Two-Dimensional Chromatography Method Applied to the Enantiomeric Determination of Lansoprazole in Human Plasma by Direct Sample Injection

RICARDO F. GOMES,[1] NEILA M. CASSIANO,[1] J. PEDRAZZOLI Jr.,[2] AND QUEZIA B. CASS[1]*

[1]*Departamento de Química, Universidade Federal de São Carlos, São Carlos, São Paulo, Brazil*

[2]*Faculdade de Medicina, Unidade Integrada de Farmacologia e Gastroenterologia, Universidade São Francisco, Bragança Paulista, São Paulo, Brazil*

ABSTRACT A two-dimensional HPLC method based on the direct injection of biological samples has been developed and validated for the determination of lansoprazole enantiomers in human plasma. The lansoprazole enantiomers were extracted from the biological matrix using an octyl restricted access media bovine serum albumin column (C_8 RAM BSA) and the enantioseparation was performed on an amylose tris(3,5-dimethoxyphenylcarbamate) chiral column using acetonitrile:water (35:65 v/v) and UV detection at 285 nm. Analysis time was 25 min with no time spent on sample preparation. The method was applied to the analysis of the plasma samples obtained from nine Brazilian volunteers who received a 30 mg oral dose of racemic lansoprazole and was able to quantify the enantiomers of lansoprazole in the clinical samples analyzed. *Chirality 22:35–41, 2010.* © 2009 Wiley-Liss, Inc.

KEY WORDS: enantioseparation; restricted access media (RAM); column-switching; biological fluid; polysaccharide chiral phases

INTRODUCTION

The chiral compound lansoprazole (see Fig. 1), 2-[(3-methyl-4-(2,2,2-trifluoethoxy)-2-pyridinyl)methyl]sulfinylbenzimidazole is one of the proton pump inhibitors (PPIs) that inhibits gastric acid secretion through an interaction with (H^+/K^+)-ATPase in gastric pariental cells.[1] This drug, although it has an asymmetric sulfur in its chemical structure, is clinically administered as a racemic mixture of (*R*)-(+)- and (*S*)-(−)-enantiomers and is frequently prescribed for the treatment of acid-related conditions such as gastric and duodenal ulcers, Zollinger–Ellison syndrome, and other hypersecretory diseases.[2] Following oral administration, lansoprazole is extensively metabolized in the liver to 5-hydroxylansoprazole and lanzoprazole sulfone, which are mainly catalyzed by CYP2C19 and CYP3A4, respectively.[3]

A significant number of analytical HPLC methods have been described for the determination of lansoprazole enantiomers in biological fluids.[4–9] All of them, however, used off-line sample clean-up procedures before analysis such as liquid–liquid or solid-phase extraction. The main restriction of these traditional methods is the time spent on sample preparation.

This work reports the first HPLC method for the analysis of plasma levels of lansoprazole's enantiomers by direct sample injection. This was achieved by the use of a two-dimensional chromatography system using an octyl restricted access media bovine serum albumin column (C_8 RAM BSA) in the first dimension for extraction and clean-up and a polysaccharide column, under reversed-phase mode of elution, in the second dimension for the enantioseparation.

The validated method was applied to the analysis of the plasma samples obtained from nine Brazilian volunteers who ingested 30 mg capsules of racemic lansoprazole.

© 2009 Wiley-Liss, Inc.

MATERIALS AND METHODS

General

Acetonitrile was HPLC grade and purchased from Mallinckrodt Baker (St. Louis, MO). Water used for the mobile phase was purified using a Milli-Q system (Millipore, Ribeirão Preto, SP, Brazil).

Bovine serum albumin was purchased from Sigma (fraction V powder minimum 98%; St. Louis, MO). Glutaraldehyde, potassium dihydrogen phosphate, and sodium borohydride were from Merck (Darmstadt, Germany).

The racemic lansoprazole standard was supplied by Boehringer Ingelheim do Brasil (São Paulo, SP, Brazil).

Contract grant sponsors: Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES).

*Correspondence to: Quezia B. Cass, Departamento de Química, Universidade Federal de São Carlos, Cx. Postal 676, São Carlos 13565-905, SP, Brazil. E-mail: quezia@pq.cnpq.br

Received for publication 4 July 2008; Accepted 13 December 2008
DOI: 10.1002/chir.20701
Published online 19 February 2009 in Wiley InterScience (www.interscience.wiley.com).

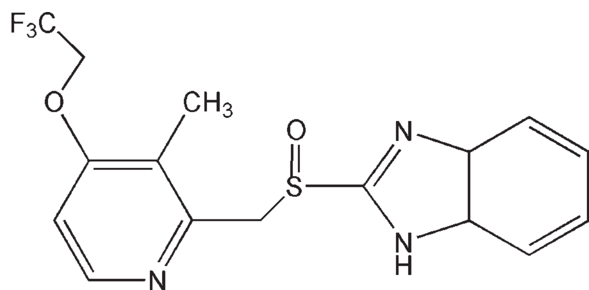

Fig. 1. Chemical structure of lansoprazole.

