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Original Article

Development of a UHPLC-MS/MS method for the quantification of ilaprazole enantiomers in rat plasma and its pharmacokinetic application

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ABSTRACT

In Korea and China, ilaprazole is a widely used proton pump inhibitor in the treatment of gastric ulcers. In this study, a specific and sensitive LC-MS/MS method has been developed and validated for the quantification of ilaprazole enantiomers in the rat plasma, using *R*-lansoprazole as the internal standard. The enantioseparation was achieved on a CHIRALPAK AS-RH column (4.6 mm × 150 mm, i.d. 5 μm), with a mobile phase composed of 10 mM ammonium acetate aqueous solution and acetonitrile (60:40, *V/V*), at a flow-rate of 0.5 mL/min. The method was validated over the concentration range of 0.5–300 ng/mL for both, *R*- and *S* -ilaprazole. The lower limit of quantification was 0.5 ng/mL for both enantiomers. The relative standard deviation (RSD) of intra- and inter-day precision of *R*-ilaprazole and *S*-ilaprazole was less than 10.9%, and the relative error accuracy (RE) ranged from −0.5%–2.0%. Finally, the method was successfully evaluated in rats in a stereoselective pharmacokinetic study of the ilaprazole racemate.

1. Introduction

Proton pump inhibitors (PPIs) are widely regarded as an effective revolution to treat gastric and duodenal ulcers through the inhibition of hydrogen potassium adenosine triphosphatase (H^+K^+-ATPase) to relieve gastric acid secretion [1]. The first-generation PPIs, including omeprazole and pantoprazole, had noticeable limitations, whereas the second-generation PPIs, such as rabeprazole, made several breakthroughs but still failed to obtain good clinical results [2]. In clinical studies, ilaprazole was utilized to treat reflux esophagitis and gastric secretion, and demonstrated fewer side effects on the nervous and cardiovascular systems, compared with other similar drugs [3–5]. Compared with previously evaluated PPIs, the half-life of ilaprazole is prolonged, with a similar safety profile. Ilaprazole is mainly metabolized by CYP3A, and demonstrates good clinical efficacy [6,7]. The pharmacokinetics of ilaprazole and its two metabolites, ilaprazole sulfone and ilaprazole thiol ether, have been determined in healthy humans following a single oral dose of 5 mg [8]. Additionally, the pharmacokinetics and pharmacodynamics of intravenous ilaprazole in healthy subjects, after single ascending doses, has also been reported [9]. The pharmacokinetic profile of ilaprazole after 7 days of a 10 mg oral dose, and following 7 consecutive days of a 10 mg intravenous injection, in humans, was also evaluated [10,11].

Enantiomers may have different stereoselective properties, which could influence the pharmacokinetic characteristics and therapeutic effects of drugs [12–15]. Dexlansoprazole modified-release is the *R*-enantiomer of lansoprazole, and is the only PPI with a novel dual delayed release formulation. Compared with lansoprazole racemate, dexlansoprazole has a similar safety and side effect profile; however, the therapeutic effect is enhanced [16]. According to the structure of ilaprazole, the compound is a chiral molecule composed of *R*- and *S*-ilaprazole enantiomers, which may exhibit various pharmacokinetic activities. In order to study the stereoselective pharmacokinetics of *R*- and *S*-ilaprazole

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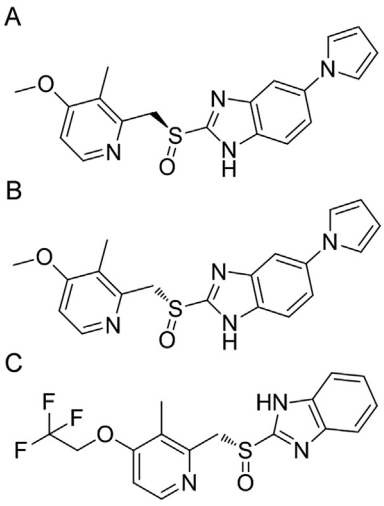

Fig. 1. Chemical structure of (A) *R*-, (B) *S*-ilaprazole and (C) internal standard.

enantiomers, a highly sensitive and selective chiral separation method needs to be developed.

