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Simultaneous determination of lansoprazole and its metabolites 5′-hydroxy lansoprazole and lansoprazole sulphone in human plasma by LC–MS/MS: Application to a pharmacokinetic study in healthy volunteers

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ABSTRACT

A highly sensitive and specific liquid chromatography coupled with tandem mass spectrometric (LC–MS/MS) method has been developed and validated for the simultaneous determination of lansoprazole and its metabolites 5′-hydroxy lansoprazole and lansoprazole sulphone. The detection was operated with multiple reaction-monitoring (MRM) using the electrospray ionization technique. The assay procedure involved precipitation of plasma samples with acetonitrile after indapamide was added as internal standard (IS). The chromatographic separation was achieved with a mixture of methanol–0.2% ammonium acetate and 0.1% methanoic acid in water (75:25, v/v) as mobile phase on an Inertsil ODS-3 column. The method was proved to be accurate and precise with linearity ranges of 10–4000 ng/ml, 5.0–400 ng/ml, and 1.0–400 ng/ml for lansoprazole, 5′-hydroxy lansoprazole and lansoprazole sulphone, respectively, with the correlation coefficients (r) better than 0.999. The lower limits of quantification (LLOQ) were 2.0 ng/ml, 2.0 ng/ml, and 0.5 ng/ml for lansoprazole, 5′-hydroxy lansoprazole and lansoprazole sulphone, respectively. The intra- and inter-day precision and accuracy values were found to be within the assay variability limits (R.S.D.% within ±15) in accordance with FDA guidelines. The validated LC–MS/MS method has been successfully applied for the determination of lansoprazole and its metabolites in human plasma.

1. Introduction

Lansoprazole [2-{(3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl)methyl}sulfinylbenzimidazole], a benzimidazole derivative, is a proton pump inhibitor that acts on the membrane H^+/K^+-ATP (adenosine triphosphatase) in gastric parietal cells [1]. It is effective in the treatment of various peptic diseases, including gastric and duodenal ulcer, reflux esophagitis, Zollinger–Ellison syndrome [2], and other hyperacidic-related conditions. Lansoprazole is metabolized in the liver and the main metabolites are 5′-hydroxy lansoprazole and lansoprazole sulphone. Formation of the 5′-hydroxy metabolite is mainly by cytochrome P4502C19 (CYP2C19), whereas CYP3A4 is involved in the formation of the sulphone [3,4]. It is clinically important to measure CYP2C19 activity using the hydroxylation and sulfoxidation indexes of lansoprazole, which reflects phenotype, and genotype of CYP2C19 [5].

Several high-performance liquid chromatographic (HPLC) methods for the determination of plasma lansoprazole and lansoprazole metabolites concentrations have been reported [1,6–9]. However, they were not highly selective to obtain concise pharmacokinetic parameters of lansoprazole and its metabolites. In this paper, a simple and sensitive liquid chromatography tandem mass spectrometric method for the simultaneous determination of lansoprazole and its main metabolites in human plasma is described, and the method was developed for a study of pharmacokinetic analysis.

2. Experimental

2.1. Chemicals and reagents

Lansoprazole test enteric-coated capsules (30 mg of lansoprazole per capsule; Lot No. 061001) were supplied by Takeda Pharmaceutical Co. Ltd. (Tianjin, PR China). The reference