Ogastro® capsules from Abbott Laboratórios do Brasil (São Paulo, SP, Brazil) containing 15 mg of lansoprazole were purchased at a local drugstore.

The enantiomers of lansoprazole were obtained, as enriched mixture, by chiral separation using a amylose tris[(*S*)-1-phenylethylcarbamate)] coated (20% w/w) onto APS-Nucleosil (7 μm particle size and 500 Å pore size) (150 mm × 4.6 mm I.D.) as stationary phase and hexane:ethanol (70:30 v/v) as mobile phase. The optical rotation of the separated enantiomers was measured in ethanol as reported elsewhere[10] using a Perkin–Elmer Model 241 polarimeter. The absolute configurations for the PPIs have been reported[11] and were used to correlate to the optical rotation obtained of the isolated enantiomers with the highest enantiomeric purity. Then, it was possible to determine the enantiomeric elution order by injection of (*R*)-(+) and (*S*)-(−)-lansoprazole on a chiral column of amylose tris(3,5-dimethoxyphenylcarbamate) coated (20% w/w) onto APS-Nucleosil (7 μm particle size and 500 Å pore size) (150 mm × 4.6 mm I.D.) using acetonitrile: water (35:65 v/v) at a flow rate of 1.0 ml/min.

The administration of the Ogastro® capsules and collection of blood samples from the volunteers were made at São Francisco University Hospital, Bragança Paulista, SP, Brazil. Nine healthy volunteers were used in the study. Pooled control human plasma was also supplied by the University Hospital and stored at −20°C until use.

Written consent was obtained from each volunteer before the study. The protocol was approved by the São Francisco University Medical School Ethics Committee and is in accordance with the Declaration of Helsinki.

Equipment and Columns

The HPLC system consisted of two Shimadzu LC-10ATVP pumps (Kyoto, Japan), with one of the pumps having a FCV-10AL valve for selecting solvent, a DGU-14A degasser model, an autoinjector model SIL 10AVP, a SPD–10AVP UV–vis detector, a SPD-M10AVP diode array detector, and a SCL 10AVP interface. A HPLC 7000 Nitronic EA (Sulpelco, St. Louis, MO) six-port valve was used for the automated column-switching. Data acquisition was done on a Shimadzu CLASS-VP software.

The chiral column of amylose tris(3,5-dimethoxyphenylcarbamate) coated (20% w/w) onto APS-Nucleosil (7 μm particle size and 500 Å pore size) (150 mm × 4.6 mm I.D.) and the RAM BSA-octyl column (Hypersil, 10 μm particle size and 120 Å pore size) (100 mm × 4.6 mm I.D.) were prepared at the UFSCar laboratory as previously described.[12,13]

Sample Preparation

Stock solutions of (±)-lansoprazole were prepared by dissolving the drug in methanol to a final concentration of 200 μg/ml. From these stock solutions, eight calibration standard solutions containing 2.0, 4.0, 8.0, 12.0, 20.0, 25.0, 30.0, and 40.0 μg/ml and three quality controls (QC) solutions at concentrations of 4.8, 24.0, and 36.0 μg/ml were prepared in methanol. To prepare the spiked plasma samples, aliquots (20 μl) of the appropriated solution were placed in a culture tube and the solvent was evaporated under a stream of nitrogen. The dried analytes were reconstituted using 200 μl of plasma (or mobile phase as the solvent for the extraction and transfer evaluation) and the solutions were vortex-mixed for 15 sec. A 180 μl aliquot was transferred to autosampler vials and 100 μl were injected onto the HPLC system.

Column-Switching Procedure

The column-switching system used for the coupling of the RAM and the chiral columns is illustrated in Figure 2. The position of the column-switching device alternated between Positions 1 and 2 and was controlled through the timed events using the Class-VP Software. The time sequence used is listed in Table 1.

Initially, the column-switching was set to Position 1 and the plasma sample was injected into the RAM column. The six-port valve remained in this position for 9.60 min while the macromolecules were discharged into the waste and the analytes were retained on the hydrophobic phase of RAM column. At the same time, the analytical column was conditioned with the mobile phase delivered by Pump 2. Then, the valve changed to Position 2 directing the flow from the waste to the analytical column. The transfer of the effluent fraction containing the analyte took between 9.6 and 12.5 min.