Ilaprazole enantiomers had a good separation using a CHIRALPAK IC column in HPLC [17]. However, the mobile phase used was n-hexane/EtOH/DEA/TFA (50:50:0.1:0.1, *V/V/V*), which is rarely used in an LC-MS/MS system. Tan et al. reported a chiral LC-MS/MS method with CHIRALCEL OZ-RH as the analytical column for the determination of ilaprazole in human plasma. However, the sample preparation method was time consuming, with poor sensitivity [18]. In the present study, a rapid and sensitive LC-MS/MS method has been developed for the evaluation of ilaprazole enantiomers present in rat plasma. This method exhibited a higher sensitivity with the lower limit of quantitation of 0.5 ng/mL compared with previous works. Furthermore, the present plasma sample processing method, which precipitates protein directly by adding acetonitrile, is more convenient than liquid-liquid extraction with methyl tertbutyl ether as used in previous method [18]. The present method was then successfully used to evaluate the pharmacokinetic profiles of ilaprazole enantiomer in rats.

2. Materials and methods

2.1. Instruments and other equipments

ACQUITY TQD-tandem quadrupole liquid chromatography-mass spectrometer (Waters, USA); electronic balance AL104 (Mettler Toledo Cooperation, Swiss); vortex (QL-901, Haimen City Qilinbeier Instrument Manufacturing Co., Ltd., China); and Centrifuge 5424R (Eppendorf, Germany) were used. The high performance liquid chromatography (HPLC) system consisted of photodiode array (PDA) detector (MD-4010, Jasco, Japan) and circular dichroism (CD) spectroscopy (CD-4095, Jasco, Japan).

2.2. Chemicals

Ilaprazole racemate and *R*-lansoprazole (internal standard, IS) were kindly given by Huadong Medicine Co., Ltd., China (Fig. 1). The purity of ilaprazole racemate and *R*-lansoprazole was greater than 98%. The other reagents were all purchased from commerce, such as acetonitrile (AR, JA043730, German Merck KGaA), distilled water (A.S. Watson Group (Hong Kong) Ltd., China), ammonium acetate (AR, C10099621, Shanghai Macklin Biochemical Technology Co., Ltd., China).

2.3. Solutions

A certain amount of ilaprazole racemate was dissolved in acetonitrile to prepare the concentration of 10 mg/mL stock solution. A certain amount of internal standard compound *R*-lansoprazole was added into acetonitrile and the concentration of 16.6 μg/mL IS stock solution was prepared. The stock solution was stored at −80 °C. The internal standard stock solution was then diluted by acetonitrile to 6.225 ng/mL as the IS working solution and was stored at 4 °C.

