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Journal of Chromatography B, 783 (2003) 453–459

JOURNAL OF CHROMATOGRAPHY B

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Lansoprazole quantification in human plasma by liquid chromatography–electrospray tandem mass spectrometry

Celso H. Oliveira[a], Rafael E. Barrientos-Astigarraga[b], Eduardo Abib[a], Gustavo D. Mendes[b], Débora R. da Silva[b], Gilberto de Nucci[a,b,*]

[a]*Department of Pharmacology, State University of Campinas, P.O. Box 6111, Campinas, SP, Brazil*
[b]*Cartesius Analytical Unit, Department of Pharmacology ICB-USP, Av. Prof Lineu Prestes, 1524, 05508-900 Sao Paulo, SP, Brazil*

Received 16 May 2002; received in revised form 16 September 2002; accepted 16 September 2002

Abstract

An analytical method based on liquid chromatography with positive ion electrospray ionization (ESI) coupled to tandem mass spectrometry detection was developed for the determination of lansoprazole in human plasma using omeprazole as the internal standard. The analyte and internal standard were extracted from the plasma samples by liquid–liquid extraction using diethyl-ether–dichloromethane (70:30; v/v) and chromatographed on a C_{18} analytical column. The mobile phase consisted of acetonitrile–water (90:10; v/v)+10 m*M* formic acid. The method has a chromatographic total run time of 5 min and was linear within the range 2.5–2000 ng/ml. Detection was carried out on a Micromass triple quadrupole tandem mass spectrometer by Multiple Reaction Monitoring (MRM). The intra- and inter-run precision, calculated from quality control (QC) samples, was less than 3.4%. The accuracy as determined from QC samples was less than 9%. The method herein described was employed in a bioequivalence study of two capsule formulations of lansoprazole.

Keywords: Lansoprazole

1. Introduction

Lansoprazole, 2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridinyl]methyl] sulfinyl]-1H-benzimidazole, is a gastric proton-pump inhibitor and has been demonstrated to be effective in the treatment of duodenal and gastric disorders [1]. The empirical formula is $C_{16}H_{14}F_3N_3O_2S$ with a molecular mass of 369.37 g/mol.

Lansoprazole has been determined in solutions and plasma by different methods like spectroscopy and high-performance liquid chromatography (HPLC) [2,3]. Many studies using HPLC with UV detection show higher ranges in LOQ (between 5.0 and 20 ng/ml) [4–8] and longer retention times (RT) 11 min [4,5]. Landes et al. [9] using a HPLC method with loop column, observed a LOQ of 2.0 ng/ml, but the extraction was more complex with double extraction and two evaporation steps with nitrogen. Furthermore, the RT was approximately 11 min.

In this work, we describe a rapid, sensitive and selective high-performance liquid chromatography coupled to tandem mass spectrometry (LC–MS–

*Corresponding author. Present address: 415 Jesuíno Marcondes Machado Ave., Campinas, SP 13092-320, Brazil. Tel.: +55-19-3251-6928; fax: +55-19-3252-1516.
E-mail address: denucci@dglnet.com.br (G. de Nucci).

1570-0232/02/$ – see front matter
PII: S1570-0232(02)00711-0

MS) method for the quantitation of lansoprazole using omeprazole as the internal standard. The method was developed for a study of bioequivalence of two oral formulations of lansoprazole (30 mg capsule; Lansoprazol from Medley S/A Indústria Farmacêutica, Brazil, as test formulation and Ogastro® from Abbott Laboratórios do Brasil, as reference formulation).

2. Experimental

2.1. Chemicals and reagents

Lansoprazole was provided by Medley, Indústria Farmacêutica, Brazil, lot number WS/BL/L1/4. Omeprazole was obtained from EMS Indústria Farmacêutica Ltd., Brazil, lot number 00098201. Acetonitrile and methanol (HPLC-grade) and dichloromethane and diethyl ether (analytical-grade) were purchased from Mallinckrodt (Paris, KY, USA). Formic acid (analytical-grade) was purchased from Merck (Rio de Janeiro, RJ, Brazil). Ultra-pure water was obtained from an Elga UHQ system (Elga, UK). Blank human blood was collected from healthy, drug-free volunteers. Plasma was obtained by centrifugation of blood treated with the anticoagulant sodium heparin. Pooled plasma was prepared and stored at approximately −20 °C until needed.