At the end of this time, the valve was switched back to Position 1 for cleaning and conditioning the RAM column while lansoprazole enantiomers were analyzed on the chiral column using acetonitrile:water (35:65 v/v), which was delivered by Pump 2. The flow rate used was of 1.0 ml/min and the absorbance was measured at 285 nm. Analysis time was 25 min.

Method Validation

The following validation parameters were evaluated: selectivity, linearity, recovery, accuracy and precision, limit of quantification (LOQ), limit of detection (LOD), and stability. The validation parameters were in conformity with the internationally accepted criteria.[14]

Chromatograms of drug-free plasma samples, plasma spiked with (±)-lansoprazole, and plasma samples of the volunteers were analyzed to evaluate the selectivity of the method. Plasma samples of the volunteers were assayed using a photodiode array UV–vis detector and the peak purity of each enantiomer was evaluated.

The linearity was evaluated using calibration standards (0.20, 0.40, 0.80, 1.2, 2.0, 2.5, 3.0, and 4.0 μg/ml) prepared

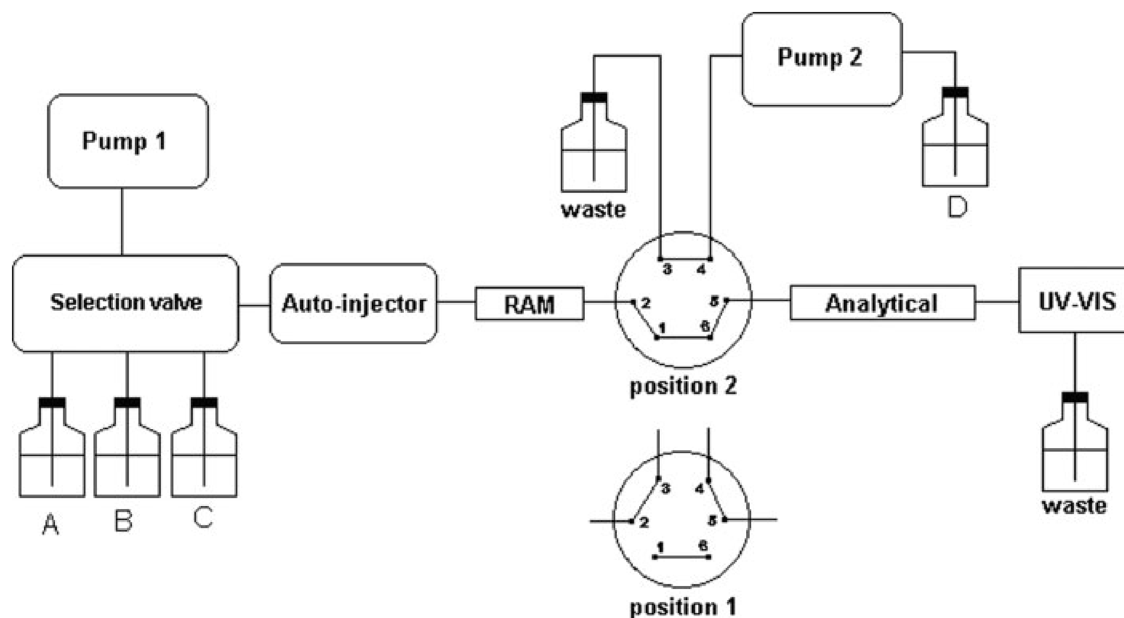

Fig. 2. Schematic diagram of the column-switching HPLC system.

in triplicate. Plasma calibration curves were constructed by plotting the peak area against the concentration of each enantiomer of lansoprazole.

The extraction and transfer efficiency of each enantiomer of lansoprazole (recovery) from human plasma by RAM column was determined by analyzing the QC samples. The efficiencies were calculated by dividing mean peak areas obtained from the spiked plasma samples by mean peak areas obtained from samples of the lansoprazole prepared in mobile phase as the solvent.

The accuracy and precision of the method were determined by quintuplicate analyses at the three QC samples (0.48, 2.4, and 3.6 μg/ml). Calibration standards and QCs were analyzed on three different days to determine the intra and interday variability.

The LOQ and the LOD were measured by preparing spiked plasma samples with serial diluted solutions. LOD was calculated taking a signal-to-noise ratio of 3 as criteria.

The acceptance criteria for the accuracy was to be within ±15% of the accepted true value, except at the LOQ where ±20% were accepted. The precision should not exceed 15% of the coefficient of variation (CV) except for the LOQ, where it should not exceed 20% of the CV.

Stability was evaluated for stock solutions stored and refrigerated at +4°C for 4 mo. The chemical stability of plasma samples containing (±)-lansoprazole and drug-free plasma samples (matrix) were tested in the following conditions: (a) sitting at room temperature for 24 h (autosampler stability) and (b) stored at −70°C for 6 mo (long-term stability). The analytes were considered stable if the variation of the concentrations between the assays were less than 15% of initial time response.

