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Short communication

Determination of lansoprazole in human plasma by rapid resolution liquid chromatography–electrospray tandem mass spectrometry: Application to a bioequivalence study on Chinese volunteers

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ABSTRACT

A simple, sensitive and rapid LC/MS/MS method was developed for the quantification of lansoprazole in human plasma. After a simple sample preparation procedure by one-step protein precipitation with acetonitrile, lansoprazole and the internal standard bicalutamide were chromatographed on a Zorbax SB-C_{18} (3.0 mm × 150 mm, 3.5 μm, Agilent) column with the mobile phase consisted of methanol–water (70:30, v/v, containing 5 mM ammonium formate, pH was adjusted to 7.85 by 1% ammonia solution). Detection was performed on a triple quadrupole tandem mass spectrometry by multiple reaction monitoring (MRM) mode via negative eletrospray ionization source (ESI^-). The lower limit of quantification was 5.5 ng/mL, and the assay exhibited a linear range of 5.5–2200.0 ng/mL. The validated method was successfully applied to investigate the bioequivalence between two kinds of preparation (test vs. reference product) in twenty-eight healthy male Chinese volunteers.

1. Introduction

Chemically lansoprazole is 2 {(3-methyl-4-(2,2,2-oroethoxy)-2-pyridyl) methyl} sulfinylbenzimidazole [1]. It is substituted benzimidazole and is a proton pump inhibitor that suppresses gastric acid secretion through an interaction with (H^+/K^+)-ATPase in gastric parietal cells [2]. A substantial number of studies had confirmed that orally 30 mg/day of lansoprazole provided effective symptoms relief and healing of duodenal ulcer in 75–100% of patients after 4 weeks of therapy in non-comparative and comparative trials [1,4]. So far, it has been widely used for the *Helicobacter pylori* eradication therapy in combination with clarithromycin and amoxicillin clinically [3].

At present, determination of lansoprazole had been established by the use of HPLC–UV spectrometry [1,3–8]. Most of them show higher ranges in LLOQ and longer retention times (RT). Recently, Oliveira et al. [9] using a liquid chromatography–electrospray tandem mass spectrometry method was observed in short run, but the preparation of the analyte in plasma using liquid–liquid extraction was complex and time-consuming. Thus in this work, we designed a method using RRLC/MS/MS for determination of lansoprazole with bicalutamide as internal standard. The assay described here required small volume of mobile phase and sample volume, short chromatographic run and was sensitive, specific and fully validated. The method was developed successfully for a study of bioequivalence of two oral formulation of lansoprazole (30 mg lansoprazole table, Zhejiang Hisun Pharmaceutical Co., Ltd., China; 30 mg Takepron capsule, Takeda Chemical Industries, Ltd., Japan).

2. Experimental

2.1. Chemicals and reagents

Standard compounds lansoprazole (>99.8%) and bicalutamide (>99.7%) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

HPLC grade reagents (methanol, acetonitrile, formic acid) were obtained from Merck (Darmstadt, Germany). Water used throughout the experiments was generated by a Milli-Q academic water purification system (Milford, MA, USA). Human plasma was obtained from the Blood Center of Zhejiang (Hangzhou, China).

The test preparation was lansoprazole tablets (batch no. 061001), which was supplied by Hisun Pharmaceutical Co. Ltd. (Zhejiang, China). The reference preparation was lansoprazole cap-

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sules (batch no. 060601), which was commercially obtained from Tianjin Takeda Pharmaceutical Co. Ltd. (Tianjin, China). Both preparations were labeled to contain 30 mg lansoprazole.

2.2. Solution preparation

A concentrated stock solution of lansoprazole (2.2 mg/mL) was prepared in methanol and was further diluted into 55–22,000 ng/mL in 80% (v/v) methanol aqueous solution for the preparation of standard samples. Bicalutamide (100 ng/mL, IS) was prepared in methanol. All the standard solutions were stored at 4 °C and brought to room temperature before use.

2.3. Sample preparation

An aliquot of 20 μL of each lansoprazole standard solution spiked with 180 μL human control plasma and vortex-mixed for 30 s to yield calibration curve samples at concentrations of 5.5, 11, 55, 110, 220, 550, 1100 and 2200 ng/mL. Quality control (QC) samples of lansoprazole at three concentrations (11, 220 and 2200 ng/mL) were also prepared in the same way. The IS solution was added to each standard sample just prior to sample processing.