2.4. Sample preparation

100 μL rat plasma sample was precisely removed into a 1.5 mL

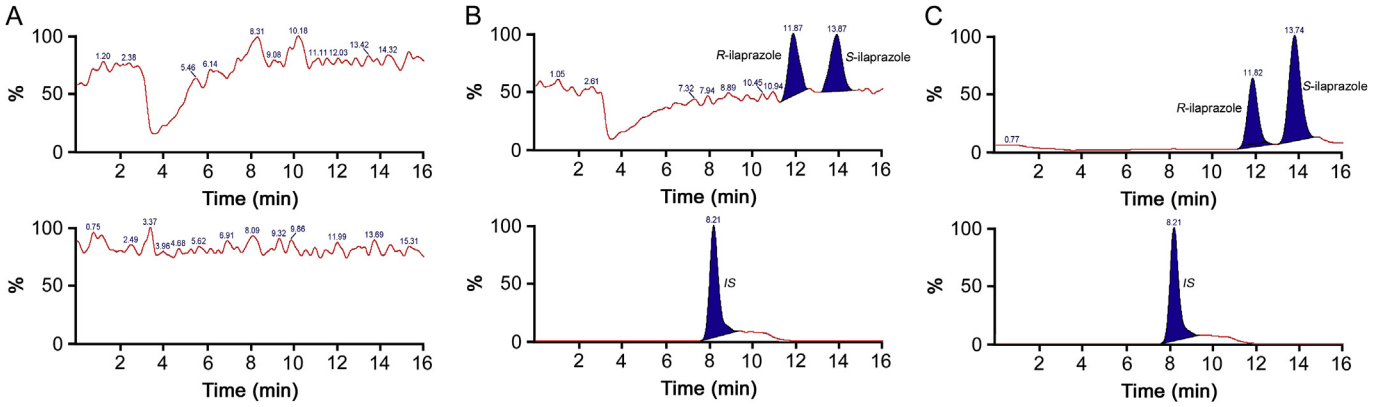

Fig. 2. Typical LC-MS/MS ion chromatograms of *R*-, *S*-ilaprazole and internal standard in (A) blank rat plasma, (B) plasma spiked with ilaprazole racemate (0.5 ng/mL) and dexlansoprazole (6.225 ng/mL), (C) rat plasma at 0.33 h after administration of ilaprazole racemate (5 mg/kg). The peak at 11.8 min was *R*-ilaprazole, and the peak at 13.7 min was *S*-ilaprazole.

Table 1
Intra-batch and inter-batch of precision and accuracy analysis of ilaprazole enantiomers ($n = 5$).

Analyte	Spiked (ng/mL)	Intra-batch								Inter-batch		
		Batch 1			Batch 2			Batch 3				
		Measured (ng/mL)	RSD (%)	RE (%)	Measured (ng/mL)	RSD (%)	RE (%)	Measured (ng/mL)	RSD (%)	RE (%)	Measured (ng/mL)	RSD (%)	RE (%)
R-ilaprazole	2.5	2.57	2.2	2.8	2.56	4.2	2.4	2.46	7.0	−1.6	2.53	4.9	1.2
	25	28.0	3.0	11.9	26.2	7.2	4.6	22.3	2.8	−10.8	25.5	10.6	1.9
	250	274.2	2.2	9.7	273.4	1.9	9.4	217.4	1.5	−13.0	255.0	10.9	2.0
S-ilaprazole	2.5	2.37	8.1	−5.2	2.7	7.4	8	2.39	5.2	−4.4	2.485	9.1	−0.5
	25	26.8	1.5	7.2	26.6	5.3	6.2	21.8	1.9	−13.0	25.1	10.1	0.2
	250	264.2	3.2	5.7	280.1	1.8	12.1	221.0	2.0	−11.6	255.1	10.4	2.0

Note: RSD = relative standard deviation; RE = relative error.

centrifuge tube, and 300 μL IS working solution was added to precipitate protein. After vortex for 15 s, the samples were centrifuged at 15,871 *g* for 10 min and the supernatant was acquired for following LC-MS/MS analysis.

2.5. LC-MS/MS analysis

2.5.1. Liquid chromatography

Chromatographic separation was performed using a CHIRALPAK AS-RH column (4.6 mm × 150 mm, i.d. 5 μm; Daicel/Chiral Technologies, Illkirch, France) at 15 °C with the injection volume of 7.0 μL. The flow rate was 0.5 mL/min and isocratic elution was applied using 10 mM ammonium acetate water solution and acetonitrile (60:40, *V/V*) as mobile phase.

2.5.2. Mass spectrometry

A triple quadrupole mass spectrometer equipped with an electron ionization (ESI) operated in positive ion mode was used. The source temperature and desolvation temperature were 150 °C and 450 °C, respectively. The capillary voltage was 3.59 kV, the cone voltage was set at 25 V for ilaprazole enantiomers and 14 V for IS. The collision energy for ilaprazole enantiomers and IS was 30 eV and 14 eV, respectively. The flow velocity of collision gas, desolvation gas and cone gas were 0.15 mL/min, 600 L/h, and 50 L/h, respectively. Multiple-reaction monitoring (MRM) was operated to quantify analytes and IS; the transition *m/z* 367 → 184 was detected for ilaprazole enantiomers and *m/z* 370 → 252 was used to detect IS.