Human Study

A single 30 mg (two capsules containing 15 mg) dose of racemic lansoprazole was administered orally to nine healthy volunteers after an overnight fast. Venous blood samples were collected in heparinized vacutainer tubes at 0 (predose) and 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, and 8.0 h after dosing. The tubes were centrifuged at 2000*g* for 10 min, the plasma collected and stored at −70°C until analysis.

TABLE 1. Time events for the switching of columns and of mobile phases

Time (min)	Pump	Event	Valve position
0.00–5.00	Pump 1 (eluent A)	Plasma proteins are excluded by RAM column	1
5.01–9.59	Pump 1 (eluent B)	Elution of retained components on the RAM	1
9.60–12.50	Pump 1 (eluent B)	Analytes are transferred to the chiral column	2
12.51–25.00	Pump 2 (eluent D)	Analysis of the lansoprazole enantiomers	1
14.01–19.00	Pump 1 (eluent C)	Washing of RAM column	1
19.01–25.00	Pump 1 (eluent A)	Conditioning of RAM column	1

Eluents used in Pump 1: (A) H_2O, (B) CH_3CN:H_2O (35:65 v/v), (C) CH_3CN.
Eluent used in Pump 2: (D) CH_3CN:H_2O (35:65 v/v). Flow rate: 1.0 ml/min, λ: 285 nm.

RESULTS AND DISCUSSION

Method Development

Sample preparation is often considered as the most vital step in HPLC analysis in the bioanalytical field. Proteins present in the matrix can precipitate or denature and adsorb onto the packing material, leading to back-pressure build-up, changes in retention time, and decreased column efficiency. Some of the most commonly used sample preparation techniques include liquid–liquid extraction, protein precipitation, and solid-phase extraction. However, these methods increase the total analysis time and reduce the recovery of the analytes of interest. To eliminate the problems such as coprecipitation of the analytes with proteins and loss of analytes during extraction and to avoid the adsorption of protein onto the analytical column, a large number of different restricted-access media (RAM) supports have been developed to allow the direct injection of biological fluid samples into HPLC system.[15,16] These columns possess the ability of excluding macromolecules, whereas analytes are, generally, retained by hydrophobic interactions.

Cass and collaborators have reported on the preparation and application of a RAM BSA column coupled to a chiral polysaccharide column phase for the analysis of pantoprazole and omeprazole enantiomers[17,18] and for the determination of metyrapol enantiomers and metyrapone[13] by achiral–chiral chromatography in human plasma.

The RAM BSA column can be characterized by a hydrophilic outer phase and a hydrophobic inner phase. Thus, large molecules such as proteins are excluded in the void volume whereas the small hydrophobic analytes are selectively retained.

The column-switching system used for the coupling of the RAM BSA and the chiral columns is the one showed schematically in Figure 2.

The role of the RAM column in this column-switching system is to remove the proteins and fractionate a zone containing the analyte. To determine the elution profile and retention times of the analyte in the plasma matrix, the RAM column was directly connected to UV detector.

The plasma samples were applied when the valve was in Position 1. Water, delivered by Pump 1, eluted mainly the proteins of the sample from the RAM column to waste whereas the analytes were retained on the hydrophobic phase of the sorbent. Five minutes after the sample injection, acetonitrile:water (35:65 v/v) was applied for compression of the lansoprazole chromatographic band, and the switching valve was rotated to Position 2, coupling the RAM column to the chiral column. The switching time was set from 9.60 to 12.5 min to transfer the lansoprazole to the analytical column. The enantioselective analyses were performed using acetonitrile:water (35:65 v/v), which was delivered by Pump 2, at a flow rate of 1.0 ml/min (Position 1; Fig. 2). The RAM column was first cleaned with 100% acetonitrile, and then, it was conditioned with water by Pump 1 while the separation was carried out on the amylose tris(3,5-dimethoxyphenylcarbamate) chiral column.

Previously, a complete study for the enantioresolution of omeprazole, lansoprazole, and pantoprazole using cellulose and amylose tris(3,5-dimethylphenylcarbamate) phases and amylose tris[(*S*)-1-phenylethylcarbamate] and tris(3,5-dimethoxyphenylcarbamate) phases, on multimodal elution, was carried out.[19] Great differences in enantioselectivity for each phase were observed on the different modes of elution for the series of benzimidazoles investi-