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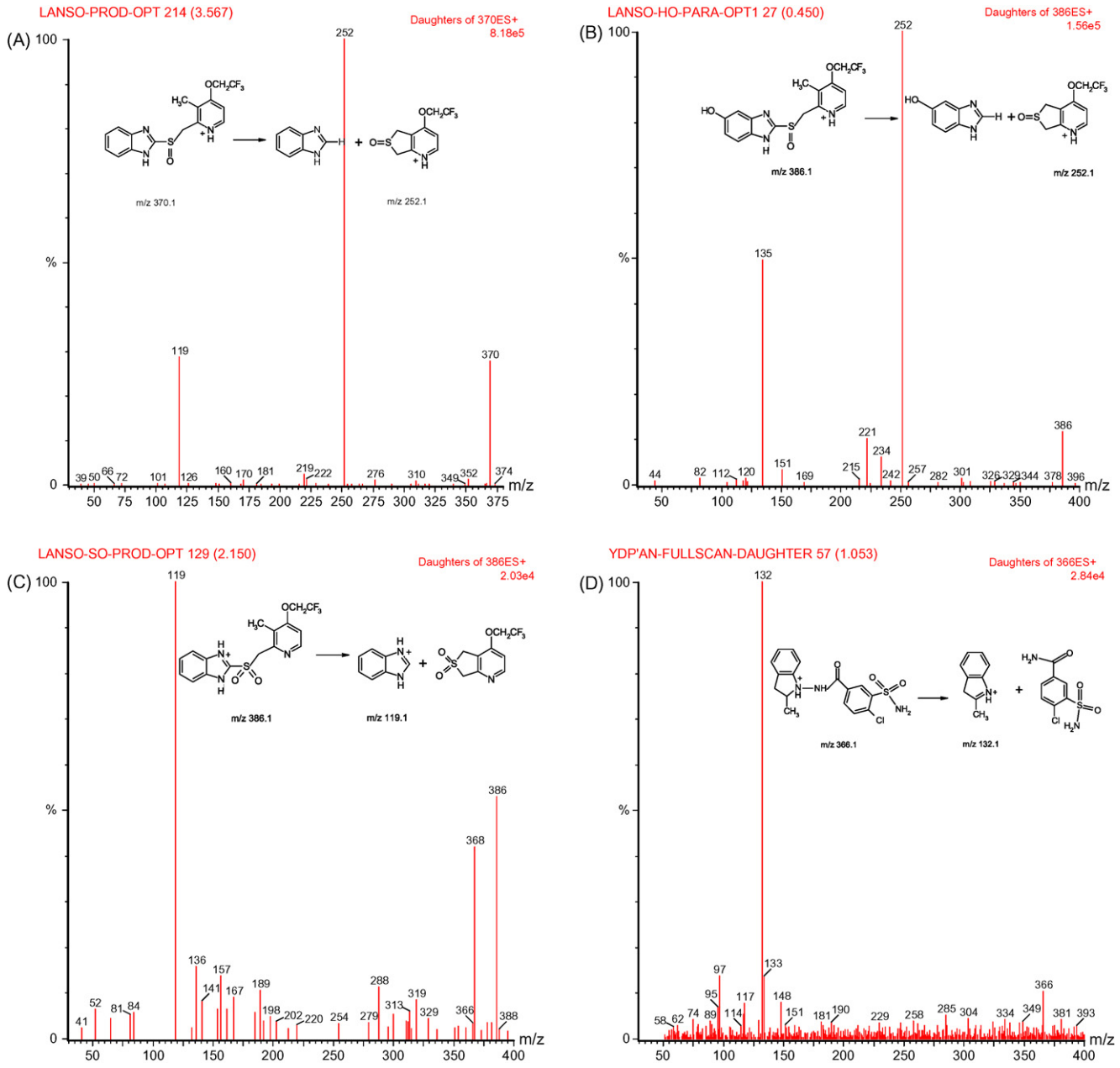

Fig. 1. Full scan positive ESI product ion mass spectra and the proposed patterns of fragmentation of: (A) lansoprazole, (B) 5′-hydroxy lansoprazole, (C) lansoprazole sulphone, and (D) indapamide.

standard of lansoprazole and its metabolites 5′-hydroxy lansoprazole and lansoprazole sulphone (>99.0% purity) were purchased from Jing Long PharmaTech (Nanjing, PR China). Indapamide (IS) reference standard (99.5% purity) was procured from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, PR China). HPLC-grade methanol and acetonitrile were purchased from Tedia Company Inc. (Fairfield, OH, USA). Methanoic acid and ammonium acetate were all purchased from Nanjing Chemical Reagent Factory (Nanjing, PR China). Water was prepared with double distillation.

2.2. Instrumentation

A Waters Alliance 2695 LC was coupled with a Micromass Quattro-micro triple-quadrupole mass spectrometer (Micromass, Manchester, UK) which was equipped with an electrospray ion source. The LC–MS/MS system was operated with MassLynx software (Version 4.0).

2.3. Chromatographic and MS/MS conditions

Liquid chromatography was performed on an Inertsil ODS-3 column (250 mm × 4.6 mm i.d., 5 μm particle size) at 35 °C temperature. A mixture of 75 volumes of methanol and 25 volumes of 0.2% ammonium acetate and 0.1% methanoic acid in water was used as mobile phase, which was pumped at a flow rate of 1.0 ml/min and 30% of the eluent was split into the inlet of the mass spectrometer.