2.6. Method validation

The method was validated according to the FDA Bioanalytical Method Validation, including selectivity, calibration curve, carryover, precision and accuracy, recovery, stability and dilution integrity.

2.6.1. Selectivity

The selectivity of this method was assessed by comparing the ion chromatograms of blank plasma samples from six rat plasma blank samples from different sources to exclude endogenous interference.

2.6.2. Calibration curve

Ilaprazole stock solution were precisely diluted using acetonitrile to prepare the standard working solution of ilaprazole racemate with the concentration of 10, 50, 100, 500, 1000, 5000, and 6000 ng/mL. Aliquot 5 μL of each standard solution was precisely added into 95 μL blank rat plasma solution to acquire the standard curve sample with concentrations of 0.5, 2.5, 5, 25, 50, 250, and 300 ng/mL for each enantiomer. Calibration curve was plotted as the peak area ratio (y) of sample and internal standard versus the sample concentrations (x). The equation was fitted by applying a

Table 2
Carryover analysis of *R*- and *S*-ilaprazole in LC-MS/MS system.

Analyte	Name	Peak area	Residual (%)	Result
R-ilaprazole	Residue 1	16.15	6.4	< 20%
	Residue 2	0	0	< 20%
	0.5 ng/mL	258.7	–	–
	0.5 ng/mL	245.4	–	–
S-ilaprazole	Residue 1	0	0	< 20%
	Residue 2	7.1	5.1	< 20%
	0.5 ng/mL	123.9	–	–
	0.5 ng/mL	152.6	–	–

Table 3
Extraction recovery and matrix effect analysis of ilaprazole enantiomers.

Sample	Concentration (ng/mL)	Extraction recovery		Matrix effect			
		Mean (%)	RSD (%)	MF for ilaprazole	MF for IS	IS-normalized MF	RSD (%)
R-ilaprazole	2.5	75.1	2.5	1.00	0.98	1.02	1.2
	25	71.6	4.3	1.02	1.04	0.98	0.3
	250	81.4	6.3	0.99	1.00	0.99	4.8
S-ilaprazole	2.5	79.1	0.9	1.15	0.99	1.16	2.5
	25	76.4	9.2	0.99	0.99	1.00	7.2
	250	81.2	5.2	1.04	1.00	1.03	4.5

Note: MF = Matrix effect.

weight factor of 1/x linear regression analysis.

2.6.3. Carryover

Carryover was estimated by injecting the lowest calibrant (0.5 ng/mL), then the highest calibrant (300 ng/mL) followed by blank solution containing 40% ACN. Carryover was expressed as the ratio of the area measured for the blank to the area measured for the lowest calibrant.

2.6.4. Precision and accuracy

Each above working solution was added into rat blank plasma at the ratio of 5:95 *(V/V)* to prepare quality controls (QC) with ilaprazole enantiomer concentrations of 0.5, 2.5, 25, and 250 ng/mL. Five replicates of each sample were detected to assess precision and accuracy of *R*- and *S*-ilaprazole ($n = 5$). Intra-batch and inter-batch of precision and accuracy analyses of ilaprazole enantiomers were acceptable when precision was assessed as the deviation within ±15% and the accuracy average was within ±15% of the nominal value of the QC sample (±20% at LLOQ).

2.6.5. Extraction recovery and matrix effect

The extraction recovery of ilaprazole enantiomer and IS was obtained by comparing the average peak response of the QC samples ($n = 6$) at the three concentration levels and the samples of the same concentration (blank plasma extracted with acetonitrile, then adding standard sample).