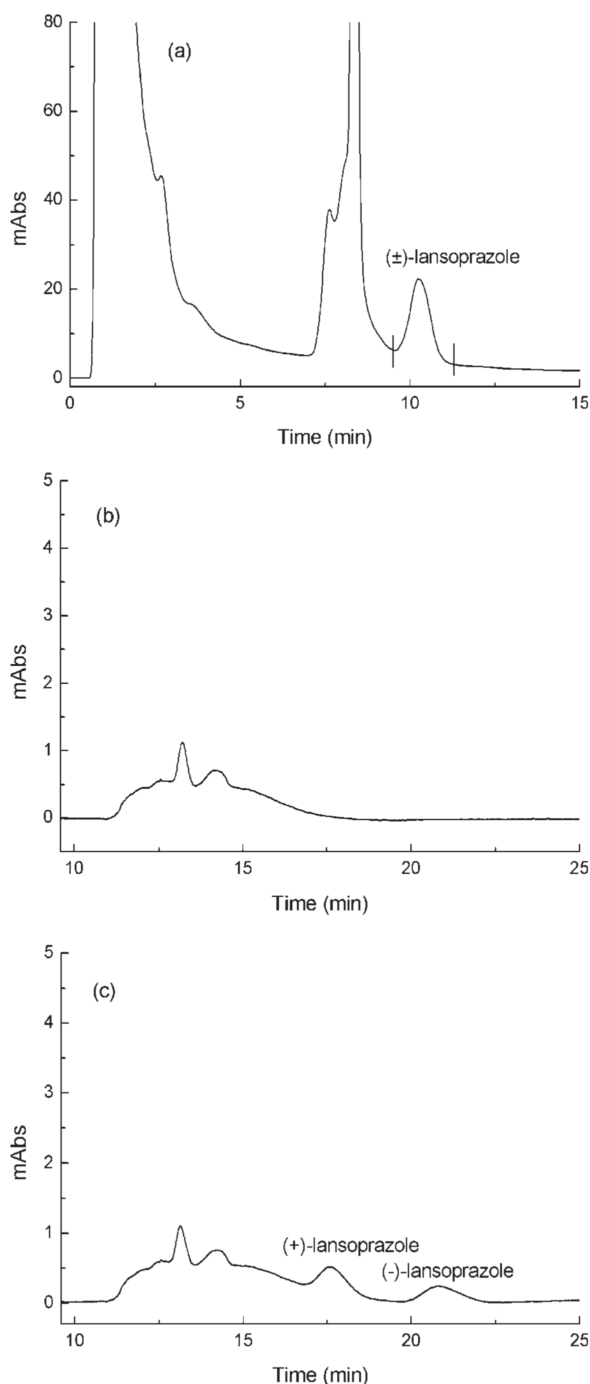

Fig. 3. Typical chromatograms of (**a**) plasma sample from one volunteer collected 1.5 h after an oral dose of 30 mg of (±)-lansoprazole showing the time-window used for transferring the (±)-lansoprazole from the RAM to the chiral column, (**b**) drug-free plasma, and (**c**) spiked plasma with (±)-lansoprazole 0.10 μg/ml.

TABLE 2. Extraction efficiencies of the lansoprazole enantiomers from human plasma ($n = 5$)

Concentration added (μg/ml)	Recovery (%)	
	(+)-Lansoprazole	(−)-Lansoprazole
0.24	90.2	98.7
1.2	92.7	94.8
1.8	89.6	90.7

gated. The amylose tris(3,5-dimethoxyphenylcarbamate) phases showed excellent enantioselectivity for lansoprazole ($\alpha = 1.72$ and $R_s = 3.57$) using acetonitrile:water (50:50 v/v) as solvent. Because reversed-phase conditions are particularly adapted to bioanalytical applications, this polysaccharide-based chiral stationary phase was selected for the work.

Method Validation

The chromatogram of plasma sample from one volunteer collected 1.5 h after an oral dose of 30 mg of (±)-lansoprazole showing the time-window used for transfer the (±)-lansoprazole from the RAM column to the chiral column is illustrated in Figure 3a. Figures 3b and 3c shows the chromatograms, respectively, of the analysis of blank plasma samples and spiked plasma with (±)-lansoprazole 0.10 μg/ml analyzed at the established conditions. No endogenous compounds interfered with the detection of the lansoprazole enantiomers.

Plasma calibration curves were constructed by plotting the peak area against the concentrations of each lansoprazole enantiomer from 0.10 to 2.0 μg/ml. The following regression equations and correlation coefficients were obtained: $y = 259337x - 15982$ ($r = 0.9985$) for the (+)-enantiomer and $y = 259596x - 12659$ ($r = 0.9984$) for the (−)-enantiomer. The CV of each calibration standard ($n = 5$) varied from 1.94 to 11.8% with accuracy that varied from 91.1 to 119% for the (+)-enantiomer and a CV of 1.98 to 8.54% with accuracy of 91.9 to 116% for the (−)-enantiomer.