2.4. Sample processing

Samples were taken out from freezer maintained at −20 °C and kept at room temperature for 30 min for thawing. The samples were vortexed adequately before pipetting. 200 μL of plasma was transferred into 1.5 mL polypropylene tubes and to which 20 μL standard working solutions of IS (100 ng/mL) was added and vortexed for 30 s. Then 500 μL acetonitrile was added to precipitate protein and vortexed for 60 s. After vortexing, all the samples were centrifuged at 12,000 rpm for 5 min. The supernatant was transferred into autosampler vials after filtering through a 0.45-μm syringe filter.

2.5. RRLC/MS/MS analysis

The RRLC analyses were performed on an Agilent 1200 series (Agilent, USA) equipped with a binary pump, micro degasser, Hi-performance well-plate autosampler and thermostated column compartment. The sample was separated on an Agilent Zorbax SB-C_{18} (3.0 mm × 150 mm, 3.5 μm) with an isocratic mobile phase consisted of methanol–water (70:30, v/v, containing 5 mM ammonia formate, pH was adjusted to 7.85 by 1% ammonia solution) at a flow of 0.4 mL/min. The column temperature was maintained at 25 °C, and the injection volume was set at 5 μL. Prior to the analytical column, a C_{18} guard column (Agilent Technologies) was placed to prevent column degradation.

Agilent Technologies 6410 Triple Quad LC/MS equipped with electrospray ionization (ESI) was run by Agilent MassHunter Workstation B.01.03. The compounds were ionized in the negative ion polarity mode. The spray voltage was set at 4000 V. Nitrogen was used as nebulizer gas and nebulizer pressure was set at 45 p.s.i with a source temperature of 105 °C. Desolvation gas (nitrogen) was heated to 350 °C and delivered at a flow-rate of 10 L/min. For collision-induced dissociation (CID), high purity nitrogen was used as collision gas at a pressure of 0.1 MPa. Quantification was performed using multiple reaction monitoring (MRM) mode at m/z 368.2 → 164.1 for lansoprazole and m/z 429.1 → 255.0 for bicalutamide (IS). The fragmentation energies of Q1 for the analytes were set at 120 V. The optimized collision energies of 20 and 10 eV were used for lansoprazole and bicalutamide, respectively. During the data acquisition, the delta potential of the electron multiplier (EMV) was set to 200 V. The peak widths of precursor and product ions were maintained at 0.7 amu at half-height in the MRM mode.

2.6. Validation of the method

Specificity was ascertained by analyzing six blank human plasma samples without adding IS to determine the interference the analyte. Matrix effects for lansoprazole were evaluated comparing the peak areas of analyte in extracted samples blank plasma from six different drug-free volunteers spiked the known concentrations with the corresponding peak areas obtained by direct injection of standard solutions. Matrix effects for the IS were also investigated. Linearity was analyzed by weighted linear regression ($1/x^2$) of analyte–internal standard peak area ratios. The calibration curves ($n = 5$) were prepared by spiking blank human plasma with standard solution of lansoprazole. The LLOQ was the smallest analytical concentration at which the precision expressed by the coefficient of variation (CV) was lower than 20% and the accuracy evaluated by the deviation of the mean from the true value (Dev) was lower than 20%. Intra-day, inter-day precision and accuracy were determined by analyzing QC samples at three different concentrations ($n = 6$) on three different days. The extraction recoveries of lansoprazole at low, medium and high concentrations were determined by comparing the results obtained from the spiked plasmas to standard solutions. Stability tests including three freeze–thaw cycles, storage for 35 days at −20 °C, the treated plasma sample dissolved in mobile phase in autosampler vials at 4 °C for 24 h and the untreated plasma sample at room temperature for 24 h were evaluated by QC samples at three different concentrations.

2.7. Application of the assay

The method described in this paper was applied to a bioequivalence study of two oral formulation of lansoprazole (30 mg lansoprazole tablet, Zhejiang Hisun Pharmaceutical Co., Ltd., China; 30 mg Takepron capsule, Takeda Chemical Industries, Ltd., Japan).

Twenty-eight healthy male volunteers were selected for the study. The study followed a single dose, two-way randomized crossover design with a 1-week washout period between doses. Blood samples were collected at 0, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10, 12 and 16 h post-dosing. Samples were stored at −70 °C until analyzed. The bioequivalence of the two formulations was assessed according FDA guidelines [10].