Quantification was achieved by MS/MS detection via an ESI interface. MS/MS conditions were optimized as follows: the spray voltage was set at 4 kV with the source tempera-

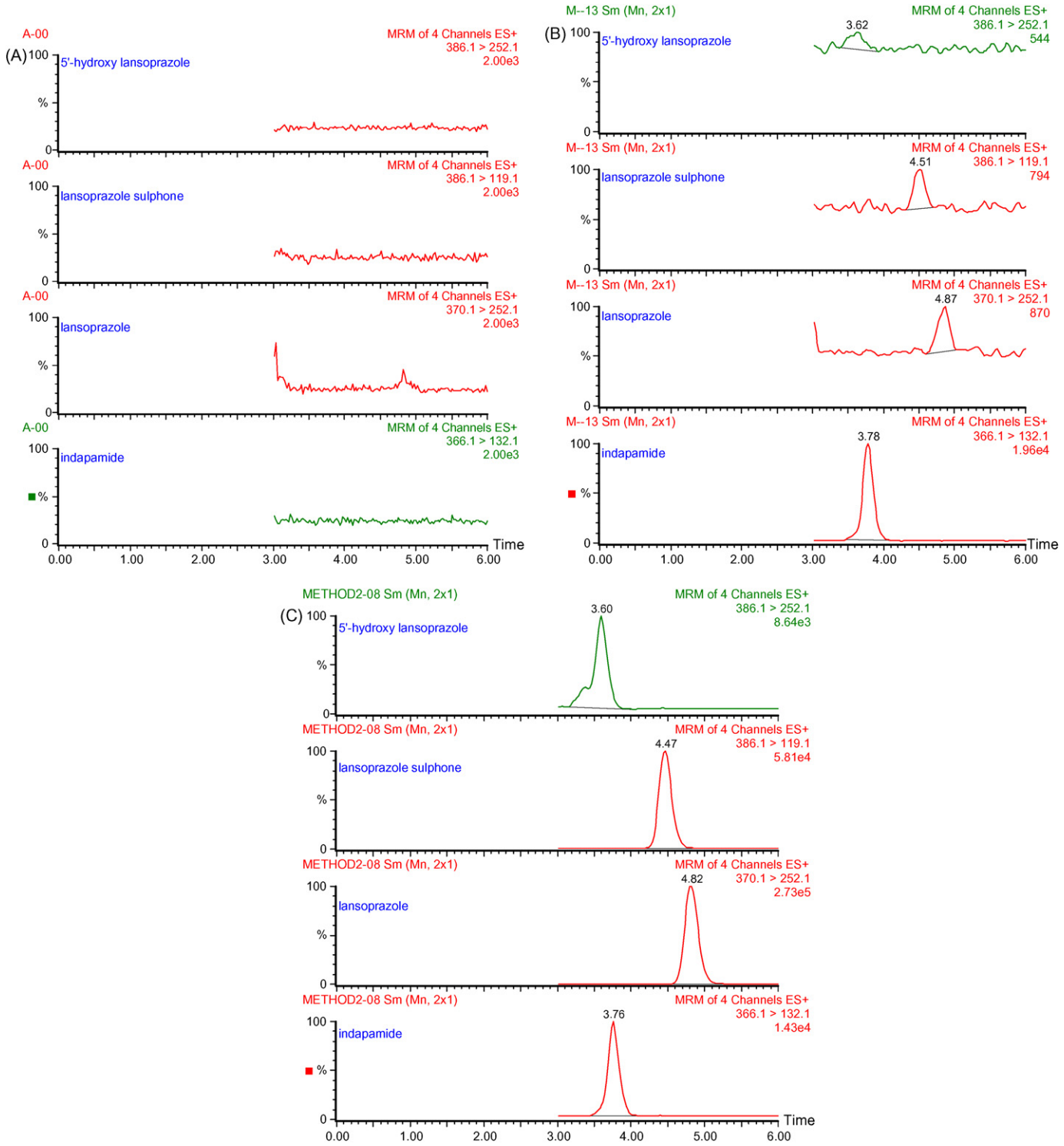

Fig. 2. Typical MRM chromatograms of lansoprazole, 5′-hydroxy lansoprazole, lansoprazole sulphone and IS (indapamide) in human plasma. (A) Drug-free plasma sample; (B) plasma sample spiked with lansoprazole (t_R = 4.80 min, C = 2.0 ng/ml), 5′-hydroxy lansoprazole (t_R = 3.60 min, C = 2.0 ng/ml), lansoprazole sulphone (t_R = 4.49 min, C = 0.5 ng/ml), and IS (indapamide, t_R = 3.76 min, C = 200 ng/ml); (C) plasma sample spiked with lansoprazole(t_R = 4.82 min, C = 1000 ng/ml), 5′-hydroxy lansoprazole (t_R = 3.60 min, C = 100 ng/ml), lansoprazole sulphone (t_R = 4.47 min, C = 100 ng/ml), and IS (indapamide, t_R = 3.76 min, C = 200 ng/ml).

ture at 120 °C, and the desolvation nitrogen gas temperature at 380 °C. In order to increase the sensitivity and selectivity, argon gas collision-induced dissociation (CID) was employed and optimized with collision energies of 11 eV, 13 eV and 22 eV for lansoprazole, 5′-hydroxy lansoprazole, and lansoprazole sulphone, respectively. The strongest multiple reaction monitoring transitions were selected with m/z 370.1–252.1 for lansoprazole, m/z 386.1–252.1 for 5′-hydroxy lansoprazole, m/z 386.1–119.1 for lansoprazole sulphone, and m/z 366.1–132.1 for IS. Fig. 1 shows the full scan positive ESI product ion mass spectra and the proposed patterns of fragmentation of the analytes.

2.4. Preparation of stock and working standard solutions

Primary stock solutions of lansoprazole, 5′-hydroxy lansoprazole, and lansoprazole sulphone were prepared by dissolving appropriate amounts of the chemical reference substances in acetonitrile, which were found to be stable for 30 days stored at −80 °C, and for at least 24 h stored at 5 °C. Appropriate dilutions were made in