2.2. Calibration standards and quality control

Stock solutions of lansoprazole and I.S. were prepared in methanol–water (50:50 v/v) at concentrations of 1 mg/ml. Calibration curves of lansoprazole were prepared by spiking the blank plasma at concentrations of 2.50, 5.00, 10.0, 20.0, 50.0, 100, 200, 500, 1000 and 2000 ng/ml and the analysis was carried out in duplicate for each concentration. The quality control samples were prepared in blank plasma at concentrations of 5.00, 600 and 1500 ng/ml (QCA, QCB and QCC, respectively). The spiked plasma samples (standards and quality controls) were extracted on each analytical batch along with the unknown samples.

2.3. Sample preparation

All frozen human plasma samples were previously thawed at ambient temperature and centrifuged at 2550 *g* for 5 min at 4 °C to precipitate solids. Fifty microliters of the internal standard solution (1 μg/ml omeprazole in 50:50; v/v methanol–water solution) were added to a 200-μl aliquot of plasma sample. The tubes were briefly vortex-mixed and the compounds of interest were extracted with 4 ml of a mixture of diethyl-ether/dichloromethane (70:30; v/v). The mixture was vortex-mixed for approximately 40 s, and the organic phase was evaporated under N_2 at 37 °C. The dry residues were reconstituted to 200 μl with a solution of CH_3CN and H_2O (90:10; v/v) containing 2.5 m*M* of ammonia and vortex-mixed for 15 s. The solutions were then transferred to the auto-injector microvials.

2.4. Chromatographic conditions

An aliquot (20 μl) of each plasma extract was injected into a Genesis C_{18} 4 μm analytical column (150 mm×4.6 mm I.D.) operating at 40 °C. The compounds were eluted by pumping the mobile phase (CH_3CN and H_2O (90:10; v/v) containing 10 m*M* formic acid) at a flow-rate of 0.6 ml/min. Under these conditions, typical standard retention times were 3.3 min for lansoprazole and 3.0 min for omeprazole, and back-pressure values of approximately 50–70 bar were observed.

A split of the column eluant of approximately 1:10 was included so that only 60 μl/min entered the mass spectrometer. The temperature of the autosampler was kept at 5 °C and the run-time was 5.0 min.

2.5. Mass-spectrometric conditions

The mass spectrometer (Micromass model Quattro II) equipped with an electrospray source using a crossflow counter electrode run in positive mode (ES^+), was set up in Multiple Reaction Monitoring (MRM), monitoring the transitions 369.8>251.7 and 346.0>197.7, for lansoprazole and omeprazole, respectively. Fig. 1 shows the full scan spectra (upper trace) and the product ion spectra (lower trace)