The matrix effect (MF) was evaluated by comparing the peak response of the ilaprazole enantiomer and IS in the extracted sample with the peak response of the ilaprazole enantiomer and IS in the same concentration of acetonitrile. IS-normalized MF = MF for ilaprazole/MF for IS.

2.6.6. Stability

Each different working solution was added into rat blank plasma at the ratio of 5:95 *(V/V)* to gain stability analytes with ilaprazole enantiomer concentration of 2.5, 25, and 250 ng/mL. The evaluation of stability included short-term stability (stored at room temperature for 4 h), long-term stability (frozen at −80 °C for 20 days), freeze-thaw stability (three cycles), auto sampler stability (4 °C for 24 h) and stability of ilraprazol racemate stock solution at −80 °C for 15 days. The initial and final concentrations of the ilaprazole enantiomer under the respective storage conditions were determined and calculated to evaluate the stability of the ilaprazole enantiomer during storage and handling.

2.6.7. Dilution integrity

The two ilaprazole enantiomer stock solutions were diluted with acetonitrile to give a working solution of 12000 ng/mL. The two enantiomer working solutions were separately added to the blank rat plasma at a ratio of 5:95 *(V/V)* to obtain plasma samples of two ilaprazole enantiomers at a concentration of 600 ng/mL. Both ilaprazole enantiomer dilution concentrations were 2.0 times the upper limit of quantitation (ULOQ) concentration. Six replicates each with 4- and 10-fold diluted concentrations were prepared and their concentrations were calculated by applying the dilution factor 4- and 10-fold, respectively.

2.7. Pharmacokinetics studies

Sprague Dawley rats (Male, 200–220 g, obtained from the Animal Center of Zhejiang Academy of Medical Sciences, Hangzhou, China) were fasted for 12 h before experiment. Eighteen rats were divided randomly into three groups, and were intragastrically administered ilaprazole racemate at a dose of 1, 5, and 10 mg/kg, respectively. Rats were bled at each of the following time points: 0 (pre-dose), 0.016, 0.05, 0.117, 0.167, 0.25, 0.33, 0.50, 0.75, 1, 2, and 4 h after intragastric administration. The blood was immediately obtained by centrifugation at 4000 rpm for 10 min at 4 °C. Plasma samples were stored at −80 °C and analyzed by LC-MS/MS. The animals used in this trial were approved by the Zhejiang University Laboratory Animal Management and Use Committee (No. 11252).

2.8. Data analysis

All data were collected and analyzed by MassLynx software, and calculated using Microsoft Excel. Pharmacokinetics parameters were calculated with the DAS 2.1 software (Mathematical Pharmacology Professional Committee of China, Shanghai, China) using non-compartment model. Results were expressed as mean ± standard deviation (SD).The Student's *t*-test (Prime 5 statistical software) was used to compare the pharmacokinetic parameters of each group except T_{max} with non-parametric test. $P < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Ilaprazole enantiomeric configuration

As the enantiomers of ilaprazole were difficult to procure, we prepared a single ilaprazole enantiomer using HPLC. The chromatographic separation was performed on the CHIRALPAK AS-RH column (4.6 mm × 150 mm, i.d. 5 μM) using acetonitrile and water as the mobile phase. The eluent for each peak of the ilaprazole enantiomer was collected and dried at 15 °C, with the eluent peaks named eluent 1 and eluent 2, respectively. The residue was dissolved using methanol. The conformation of the enantiomer was identified by the combined means of an HPLC-PDA detector and CD spectroscopy. According to the HPLC-PDA-CD data (The HPLC-PDA-CD data are shown in the supplementary data file), the peaks indicated at the retention time of 11.8 and 13.7 min (Fig. 2) were *R*-ilaprazole and *S*-ilaprazole, respectively.

Table 4
Stability analysis of ilaprazole enantiomers in rat plasma ($n = 3$).