3. Results and discussion

3.1. Mass spectrometry

Analyte and internal standard responded best to negative ionization, so deprotonated molecular ions $[M-H]^-$ were present as major peaks for both compounds. The full-scan product ion spectra of $[M-H]^-$ showed fragment ions at m/z 368.2 for lansoprazole and at m/z 429.0 for bicalutamide (Fig. 1).

3.2. Chromatography

The chromatographic conditions were investigated to optimize sensitivity, speed, and peak shape. The mobile phase consisting of methanol–water (70:30, v/v, containing 5 mM ammonia formate, pH was adjusted to 7.85 by 1% ammonia solution) was used in the experiment. This was quite different from the detection in positive electrospray described before [9]. They had to contain acid in mobile phase in order to improve the detection in positive electrospray. Although no perceivable degradation of lansoprazole was

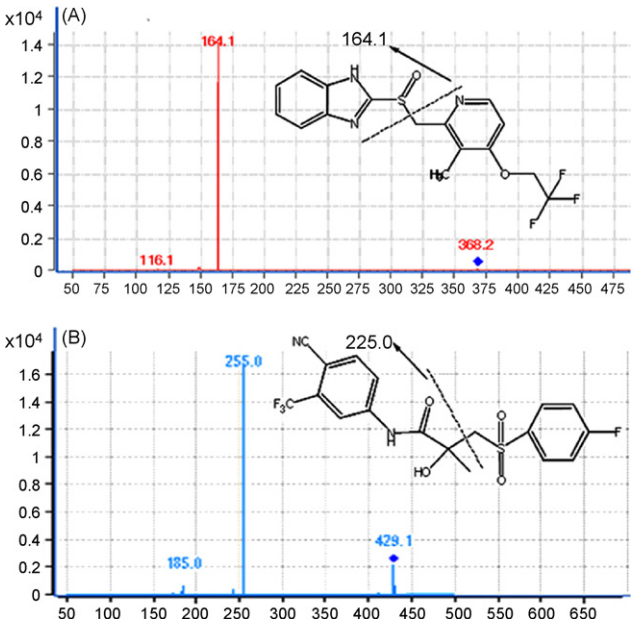

Fig. 1. Chemical structures with fragmentation and produce ion spectrum of $[M-H]^-$ of lansoprazole (A) and bicalutamide (B, IS).

observed under the described conditions in their method, it is well known that lansoprazole is not stable at low pH.

In addition, only a small fraction (5 μL) of the sample after preparation was injected into the column with a flow-rate of 0.4 mL/min to maintain high efficiency and resolution. Under the optimum conditions, analyte and internal standard gave retention times of 3.3 and 3.4 min, respectively, which allowed a high sample throughput.

Appropriate choice of internal standard was important to achieve acceptable method performance, especially in negative ion polarity mode. Bicalutamide was found to be suitable for the quantification of lansoprazole and was easily obtained commercially.

3.3. Assay validation

3.3.1. Specificity

The analysis of analyte and internal standard using the MRM function was highly selective. Representative chromatograms obtained from blank plasma, analyte with a low concentration and internal standard spiked blank plasma and a study plasma sample of volunteer 2.5 h after an oral administration of 30 mg lansoprazole were shown in Fig. 2. Furthermore, there was no interference and significant ion suppression from endogenous substances in matrix.

3.3.2. Calibration curves and LLOQ

The calibration standards were assayed using the method described above. The calibration curves were linear in the concentration range 5.5–2200 ng/mL $Y = 0.0163X + 0.00023$ ($r^2 = 0.9972$).