The lower concentration calibration standard (0.10 μg/ml) was taken as the LOQ in both cases (accuracy within ±20% and CV < 20%). The LOD was 0.025 μg/ml for each enantiomer.

The extraction and transfer efficiencies of each enantiomer of lansoprasole obtained at the three QC levels are given in Table 2. The results show that the extractions for both enantiomers were efficiently performed by RAM column.

The intra and interday accuracy and precision of the method were determined for lansoprazole enantiomers by analyzing five replicates of the three QC plasma samples representing the entire range of the calibration curves (low, medium, and high concentrations) on three different days. The accuracies were evaluated by back-calculation and expressed as the percent deviation between found and added concentrations of each enantiomer and the precision are expressed as CV. Table 3 shows that CV was 2.40–10.0% for (+)-lansoprazole and 2.95–9.18% for (−)-lansoprazole at different concentrations. In addition, the accuracy was estimated to be within ±15% for both enantiomers. These results suggest that the method is accurate and reproducible for the determination of lansoprazole enantiomers in human plasma.

The stock solutions of (±)-lansoprazole prepared in methanol proved to be stable for at least 4 mo when stored at +4°C and no evidence of degradation of the analytes was observed on the chromatograms during this period. The autosampler stability and the long-term storage were evaluated. The permanence in the autosampler tray for 24 h at room temperature (~22°C) had no significant effect on the quantitative determination of lansoprazole enantiomers in plasma samples. No sign of degradation were observed in drug-free plasma samples (matrix) and of plasma samples containing (±)-lansoprazole stored at −70°C for 6 mo (long-term stability).

Application of the Method

The present method was applied for the determination of the enantiomers of lansoprazole in plasma samples of nine healthy volunteers after a 30 mg oral dose of racemic lansoprazole administration. To evaluate if the selectivity of the method was maintained in the presence of lansoprazole metabolites, plasma samples from one volunteer collected at 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, and 8.0 h after the dose administration were analyzed, at the established conditions but using a photodiode array detector and the peak purity of each enantiomer was examined. No interfering metabolites were detected on the samples examined, demonstrating the selectivity of the method.

After analysis of the 70th volunteer plasma sample a compound, which coeluted with the (+)-lansoprazole was detected. Despite this, the QC controls did not reveal this interference. However, the presence of this interfering

TABLE 3. Accuracy and intra- and interday variability for the assay of lansoprazole enantiomers in human plasma

Enantiomers	Concentration (μg/ml)	First day ($n = 5$)		Second day ($n = 5$)		Third day ($n = 5$)		Pooled ($n = 15$)	
		Accuracy (%)	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)
(+)-Lansoprazole	0.24	109	5.15	113	2.98	104	3.17	108	5.06
	1.2	85.0	10.0	87.1	6.85	89.4	2.40	87.2	6.42
	1.8	91.8	2.72	92.3	7.41	88.2	7.37	90.8	5.65
(−)-Lansoprazole	0.24	112	3.21	110	3.70	105	2.95	109	3.27
	1.2	85.0	9.18	87.2	6.57	89.5	2.96	87.2	6.24
	1.8	91.8	2.99	91.8	7.47	88.1	7.80	90.6	6.09

chromatographic band was observed subsequently during the reanalysis of the volunteer and predose samples. Thus, we determined that this interference resulted from memory effect from the RAM column during analysis of the volunteer's sample. The mobile phase used in the chiral column was altered from acetonitrile:water (35:65 v/v) to acetonitrile:water (30:70 v/v) and the interfering band was eliminated. Then, the method was revalidated. A new plasma calibration curve was constructed for each enantiomer ($y = 146290x - 5558.6$; $r = 0.9993$ for the (+)-enantiomer and $y = 144752x - 7756.3$; $r = 0.9979$ for the (−)-enantiomer) and one intra-assay accuracy and precision was determinate by quintuplicate analyses at the three QC samples. All accuracy and precision results led to satisfactory intraday accuracy and precision, with CV $\leq$ 5.82% and accuracy within ±15% for both enantiomers. The analysis of the other clinical samples was performed in this new condition.

The same C_8 RAM BSA and analytical chiral columns were used during the method development and validation and also for the analysis of the clinical samples collected from nine volunteers. The chromatographic performance of both columns was maintained during the complete work. The upper limit of plasma samples has not yet been determined for the RAM BSA columns.[13,17,18,20]

The assay proved to be adequate for establishing pharmacokinetic parameters in all samples evaluated.

Disposition of Lansoprazole Enantiomers in Healthy Subjects

In this study, the plasma concentrations of the (+)-enantiomer were consistently higher than those of the (−)-enantiomer in all plasma samples obtained from nine subjects after oral administration of 30 mg racemic lansoprazole.