Analyte	Spiked (ng/mL)	Short-term stability (at room temperature for 4 h)			Auto-sampler (at 4 °C for 24 h)			Long-term stability (at −80 °C for 20 days)			Freeze-thaw stability (Three cycles)		
		Measured (ng/mL)	RE (%)	RSD (%)	Measured (ng/mL)	RE (%)	RSD (%)	Measured (ng/mL)	RE (%)	RSD (%)	Measured (ng/mL)	RE (%)	RSD (%)
R-ilaprazole	2.5	2.52	7.2	4.1	2.60	4.0	1.9	2.20	12.0	0	2.32	7.2	14.4
	250	268.9	2.8	3.21	238.3	4.7	5.6	268.3	7.3	0.9	257.1	2.8	4.4
S-ilaprazole	2.5	2.55	2.4	2	2.52	0.8	12.8	2.34	6.4	8.1	2.44	2.4	8.6
	250	255.8	4.2	5.3	244.6	2.2	3.34	262.6	5.0	3.7	260.5	4.2	4

Note: RSD = relative standard deviation; RE = relative error.

3.2. Method validation

3.2.1. Selectivity

The selectivity of this method was assessed by analyzing the ion chromatograms of blank plasma, from six different rats. Fig. 2 demonstrates the ion chromatograms of the blank rat plasma (A), blank plasma spiked with ilaprazole racemate and IS (B), and plasma samples collected 15 min after the administration of ilaprazole racemate (C). The result indicated that the amount of endogenous substances in the rat plasma was lower than 20% of the lower limit of quantitation (LLOQ), indicating that the existence of endogenous substances did not influence the detection of samples.

3.2.2. Linearity and sensitivity

The calibration curve of the ilaprazole enantiomer was acquired by plotting the peak area ratio (y) of each analyte to the IS against the analyte concentration (ng/mL, x) within the range of 0.5−300 ng/mL, regressing by a weight factor of 1/x. The result presented an excellent linearity of *R*- and *S*-ilaprazole within the concentration range, with good regression coefficients (r) of more than 0.999. The representative curves of *R*- and *S*-ilaprazole were $y = 0.00856184x+0.00954081$ ($r^2 = 0.997$) and $y = 0.00918857x+0.00061505$ ($r^2 = 0.997$), respectively. The precision values of 0.5 ng/mL (LLOQ) were 15.8% and 4.8% for *R*- and *S*-ilaprazole, respectively, and the accuracy given as bias were −4.0% and 14% for *R*- and *S*-ilaprazole, respectively, indicating that LLOQ analyte obtained good precision and accuracy. The other calibrators were ±15% of nominal (theoretical) concentrations in each validation run.

3.2.3. Precision and accuracy

According to Table 1, the results presented the RSD and RE of the intra-batch and inter-batch for *R*- and *S*-ilaprazole, determined by the QC samples at three different concentrations (2.5, 25, 250 ng/mL), with 5 replicates at each level. The RSD values of intra- and inter-day precision of *R*-ilaprazole and *S*-ilaprazole were less than 10.9%, and the RE of accuracy ranged from −0.5%−2.0%. Furthermore, the inter-batch RSD and RE were lower than 10.9% and 2.0% in all cases, respectively. The result showed that the precision and accuracy of *R*- and *S*-ilaprazole were both acceptable.

3.2.4. Carryover

The blank samples were continuously analyzed after the ULOQ concentration (300 ng/mL), and the amount of both the residual enantiomers was less than 20% of the total amount of *R*- and *S*-ilaprazole, indicating that the residual effect of LC-MS/MS system was acceptable (Table 2).

3.2.5. Recovery and matrix effect

Table 3 presents the results of the extraction recovery and the matrix effect. The results showed that the extraction recovery of *R*- and *S*-ilaprazole in the analytes were ranged between 75.1% and 81.4%. The internal standard-normalized matrix factors (MF) of low, medium and high concentrations were all within 2.0%, and were deemed suitable as they were lower than 15%.