acetonitrile to produce a series of working standard solutions in the range of 100–40,000 ng/ml, 50–4000 ng/ml, and 10–4000 ng/ml for lansoprazole, 5′-hydroxy lansoprazole, and lansoprazole sulphone, respectively. The IS solution was prepared in the same way with a concentration of 2000 ng/ml. All the working standard solutions were freshly prepared, stored at 5 °C and shaded from light.

2.5. Sample preparation

All experiments including sample preparation and instrumental analysis were performed under feeble red light to prevent photodegradation of the metabolites of lansoprazole. An aliquot of 0.5 ml drug-free plasma in plastic centrifuge tube was mixed with 50 μl standard solutions of lansoprazole, 5′-hydroxy lansoprazole, lansoprazole sulphone, and IS solution, respectively, and the protein precipitation was achieved by vortex-mixing with 1.0 ml of acetonitrile for 1 min. An aliquot of 20 μl of the supernatant obtained after centrifuging at 15,000 × *g* for 10 min at 5 °C was injected into the LC–MS/MS system.

The same sample handling process was carried out for linearity, recovery, precision, accuracy, and stability tests.

Three different plasma concentration levels (20 ng/ml, 200 ng/ml, and 2000 ng/ml of lansoprazole; 10 ng/ml, 50 ng/ml, and 200 ng/ml of 5′-hydroxy lansoprazole; 2.0 ng/ml, 20 ng/ml and 200 ng/ml of lansoprazole sulphone) were selected as QC concentrations to cover the entire range of calibrations.

2.6. Method validation

2.6.1. Assay specificity

The specificity of the method was evaluated by analyzing human plasma samples from at least six different sources to investigate the potential interferences at the LC peak region for the analytes and IS.

2.6.2. Matrix effect

To evaluate the matrix effect, chromatographic peak areas of lansoprazole and its two metabolites from the spike-after protein precipitated samples were compared to the neat standards at the QC concentrations.