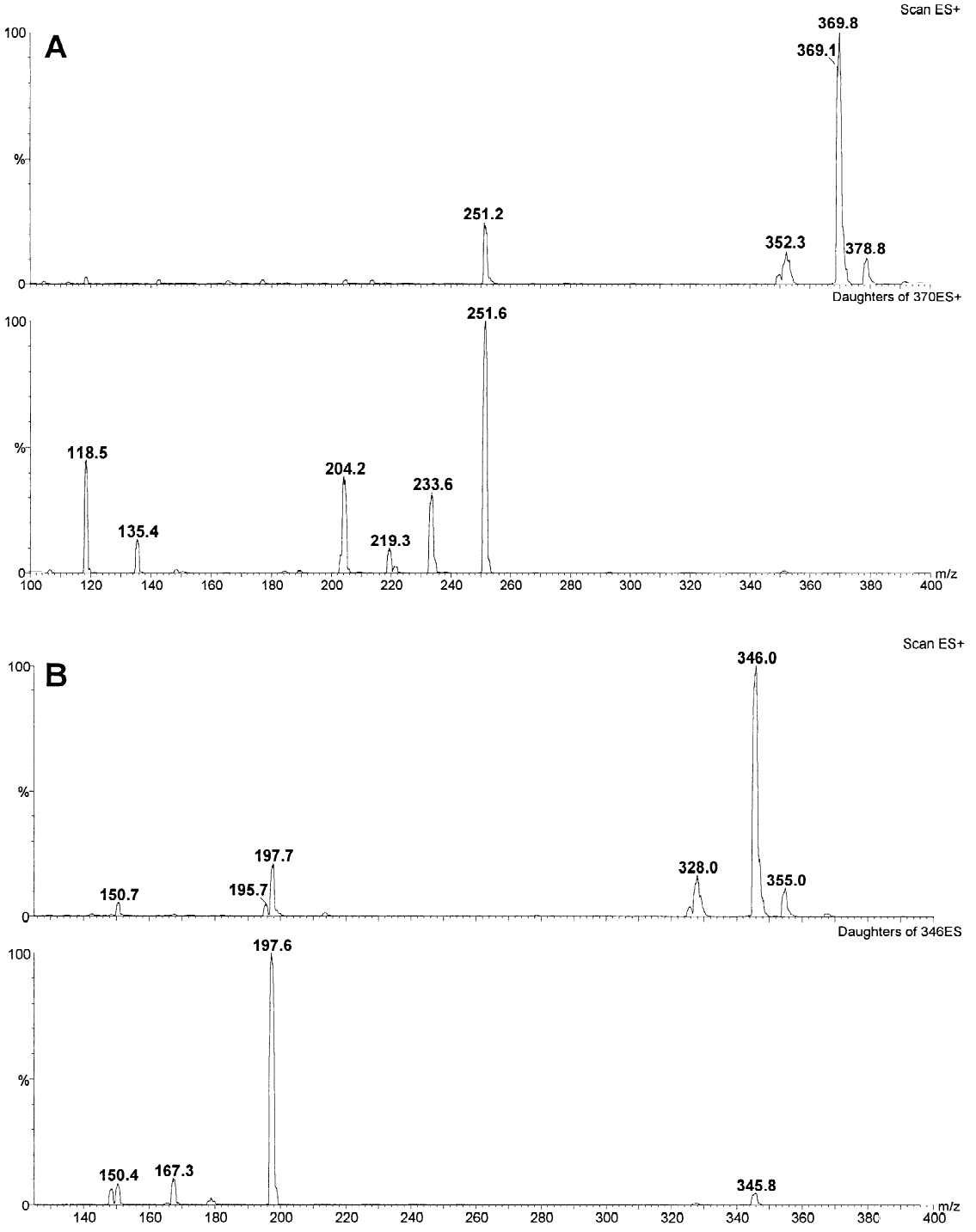

Fig. 1. Full scan mass spectra in upper trace and product ion spectra in lower trace of (panel A) lansoprazole and (panel B) omeprazole.

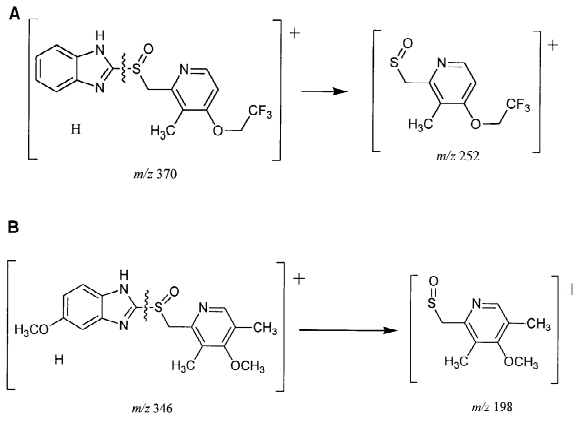

Fig. 2. Chemical structures and fragmentation pathways proposed for lansoprazole (A) and the internal standard omeprazole (B).

obtained for lansoprazole (panel A) and omeprazole (panel B). The proposed fragmentation route for lansoprazole is shown in Fig. 2. The proposed fragmentation for omeprazole has already been described [11].

In order to optimize all the MS parameters, a standard solution of the analyte and I.S. was infused into the mass spectrometer. For both lansoprazole and omeprazole, the following optimized parameters were obtained: the dwell time and the collision gas pressure (argon) were 0.5 s and 1.0×10^{-3} mbar, respectively. The cone voltage and the collision energy were 10 V and 11 eV for lansoprazole and 20 V and 10 eV for omeprazole, respectively. Data acquisition and analysis were carried out using the software MassLynx (v 3.2) running under Windows NT (v 4.0) on a Digital Celebris GL 6200 PC.

2.6. Stability

Stability quality control plasma samples (5.0, 50.0 and 500.0 ng/ml) were subjected to short-term (6 h) room temperature, three freeze/thaw (−20 to 25 °C) cycles and 12 h autosampler (5 °C) stability tests. Subsequently, the lansoprazole concentrations were measured compared with freshly prepared samples and the significance of the results obtained was analyzed by Student's *t*-test ($P<0.05$).