3.2.6. Stability

The stability data are shown in Table 4, indicating that the *R*- and *S*-ilaprazole were stable on bench-top (4 h) at room temperature, in the autosampler (24 h) at 4 °C, through three repeated freeze/thaw cycles, and cryopreservation at −80 °C for 20 days. In addition, the ilaprazole racemate stock solution showed that the RE of both, *R*- and *S*-ilaprazole, was within 15%, and the RSD was

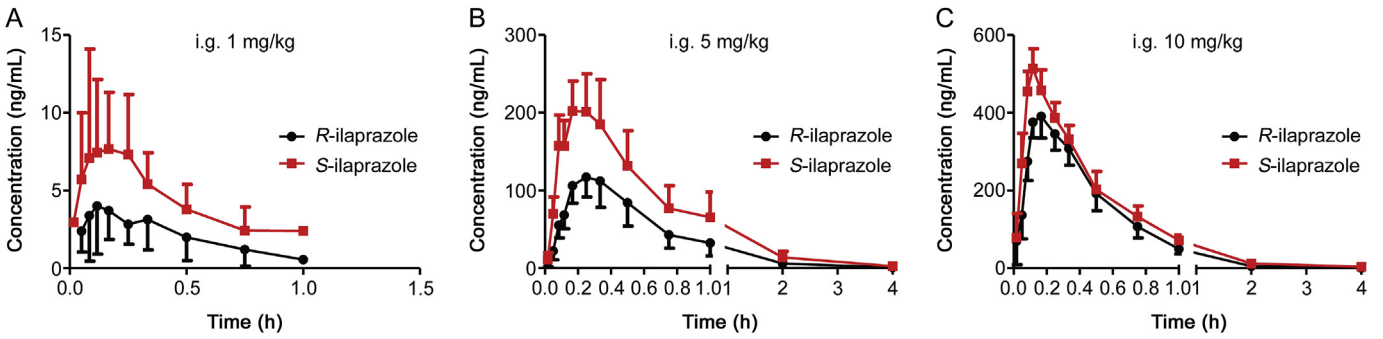

Fig. 3. Mean concentration-time profiles of ilaprazole enantiomers in rat plasma after oral administration of ilaprazole racemate.

Table 5
The main pharmacokinetics parameters of *S*- and *R*-ilaprazole in rat after oral administered ilaprazole racemate ($n = 6$).

Pharmacokinetic parameters	1 mg/kg			5 mg/kg			10 mg/kg		
	R-ilaprazole	*S*-ilaprazole	*P*	*R*-ilaprazole	*S*-ilaprazole	*P*	*R*-ilaprazole	*S*-ilaprazole	*P*
$AUC_{0\text{-}t}$ (μg./mL*h)	1.56 ± 0.61	3.80 ± 1.14	0.001	93.9 ± 64.7	183.2 ± 137.0	0.042	231.2 ± 98.0	296.7 ± 101.1	0.001
$AUC_{0\text{-}\infty}$ (μg/mL*h)	2.25 ± 0.99	5.41 ± 1.31	0.003	96.2 ± 67.3	184.0 ± 136.1	0.046	231.4 ± 98.0	296.9 ± 100.9	0.001
$t_{1/2}$ (h)	0.33 ± 0.25	0.29 ± 0.18	0.770	0.99 ± 0.87	0.95 ± 0.92	0.658	0.38 ± 0.10	0.58 ± 0.36	0.362
T_{max} (h)	0.25 ± 0.15	0.24 ± 0.16	0.363	0.25 ± 0.08	0.18 ± 0.11	0.033	0.15 ± 0.03	0.11 ± 0.02	0.001
$V_{z/F}$ (L/kg)	101.3 ± 57.0	38.0 ± 18.4	0.004	76.3 ± 112.3	42.7 ± 68.3	0.022	14.5 ± 9.0	16.6 ± 13.7	0.001
$Cl_{z/F}$ (L/h/kg)	250.8 ± 83.5	97.7 ± 26.4	0.066	39.2 ± 25.9	21.7 ± 15.3	0.166	25.1 ± 10.2	37.2 ± 12.6	0.732
C_{max} (ng./mL)	5.73 ± 2.05	11.2 ± 5.3	0.024	135.7 ± 65.7	242.4 ± 108.5	0.005	409.0 ± 123.6	522.6 ± 131.9	0.013

between 0.71% and 5.0%. Therefore, the ilaprazole racemate stock solution was stable at −80 °C for 15 days. The results demonstrated that the established method for sample extraction, storage, and intermittent analysis had been validated, and was suitable for large scale sample analysis.