2.6.3. Linearity and lower limit of quantification

The linearity of the method was determined by analysis of a series of standard samples with concentrations from 10 ng/ml to 4000 ng/ml for lansoprazole, 5.0 ng/ml to 400 ng/ml for 5′-hydroxy lansoprazole, and 1.0 ng/ml to 400 ng/ml for lansoprazole sulphone. The lower limit of quantifications (LLOQ) for lansoprazole, 5′-hydroxy lansoprazole, and lansoprazole sulphone were determined each with five lowest calibration standards of 2.0 ng/ml, 2.0 ng/ml, and 0.5 ng/ml, respectively. The analytes to IS peak area ratios obtained were plotted against the corresponding concentrations of the analytes (expressed as ng/ml) and the calibration curves were constructed by means of the least-squares linear regression method. The criteria for the calibration included a correlation coefficient (r) of 0.998 or better, and the found value for each plasma calibration standards being within ±15% deviation from the nominal value except for the LLOQ within ±20%.

2.6.4. Accuracy, precision and recovery

The intra-day accuracy and precision were evaluated by analyzing five replicates at three QC concentration levels on the same day. The inter-day assay precision was determined by analyzing the three-level QC samples on three different runs.

The criteria for acceptability of the data included accuracy within ±15% deviation from the nominal values and a precision of within 15% relative standard deviation (R.S.D.), except for at the LLOQ, where it should not exceed ±20%.

Recoveries of lansoprazole, 5′-hydroxy lansoprazole, and lansoprazole sulphone from plasma with protein precipitation by acetonitrile were determined by comparison of the peak areas of them in spiked plasma samples at three QC concentrations with those in samples prepared by spiking precipitated drug-free plasma samples with the same amounts of lansoprazole and its two metabolites at the step immediately prior to chromatography.

2.6.5. Stability test

Auto-sampler rack stability: due to the need for occasional delayed injection of the extracted samples, stability of lansoprazole and its metabolites in the auto-sampler rack was evaluated. Samples at moderate concentration (200 ng/ml of lansoprazole, 50 ng/ml of 5′-hydroxy lansoprazole, and 20 ng/ml of lansoprazole sulphone) were extracted, loaded onto the auto-sampler rack and kept for 24 h at 5 °C before injection.

Freeze–thaw stability: it was assessed by exposing samples at moderate concentration level to three freeze–thaw cycles, each cycle consisted of removing the samples from the freezer, thawing them unassisted to room temperature, kept at room temperature for 2 h and re-freezing at −20 °C.

Long-term stability: it was evaluated after storage of duplicate samples of moderate concentration level at −20 °C for 5, 15 and 30 days respectively, a span of time long enough to enclose the entire procedure for the plasma samples from collection to determination.

2.7. Pharmacokinetic analysis

The method was applied to determine the plasma concentration of lansoprazole and its metabolites following a single 30 mg oral dose administration of lansoprazole enteric capsules to 20 healthy subjects. Venous blood samples each about 3.5 ml were drawn before dosing (0 h) and at 0.33 h, 0.67 h, 1.0 h, 1.5 h, 2.0 h, 2.5 h, 3.0 h, 4.0 h, 6.0 h, 8.0 h, 10 h, 12 h, 15 h and 24 h after dosing. All blood samples were collected in heparinized tubes and centrifuged at 1000 × *g* force immediately. The separated plasma samples were stored at −80 °C until analysis.

An aliquot of 0.5 ml of thawed plasma samples was spiked with IS and processed as mentioned in sample preparation section. Along with study samples, QC samples at low, medium and high concentrations were assayed in duplicate and were distributed among unknown samples in the analytical run.

The pharmacokinetic parameters of lansoprazole and its metabolites were calculated by the non-compartmental method with the aid of the DAS program (Drugs and Statistics version 2.0, Chinese Pharmacological Society, China).

3. Results and conclusion

3.1. Sample preparation and LC–MS/MS optimization

Both liquid extractions coupled with column switching [10] and solid-phase extraction [11] were exploited for sample preparation with HPLC determination, which were not highly selective for

detailed pharmacokinetic study. In this paper, the plasma samples were treated with a simple protein precipitation, and acetonitrile gave the best result for lansoprazole and its metabolites with regard to both recovery and specificity.