The mean ± SD plasma concentration-time profile of both enantiomers in the plasma samples of the nine volunteers are shown in Figure 4 and the chromatogram at Figure 5 shows the analysis of one sample of the same volunteer collected 1.5 h after an oral dose of 30 mg of racemic lansoprazole.

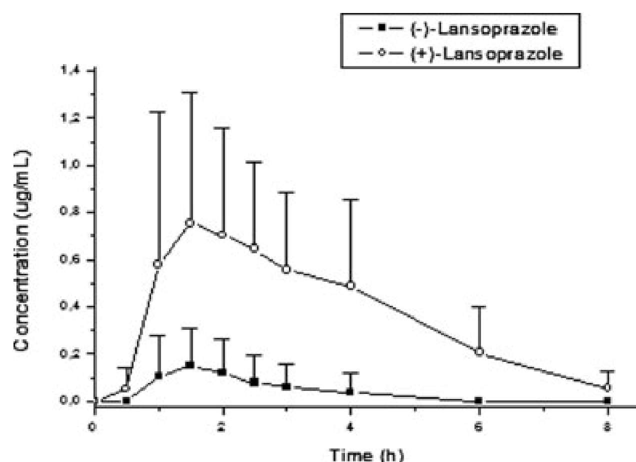

Fig. 4. Mean ± SD plasma concentration-time profiles of (+)-lansoprazole and (−)-lansoprazole after a single oral dose of 30 mg of (±)-lansoprazole.

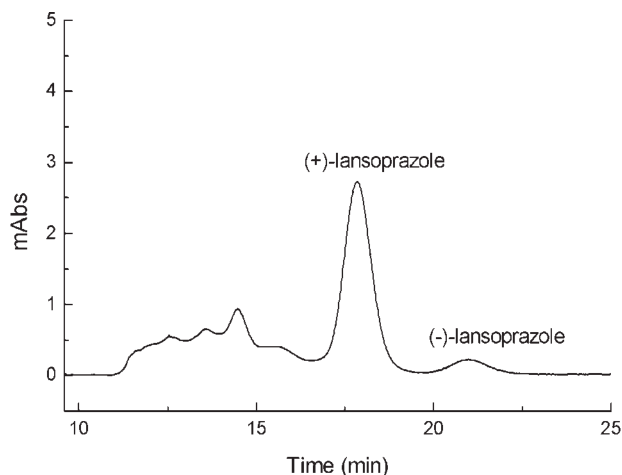

Fig. 5. Chromatograms of plasma sample from one volunteer collected 1.5 h after an oral dose of 30 mg of (±)-lansoprazole.

On the enantioselective pharmacokinetics of lansoprazole, it was reported that the plasma concentrations of the (+)-lansoprazole after oral administration of racemic lansoprazole to rats,[21] dogs,[7] and humans[4,8] were higher than those of the (−)-lansoprazole. In these works, the authors reported that the disposition of the enantiomers of lansoprazole appears to be influenced by enantioselective protein binding and by enantioselective metabolism of lansoprazole in liver microsomes.

The (+)-lansoprazole, which is more extensively bound to serum proteins, is expected to be poorly distributed and slowly metabolized, resulting in higher serum concentrations than those of (−)-lansoprazole. Consequently, enantioselective protein binding might partially influence the enantioselective disposition of lansoprazole after oral administration.[7,8]

The results of the work reported here is in agreement with previously observed results of lansoprazole[8] showing higher concentration of (+)-lansoprazole in the plasma samples of all nine volunteers in the study. Thus, the use of lansoprazole was not able to discriminate the volunteers as extensive or poor metabolizers as happened when pantoprazole[17] and omeprazole[18] were given to this same volunteer group.

CONCLUSIONS

A rapid, selective, and precise HPLC method based on the direct injection of biological samples was developed and validated for the determination of the enantiomers of lansoprazole in human plasma. The HPLC method with automated column-switching and UV detection was performed by coupling a C_8 RAM BSA column to a chiral polysaccharide column. The total analysis time was only 25 min per sample. The RAM BSA column had excellent lifetime and at least 350 plasma samples of 100 μl each were analyzed.

The method was able to quantify the lansoprazole enantiomers in a series of nine Brazilian healthy volunteers who received an oral dose of 30 mg of racemic lansoprazole. The main advantage of this new method over previous published ones is the possibility of performing sample cleanup and determination in a completely automatized system.