2.7. Recovery

The recovery was evaluated by calculating the mean of the response of each concentration and dividing the extracted sample mean by the unextracted (spiked blank plasma extract) sample mean of the corresponding concentration. Comparison with the unextracted samples, spiked on plasma residues, was done in order to eliminate matrix effects, giving a true recovery. The matrix effect experiments were carried out using the ratio between spiked mobile phase solutions and unextracted samples, spiked on plasma residues.

2.8. Bioequivalence study

The method was applied to evaluate the bioequivalence of two capsule formulations of lansoprazole in healthy volunteers: Lansoprazol (test formulation from Medley Indústria Farmacêutica, Brazil; lot N° 0012016, expiry date December 2002) and Ogastro (standard reference formulation from Abbott Laboratórios do Brasil; lot N° 72167, expiry date February 2003).

Twenty-four healthy volunteers of both sexes were selected for the study. The study was a single dose, two-way randomized crossover design with a 2-week washout period between the doses. Blood samples were collected before and 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12 and 24 h post-dosing.

The bioequivalence between the two formulations was assessed according to US-FDA methodology [10].

3. Results

As shown in Fig. 3, no endogenous peak was observed in the mass chromatogram of blank plasma. The chromatogram for the standard LOQ sample is shown in Fig. 4, in which the retention times for I.S. and lansoprazole were 3.0 and 3.3 min, respectively.

Linearity, precision and accuracy were determined to assess the performance of the method. A linear least-squares regression with a weighting index of $1/x$ was carried out on the peak area ratios of lansoprazole and I.S. versus lansoprazole concen-

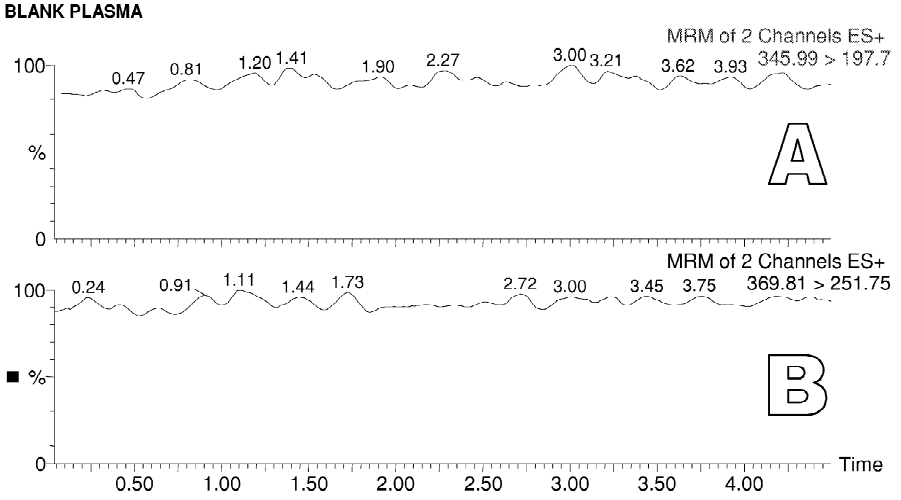

Fig. 3. MRM chromatograms of blank normal human plasma: (A) omeprazole and (B) lansoprazole.

trations of the 10 human plasma standards (in duplicate) to generate a calibration curve. The calibration curves showed good linearity within the range 2.5–2000 ng/ml.

The recoveries observed (value±SD, $n=5$) were 82±5, 92±7 and 82±11% (5.0, 50 and 500 ng/ml, respectively) for lansoprazole, and 74±11% for I.S. (200 ng/ml). No significant (less than 10%) matrix effect was observed.

The lower limit of quantification (LOQ), defined as the lowest concentration at which both precision and accuracy were less than or equal to 20%, was 2.5 ng/ml. Table 1 shows the between-run calibration quality report.