Method development began with the optimization of chromatographic conditions including mobile phase composition and column type. The feasibility of various mixtures of solvents, such as acetonitrile and methanol, using different buffers, such as ammonium acetate, ammonium formate and methanoic acid with variable pH range of 4.0–7.0, along with altered flow-rates were tested for optimum chromatographic retention of the analytes and IS. A mixture of 75 volumes of methanol and 25 volumes of 0.2% ammonium acetate and 0.1% methanoic acid in water was used as mobile phase and an Inertsil ODS-3 column (250 mm × 4.6 mm, 5 μm) was selected for the separation since good chromatographic resolution, and symmetrical peak shapes of the interest analytes were obtained. They were also compatible with the determination of the analytes by positive electrospray ionization MS detection.

Mass spectrometric conditions were optimized so as to achieve the maximum stable response of the parent and the major product ions of the analytes. Parameters such as spray voltage, desolvation gas flow and temperature were all tuned. Following detailed optimization of mass spectrometry conditions (provided in the Chromatographic and MS/MS conditions section), the predominately protonated molecule ions were obtained by the positive ESI scan at *m*/*z* 370.1, *m*/*z* 386.1, and *m*/*z* 386.1 for lansoprazole, 5′-hydroxy lansoprazole, and lansoprazole sulphone, respectively. They were apt to form major product ions at *m*/*z* 252.1, 252.1, and 119.1 with the optimum collision energies of 11 eV, 13 eV, and 22 eV, respectively.

3.2. Method validation

3.2.1. Selectivity and matrix effect

Selectivity was assessed by comparing the chromatograms of drug-free human plasma with the corresponding spiked plasma. Fig. 2 shows the typical chromatograms of drug-free plasma, drug-free plasma spiked with analytes and the IS. No interference from endogenous substances with analytes or the IS was detected. The typical retention times for lansoprazole, 5′-hydroxy lansoprazole, lansoprazole sulphone and indapamide (IS) were 4.82 min, 3.60 min, 4.47 min, and 3.67 min, respectively.

In this study, the matrix effect was evaluated by analyzing QC samples. Average matrix effect values obtained were varied from 96.2% to 114.9%, which were found to be within the acceptable limits (85–115%). The same evaluation was performed on IS and no significant peak area differences were observed.

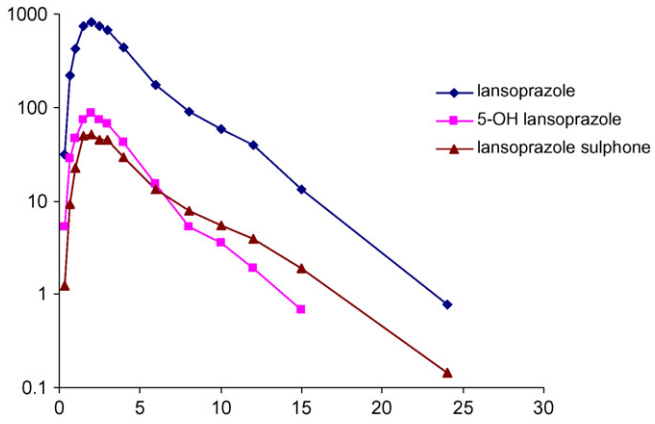

Fig. 3. The mean plasma concentration–time profiles of lansoprazole, 5′-hydroxy lansoprazole, and lansoprazole sulphone after a single oral dose of lansoprazole enteric capsules (30 mg) in 20 volunteers.

Table 1
Precision and accuracy for the analysis of lansoprazole, 5′-hydroxy lansoprazole, and lansoprazole sulphone in human plasma (n = 3 days, 5 replicates per day).

Lansoprazole				
Concentration (ng/ml)	2.0	20	200	2000
Day 1	1.82	19.73	188.8	1884
Day 2	1.85	17.31	212.5	1962
Day 3	1.90	17.49	194.3	1955
Grand mean	1.86	18.18	198.5	1934
Dev. (%)	−7.17	−9.11	−0.74	−3.33
Inter-day precision (R.S.D., %)	12.98	14.59	13.94	5.02
Intra-day precision (R.S.D., %)	3.11	0.90	2.01	1.36
5′-Hydroxy lansoprazole				
Concentration (ng/ml)	2.0	10	50	200
Day 1	1.95	10.58	49.54	215.4
Day 2	2.12	9.32	51.41	219.8
Day 3	2.04	10.04	49.51	224.5
Grand mean	2.04	9.98	50.15	219.9
Dev. (%)	1.83	−0.19	0.31	9.94
Inter-day precision (R.S.D., %)	16.77	14.17	4.85	4.66
Intra-day precision (R.S.D., %)	1.98	2.39	2.22	0.73
Lansoprazole sulphone				
Concentration (ng/ml)	0.5	2.0	20	200
Day 1	0.45	1.970	18.01	181.3
Day 2	0.44	1.854	19.74	193.6
Day 3	0.52	2.024	20.46	204.2
Grand mean	0.47	1.949	19.40	193.0
Dev. (%)	−6.00	-2.53	−2.98	−3.49
Inter-day precision (R.S.D., %)	14.23	9.98	14.53	13.24
Intra-day precision (R.S.D., %)	2.86	1.99	0.85	1.01