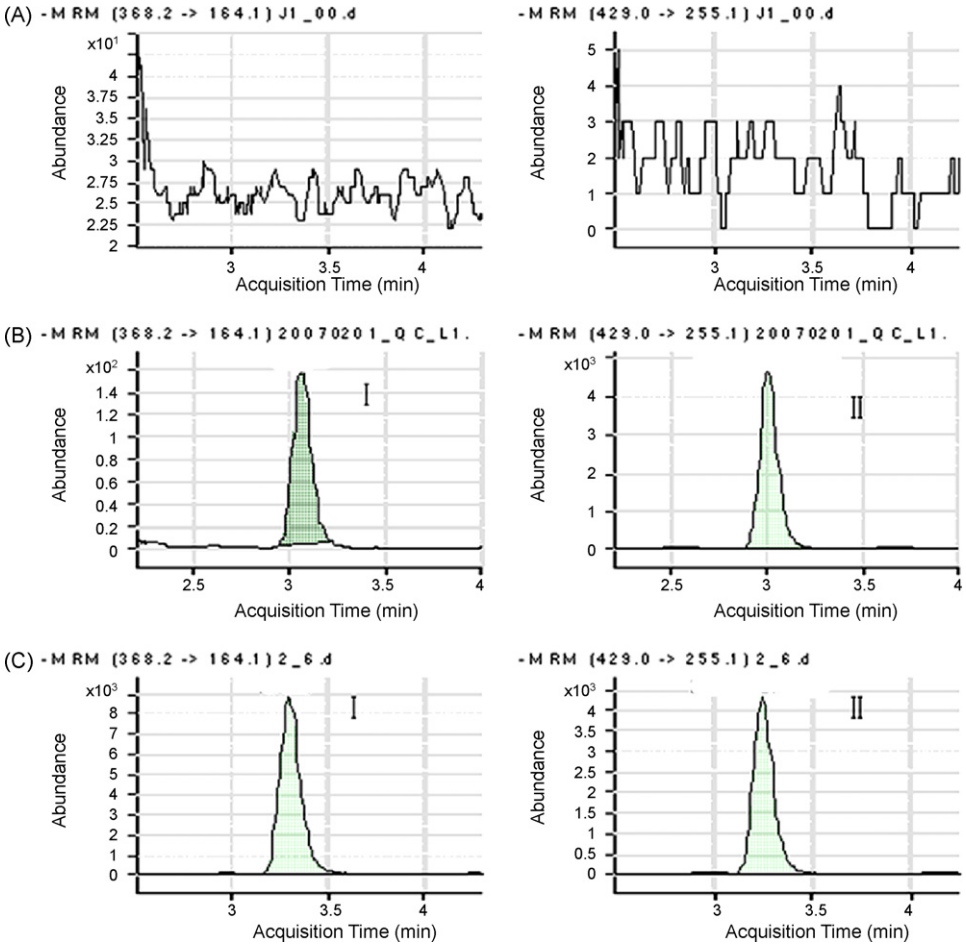

Fig. 2. Representative MRM chromatograms of lansoprazole and bicalutamid (IS) in human plasma samples. (A) Blank plasma; (B) the standard sample at LLOQ (5.5 ng/mL); (C) a plasma sample from a volunteer 2.5 h after an oral administration of 30 mg lansoprazole. Peak |, lansoprazole; peak □, bicalutamide.

Table 1
Precision and accuracy of the developed method for the determination of lansoprazole in human plasma (data were based on assay of six replicates per day, on three different days).

	Spiked concentration (ng/mL)		
	11.0	220.0	2200.0
Intra-assay			
Mean ± S.D.	11.10 ± 0.19	230.19 ± 7.22	2020.29 ± 40.43
CV (%)	1.68	3.13	2.00
Dev (%)	0.94	4.6	−8.16
Inter-assay			
Mean ± S.D.	10.99 ± 0.23	224.94 ± 5.79	2034.28 ± 17.16
CV (%)	2.06	2.57	0.84
Dev (%)	0.09	2.24	−7.53

The LLOQ was 5.5 ng/mL. This ensured the determination of the concentrations of lansoprazole in human plasma 16 h after oral administration of 30 mg lansoprazole in later bioequivalence study.

3.3.3. Precision and accuracy

The QC samples at three concentration levels were analyzed with the method mentioned above. The results of precision and accuracy of the assay were both summarized in Table 1. Intra-day and inter-day precisions were 1.68–3.13% and 0.84–2.57%, respectively, and deviation was below 8.16%. These results indicated that the method was reliable and reproducible within its analytical range.

3.3.4. Recovery and stability

The mean extraction recoveries of lansoprazole from human plasma were 78.5 ± 1.3, 87.5 ± 2.2 and 91.2 ± 2.4% for the final spiked lansoprazole concentrations of 11, 220 and 2200 ng/mL, respectively.