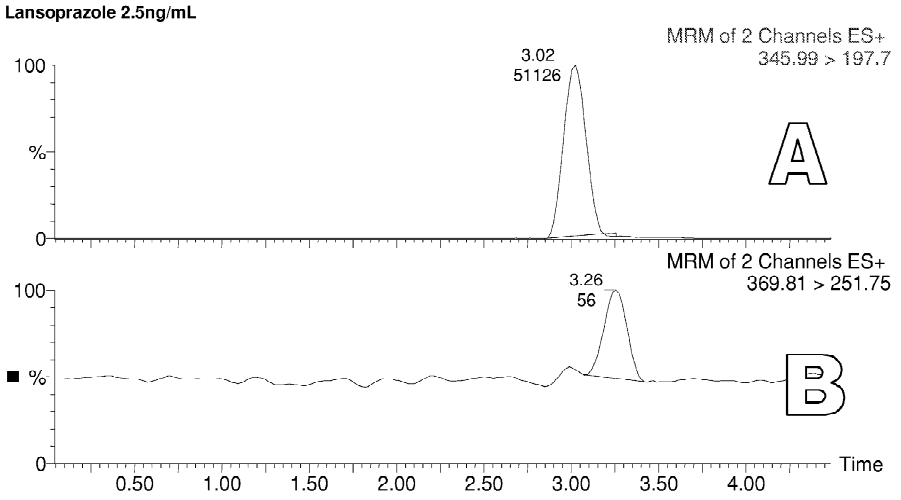

Fig. 4. MRM chromatogram of the LOQ sample (2.5 ng/ml): (A) omeprazole and (B) lansoprazole.

Table 1
Data for quantified concentration (ng/ml) of individual QC samples for intra-batch and inter-batch validation

	Nominal concentration (ng/ml)		
	5.00	600	1500
Intra-batch (n=8)			
Accuracy (%)	−8.3	−7.5	−8.5
Precision (%)	1.9	3.4	1.9
Inter-batch (n=3)			
Accuracy (%)	−7.9	−4.3	−5.0
Precision (%)	0.9	2.8	3.2

Stability analysis was carried out with plasma quality control samples (5.0, 50 and 500 ng/ml). All samples showed no significant degradation under the conditions previously described in the Experimental section.

The geometric mean and respective 90% confidence interval (CI) of Lansoprazol/Ogastro percent ratios were 92.2% (81.6–104.1%) for C_{max}, 93.5 (85.9–101.8%) for AUC_{last}, and 93.4% (85.8–101.7%) for $AUC_{0\text{-inf}}$. T_{max} was also statistically analyzed and the point estimate for individual differences (Lansoprazol/Ogastro) was −0.2 h (90% confidence interval of −0.5 to 0.1 h).

4. Discussion

The fact that the mobile phase contained a low amount of formic acid did not interfere with the analysis since the total run time was relatively short (5.0 min). Although it is well known that lansoprazole and omeperazole are not stable at low pH, no perceivable degradation of the analyte and I.S. was observed under the described conditions. Therefore we should conclude that this time was insufficient for the decomposition of the analyte and I.S. The presence of the acid was necessary in order to improve the detection of the compounds in positive electrospray.

This is the first reported study of plasma level lansoprazole determination using HPLC coupled to tandem mass spectrometry (LC–MS–MS). This method permits an increase in sensitivity and specificity and can be carried out in a shorter time (RT of 2.9 min for lansoprazole). Also, the LOQ observed (2.5 ng/ml) is sufficient for bioequivalence studies. Although deuterium-labelled lansoprazole would be the ideal I.S., omeprazole is commercially available, presents a high chemical similarity to lansoprazole, and it did not affect the good performance of the assay.

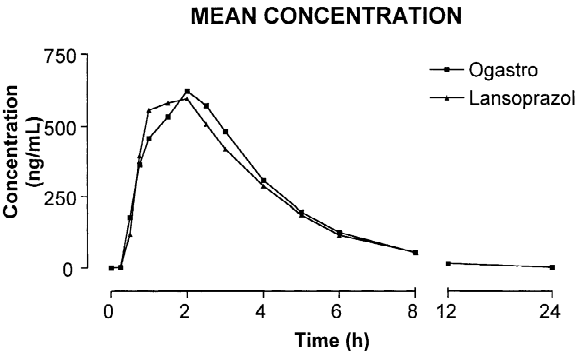

Fig. 5. Mean plasma concentrations versus time curve for two lansoprazole capsule formulations ($n=23$).

After the oral administration of the lansoprazole capsules to the volunteers, the observed lansoprazole peak plasma concentration (C_{max}) values and the time values taken to be achieved (T_{max}) were similar to those reported in the literature [5,9] and equivalent between the formulations (Fig. 5). In addition, the calculated 90% CI for mean C_{max}, AUC_{last} and $AUC_{0\text{-inf}}$ Lansoprazol/Ogastro individual ratios were within the 80–125% interval defined by the US Food and Drug Administration [10].

5. Conclusion

A fast and sensitive LC–MS–MS method for the quantification of lansoprazole in human plasma was developed and validated. The method satisfied the requirements of high sensitivity, specificity and rapid sample throughput required for pharmacokinetic studies.

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