3.2.2. Linearity and LLOQ

The calibration curve was linear over the concentration range of 10–4000 ng/ml, 5.0–400 ng/ml, and 1.0–400 ng/ml for lansoprazole, 5′-hydroxy lansoprazole and lansoprazole sulphone in human plasma, respectively. And a better fit of the data and higher accuracy could be obtained by dividing the calibration curve of lansoprazole and lansoprazole sulphone into two segments: from 10 ng/ml to 200 ng/ml, 200 ng/ml to 4000 ng/ml, and from 1.0 ng/ml to 20 ng/ml, 20 ng/ml to 400 ng/ml, respectively. Typical equations of the calibration curves within each concentration ranges are as follows: $R_1 = 0.0215 \times C_1 + 0.0279$ (10–200 ng/ml, $r^2 = 0.999$), $R_1 = 0.0233 \times C_1 - 1.445$ (200–4000 ng/ml, $r^2 = 0.999$); $R_2 = 0.0480 \times C_2 + 0.00360$ (1.0–20 ng/ml, $r^2 = 0.999$), $R_2 = 0.0485 \times C_2 - 0.0974$ (20–400 ng/ml, $r^2 = 0.999$); $R_3 = 0.00504 \times C_3 + 0.00575$ (5.0–400 ng/ml, $r^2 = 0.999$); where R_1, R_2, R_3 are the peak area ratios of lansoprazole, lansoprazole sulphone, and 5′-hydroxy lansoprazole to IS and C_1, C_2, C_3 are the plasma concentration of lansoprazole, lansoprazole sulphone, and 5′-hydroxy lansoprazole, respectively.

The LLOQs with the R.S.D. within ±20% and deviation within ±20% were found to be 2.0 ng/ml, 2.0 ng/ml, and 0.5 ng/ml for lansoprazole, 5′-hydroxy lansoprazole, and lansoprazole sulphone, respectively, which are highly sensitive for the clinical pharmacokinetic study of this drug.

3.2.3. Precision and accuracy

Accuracy and precision data for intra- and inter-day plasma samples are presented in Table 1. In this determination, the intra- and inter-day precisions ranged from 0.7% to 2.4% and 4.7% to 14.6% for each analyte at the corresponding QC levels, respectively. The accu-

Table 2
Recovery for the analysis of lansoprazole, 5′-hydroxy lansoprazole, and lansoprazole sulphone in human plasma ($n = 5$).

Lansoprazole				
Concentration (ng/ml)	2.0	20	200	2000
Recovery (%)	89.2	105.3	94.2	96.1
	85.9	99.6	93.7	98.2
	93.3	97.3	94.8	95.3
	90.0	97.7	94.4	86.4
	88.9	93.5	95.0	95.0
Mean (%)	89.5	98.7	94.4	94.2
S.D. (%)	2.65	4.33	0.51	4.55
R.S.D. (%)	2.96	4.39	0.54	4.83
5′-Hydroxy lansoprazole				
Concentration (ng/ml)	2.0	10	50	200
Recovery (%)	102.3	108.9	94.9	107.5
	105.9	103.8	102.8	109.6
	98.7	107.4	95.3	109.6
	112.2	109.4	101.0	103.2
	108.4	99.4	101.5	108.6
Mean (%)	105.5	105.8	99.1	107.7
S.D. (%)	5.24	4.16	3.71	2.66
R.S.D. (%)	4.97	3.94	3.74	2.47
Lansoprazole sulphone				
Concentration (ng/ml)	0.5	2.0	20	200
Recovery (%)	82.3	107.8	88.7	92.9
	85.5	98.6	90.7	95.3
	90.2	101.4	90.8	87.2
	91.4	92.4	90.3	90.0
	89.7	91.9	89.8	87.9
Mean (%)	87.8	98.4	90.0	90.7
S.D. (%)	3.80	6.61	0.87	3.40
R.S.D. (%)	4.33	6.71	0.96	3.75

racy expressed as deviation percentage was all found to be within the criteria limit.