The stability of lansoprazole in human plasma and mobile phase was investigated. The analytes were found to be stable in human plasma stored for 35 days at −20 °C and in mobile phase at room temperature for 24 h (<10% reduction). The analytes were also found to be stable after three freeze–thaw cycles with a reduction of less than 5.66%. The results of the stability tests were shown in Table 2.

3.4. Application of the developed LC/MS/MS method

Safety was evaluated by monitoring adverse events during the whole bioequivalence study in the clinic. The developed LC/MS/MS method had been successfully used in a pilot bioequivalence study of lansoprazole preparations in humans following oral administration. The mean plasma concentration–time curves for two kinds of lansoprazole preparations (test and reference) were shown in Fig. 3. The main pharmacokinetic parameters of lansoprazole in human plasma were shown in Table 3.

Table 2
Stability of lansoprazole in human plasma ($n = 6$).

	Spiked concentration (ng/mL)		
	11.0	220.0	2200.0
Untreated plasma sample at room temperature for 24 h			
Mean ± S.D.	11.28 ± 0.52	214.40 ± 8.17	2236.52 ± 55.82
CV (%)	4.61	3.81	2.50
Dev (%)	2.59	−2.54	1.65
Treated plasma sample in autosampler at 4 °C for 24 h			
Mean ± S.D.	11.42 ± 0.43	232.38 ± 5.56	2023.80 ± 37.19
CV (%)	3.79	2.40	1.83
Dev (%)	3.87	5.63	−8.01
Storage at −20 °C for 35 days			
Mean ± S.D.	10.93 ± 0.52	215.61 ± 12.52	2249.90 ± 174.35
CV (%)	4.84	5.80	7.74
Dev (%)	−0.59	−1.99	2.26
Three freeze–thaw cycles at −20 °C			
Mean ± S.D.	11.18 ± 0.37	232.46 ± 2.70	2083.39 ± 1.48
CV (%)	3.34	2.69	1.48
Dev (%)	1.64	5.66	−5.30

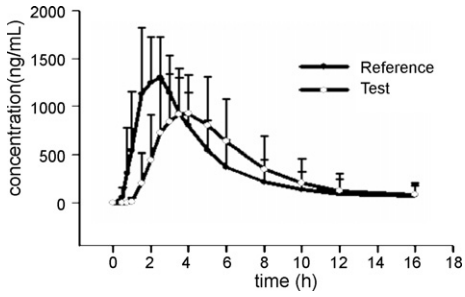

Fig. 3. Mean plasma concentration–time profile of lansoprazole after oral administration of 30 mg test and reference preparations ($n = 28$).

Table 3
Pharmacokinetic parameters of lansoprazole in 28 volunteers after an oral administration 30 mg test and reference preparations (mean value ± S.D., $n = 28$).

Parameters	Test preparation	Reference preparation
$t_{1/2}$ (h)	1.87 ± 0.89	1.94 ± 1.22
T_{max} (h)	3.64 ± 1.21	2.00 ± 0.62
C_{max} (ng/mL)	1274.58 ± 397.26	1560.89 ± 423.22
MRT (h)	3.86 ± 1.14	5.19 ± 1.47
AUC_{0-16} (ng/(mL h))	5645.05 ± 3119.60	5944.64 ± 3141.47
$AUC_{0-\infty}$ (ng/(mL h))	5931.04 ± 3647.03	6215.37 ± 3632.17
F (%)	95.15 ± 13.20	

Both the mean values and standard deviations of the main pharmacokinetic parameters such as C_{max}, T_{max}, AUC_{0-16} and $AUC_{0-\infty}$ were found to be close between test and reference preparations, indicating that plasma profiles generated by test preparations were comparable to those produced by reference preparations. T_{max} and $t_{1/2}$ of lansoprazole in human plasma were similar to those reported in the literature [2,11]. In addition, the calculated 90% CIs for mean C_{max}, AUC_{last} and $AUC_{0-\infty}$ of two drugs lay within the FDA's accepted range of 80–125%. Therefore, it could be concluded that the two lansoprazole preparations (test and reference) analyzed were bioequivalent in terms of rate and extent of absorption.

4. Conclusion

A sensitive LC/MS/MS method for lansoprazole quantitation in human plasma was validated. The method involved a sample preparation with adequate recovery by one-step protein precipitation with acetonitrile. This method provided superior sensitivity with the lower limit of quantitation as low as 5.5 ng/mL for lansoprazole. And it had been successfully applied to bioequivalence study.

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