Chloramphenicol	7.4	Praziquantel	ND
Chlordiazepoxide	10.5	Procainamide	8.5
Chlormezanone	ND	Propifenazone	7.3
Chlorpromazine	8.7	Propoxiphene	ND
Dexamethasone	11.2	Salbutamol	ND
Diazepam	11.9	Thioridazine	9.8
Dypirone	11.9	Triazolam	17.7
Disopyramide	ND	Trimetroprim	25.0
Fenproporex	ND	Verapamil	8.8
Phenylbutazone	6.6	Warfarin	13.7
Phenylephrine	ND		

t_R: retention time in minutes; ND: not detected by the chromatography system under the analytical conditions used.

SPE was the sample extraction procedure chosen due to the large numbers of adsorbents available, low solvent consumption and high automation capacity [16].

The method proved to be linear in the range analyzed (10–1000 ng/ml), with the typical calibration curve equation determined as $Y = -444.659 + 53.225X$ and $Y = -271.468 + 34.946X$ for the (*S*)- and (*R*)-enantiomers, respectively, and corresponding correlation coefficients of 0.99907 and 0.99922.

Excellent recoveries were obtained with the SPE procedure employed. All mean recoveries were higher than 90% for both enantiomers, with CV values lower than 5% (Table 2).

Precision and accuracy data are presented on Table 3 for within-day and between-day assays. Both assays produced very good results with CV and systematic errors values lower than 6%.

The lowest concentration analyzed to determine the quantification limit (5 ng/ml) demonstrated very good accuracy and precision with marks better than those required for this type of analysis [17,18] (Table 4).

The analysis of plasma samples spiked with pure omeprazole enantiomers did not demonstrate any racemization (Fig. 4). Among the drugs that presented similar retention times to those of omeprazole enantiomers, only flunitrazepam and triazolam were detected in the plasma samples after SPE, showing the high selectivity of the developed method (Table 5).

The results of the analysis of the samples collected from one volunteer were plotted on a graph of plasma

concentration versus time of collection for each enantiomer (Fig. 5). The profile obtained in this study is in accordance with the studies conducted by Tybring et al. [3] and suggests that the volunteer is an extensive metabolizer.

4. Conclusion

This new method using a common UV detector and a C_{18} solid phase extraction cartridge proved to be quite simple and did not require toxic solvents or sophisticated procedures. The Chiralpak AD column used in this assay is one of the most extensively used chiral columns for the resolution of enantiomers. The quantification limit was lower than those reported by Cairns et al. [11] and Tybring et al. [3] and as good as that obtained by Stenoff et al. using expensive mass spectrometry-based method [13]. Furthermore, the good precision, accuracy, and selectivity observed show that it is a suitable alternative for pharmacokinetic and metabolism studies.

Acknowledgements

The authors are grateful to Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) and to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support and for granting research fellowships.

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