3.2.4. Recovery and stability

High efficiency of recovery was found for each analyte. Mean values of absolute recovery for lansoprazole and its two metabolites were all over 80% (Table 2).

The metabolites of lansoprazole are sensitive to light, and they may undergo photodegradation. Acetonitrile was proved to be the most appropriate solvent for stock solution preparation. The stock was stable for at least 24 h at 5 °C and over 30 days when stored at −80 °C. The stability experiment confirmed that plasma samples were stable over 30 days when stored at −80 °C and through three freeze–thaw cycles. The worked out plasma sample were stable for at least 24 h at 5 °C when shaded from light.

Table 3
Pharmacokinetic parameters of lansoprazole, 5′-hydroxy lansoprazole, and lansoprazole sulphone following a single oral dose of 30 mg lansoprazole enteric capsules (mean ± S.D., $n = 20$).

Parameters	Lansoprazole	5′-Hydroxy lansoprazole	Lansoprazole sulphone
C_{max} (ng ml^{-1})	1047 ± 344	111.2 ± 41.8	66.6 ± 52.9
T_{max} (h)	2.0 ± 0.7	2.1 ± 0.8	1.9 ± 0.8
$T_{1/2z}$ (h)	1.94 ± 0.59	2.31 ± 1.18	2.52 ± 1.54
MRT (h)	3.62 ± 0.87		
AUC_{0-24} (h ng ml^{-1})	3388 ± 1484	317.0 ± 81.2	231.9 ± 241.7
$AUC_{0-\infty}$ (h ng ml^{-1})	3496 ± 1693		
CLz/F (l h^{-1})	9.96 ± 3.74		
Vz/F (l)	32.83 ± 11.74		

3.3. Pharmacokinetic application

The sensitivity and specificity of the assay were found to be sufficient for accurately characterizing the pharmacokinetics of lansoprazole, 5′-hydroxy lansoprazole, and lansoprazole sulphone in humans. Profiles of the mean plasma concentration vs time are shown in Fig. 3, and the main pharmacokinetic parameters are shown in Table 3.

3.4. Conclusion

A highly accurate, sensitive, specific and reproducible LC–MS/MS method for the quantification of lansoprazole, 5′-hydroxy lansoprazole and lansoprazole sulphone using commercially available IS from small volumes of human plasma with a simple protein precipitation process was developed and validated. The method has been successfully applied to the investigation of a preclinical pharmacokinetic study of lansoprazole with desired precision and accuracy along with high throughput.

References

[1] L.Q. Fu, F. Huang, Chin. J. Clin. Pharmacol. 8 (2003) 35–38.
[2] B.L. Barradell, D. Faulds, D. McTavish, Drugs 44 (1992) 225–250.
[3] T. Andersson, Clin. Pharmacokinet. 31 (1996) 9–28.
[4] R.E. Pearce, A.D. Rodrigues, J.A. Goldstein, A. Parkinson, J. Pharmacol. Exp. Ther. 277 (1996) 805–816.
[5] H. Katsuki, C. Nakamura, K. Arimori, S. Fujiyama, M. Nakano, Eur. J. Clin. Pharmacol. 52 (1997) 391–396.
[6] I. Aoki, M. Okumura, T. Yashiki, J. Chromatogr. 571 (1991) 283–290.
[7] B.D. Landes, G. Miscoria, B. Flouvat, J. Chromatogr. 577 (1992) 117–122.
[8] H. Katsuki, A. Hamada, C. Nakamura, K. Arimori, M. Nakano, J. Chromatogr. B 757 (2001) 127–133.
[9] M.D. Karol, G.R. Granneman, K. Alexander, J. Chromatogr. B 668 (1995) 182–186.
[10] T. Uno, N. Yasui-Furukori, T. Takahata, K. Sugawara, T. Tateishi, J. Chromatogr. B 816 (2005) 309–314.
[11] M. Miura, H. Tada, T. Suzuki, J. Chromatogr. B 804 (2004) 389–395.