LITERATURE CITED

1. Nagaya H, Satoh H, Maki Y. Possible mechanism for the inhibition of acid formation by the proton pump inhibitor AG-1749 in isolated canine parietal-cells. J Pharmacol Exp Ther 1990;252:1289–1295.
2. Barradell LB, Faulds D, McTavish D. Lansoprazole—a review of its pharmacodynamic and pharmacokinetic properties and its therapeutic efficacy in acid-related disorders. Drugs 1992;44:225–250.
3. Pichard L, Curi-Pedrosa R, Bonfils C, Jacqz-Aigrain E, Domergue J, Joyeux H, Cosme J, Guengerich FP, Maurel P. Oxidative metabolism of lansoprazole by human liver cytochromes P450. Mol Pharmacol 1995;47:410–418.
4. Katsuki H, Yagi H, Arimori K, Nakamura C, Nakano M, Katafuchi S, Fujioka Y, Fujiyama S. Determination of R(+)- and S(-)-lansoprazole using chiral stationary-phase liquid chromatography and their enantioselective pharmacokinetics in humans. Pharm Res 1996;13:611–615.
5. Borner K, Borner E, Lode H. Separation of lansoprazole enantiomers in human serum by HPLC. Chromatographia 1998;47:171–175.
6. Katsuki H, Hamada A, Nakamura C, Arimori K, Nakano M. High-performance liquid chromatographic assay for the simultaneous determination of lansoprazole enantiomers and metabolites in human liver microsomes. J Chromatogr B 2001;757:127–133.
7. Masa K, Hamada A, Arimori K, Fujii J, Nakano M. Pharmacokinetic differences between lansoprazole enantiomers and contribution of cytochrome P450 isoforms to enantioselective metabolism of lansoprazole in dogs. Biol Pharm Bull 2001;24:274–277.
8. Kim KA, Shon JH, Park JY, Yoon YR, Kim MJ, Yun DH, Kim MK, Cha IJ, Hyun MH, Shin JG. Enantioselective disposition of lansoprazole in extensive and poor metabolizers of CYP2C19. Clin Pharmacol Ther 2002;72:90–99.
9. Miura M, Tada H, Suzuki T. Simultaneous determination of lansoprazole enantiomers and their metabolites in plasma by liquid chromatography with solid-phase extraction. J Chromatogr B 2004;804:389–395.
10. Belaz KRA, Coimbra M, Barreiro JC, Montanari CA, Cass QB. Multimilligram enantioresolution of sulfoxide proton pump inhibitors by liquid chromatography on polysaccharide-based chiral stationary phase. J Pharm Biomed Anal 2008;47:81–87.
11. Andersson S, Nelander H, Ohlen K. Preparative chiral chromatography and chiroptical characterization enantiomers of omeprazole and related benzimidazoles. Chirality 2007;19:706–715.
12. Matlin SA, Tiritan ME, Crawford AJ, Cass QB, Boyd DR. HPLC with carbohydrate carbamate chiral phases—influence of chiral phase-structure on enantioselectivity. Chirality 1994;6:135–140.
13. Cassiano NM, Cass QB, Degani ALG, Wainer IW. Determination of the plasma levels of metyrapone and its enantiomeric metyrapol metabolites by direct plasma injection and multidimensional achiral-chiral chromatography. Chirality 2002;14:731–735.
14. U.S. Food and Drug Administration. Bioanalytical method validation. In: Guidance for industry. Rockville, MD: U.S. Food and Drug Administration; 2001. p 1–22.
15. Souverain S, Rudaz S, Veuthey JL. Restricted access materials and large particle supports for on-line sample preparation: an attractive approach for biological fluids analysis. J Chromatogr B 2004;801:141–156.
16. Cassiano NM, Lima VV, Oliveira RV, de Pietro AC, Cass QB. Development of restricted-access media supports and their application to the direct analysis of biological fluid samples via high-performance liquid chromatography. Anal Bioanal Chem 2006;384:1462–1469.
17. Cass QB, Degani ALG, Cassiano NM, Pedrazolli J Jr. Enantiomeric determination of pantoprazole in human plasma by multidimensional high-performance liquid chromatography. J Chromatogr B 2002;766:153–160.
18. Cass QB, Lima VV, Oliveira RV, Cassiano NM, Degani ALG, Pedrazolli J Jr. Enantiomeric determination of the plasma levels of omeprazole by direct plasma injection using high-performance liquid chromatography with achiral-chiral column-switching. J Chromatogr B 2003;798:275–281.
19. Cass QB, Degani ALG, Cassiano NM. Effects on enantioselectivity by the use of polysaccharide-based columns by multimodal elution. J Liquid Chromatogr Relat Technol 2003;26:2083–2101.
20. Cass QB, Gomes RF, Calafatti SA, Pedrazolli J Jr. Determination of amoxycillin in human plasma by direct injection and coupled-column high-performance liquid chromatography. J Chromatogr A 2003;987:235–241.
21. Arimori K, Yasuda K, Katsuki H, Nakano M. Pharmacokinetic differences between lansoprazole enantiomers in rats. J Pharm Pharmacol 1998;50:1241–1245.