3.2.7. Dilution integrity

The concentration was measured after the rat plasma sample spiked with ilaprazole racemate (600 ng/mL) was diluted 4- and 10-fold with the blank rat plasma. The results indicated that RE of both *R*- and *S*-ilaprazole was within 15%, and RSD was in the range of 2.2%–3.3%. Therefore, samples containing high concentrations of the analytes could be diluted 4 and 10 times, and accuracy and precision would be maintained.

3.3. Pharmacokinetics of ilaprazole enantiomers

The validated method has been successfully applied to the stereoselective pharmacokinetic studies of *R*- and *S*-ilaprazole following the oral administration of 1, 5, and 10 mg/kg ilaprazole racemate in rats. The assay was capable of measuring the concentrations of ilaprazole enantiomers, with all QC samples within 15% of their respective nominal value in the analytical run. The drug concentration-time curves are shown in Fig. 3, and the main pharmacokinetic parameters are listed in Table 5.

The results demonstrated that following the oral administration of 1, 5, and 10 mg/kg of the ilaprazole racemate, the concentration of *S*-ilaprazole in the rat plasma was always higher than that of *R*-ilaprazole. According to Table 5, the $AUC_{0\text{-}t}$ of *S*-ilaprazole were 2.44 times, 1.95 times and 1.28 times that of *R*-ilaprazole, respectively; the $V_{z/F}$ of *S*-ilaprazole were 0.38 times, 0.56 times and 1.14 times that of *R*-ilaprazole, respectively; *S*-ilaprazole C_{max} were 1.96 times, 1.79 times and 1.28 times that of *R*-ilaprazole, respectively. The above results indicated that there was a stereoselective difference in the plasma pharmacokinetic behavior of the ilaprazole enantiomer in SD rats ($P < 0.05$). At the same time, the enantiomeric differences of ilaprazole observed in the pharmacokinetic profile were opposite to those of lansoprazole and rabeprazole [19], and consistent with those of omeprazole and pantoprazole [20–22]. As demonstrated in Table 5, the AUC/dose of the ilaprazole enantiomer showed different ratios in the range of 1–10 mg/kg, and the C_{max}/dose of the ilaprazole enantiomer was not linear. In addition, other pharmacokinetic parameters also varied significantly with dose. This indicated that the ilaprazole enantiomer demonstrated nonlinear dynamic properties at doses ranging between 1 and 10 mg/kg in rats.

The pharmacokinetic profile of ilaprazole enantiomers was studied in healthy human volunteers using an oral dose of 5 mg/kg, with no significant stereoselectivity reported between the pharmacokinetics of *R*- and *S*-ilaprazole [18]. The results demonstrated that the pharmacokinetic profile of ilaprazole was significantly different between the rat and human species. This phenomenon indicates that the extrapolation of pharmacokinetic results of chiral drug enantiomers, from rats to humans, requires careful consideration.

4. Conclusion

In conclusion, an effective chiral LC-MS/MS method for the determination of ilaprazole enantiomers in rat plasma was established. Good chiral separation was achieved on the CHIRALPAK AS-RH column under the reversed-phase condition. The current method successfully revealed the stereoselective pharmacokinetics of ilaprazole enantiomers in rats. To our knowledge, this is the first study reporting the significantly higher bioavailability of the *S*-ilaprazole than that of *R*-ilaprazole in rats. This work would serve useful for monitoring the blood concentration of ilaprazole enantiomers during clinical treatment.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpha.2019.09.002.

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