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Development of a novel LC–MS/MS method for the determination of letosteine in human plasma and its application on pharmacokinetic studies

Xinxia Liu[a], Heng Zheng[b], Wei Tang[a], Zhenyu Qian[b], Yinsong Zhu[a], Jing Wang[a], Shiying Yuan[a], Xin Wen[a], Cuiling Cao[c], Hui Chen[a,*]

[a] Institute of Clinical pharmacology, Department of Pharmacology, Tongji Medical College of Huazhong University of Science and Technology, Wuhan, Hubei 430030, People's Republic of China
[b] Tongji Hospital, Tongji Medical College of Huazhong University of Science and Technology, Wuhan, Hubei 430030, People's Republic of China
[c] Union Hospital, Tongji Medical College of Huazhong University of Science and Technology, Wuhan, Hubei 430030, People's Republic of China

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ABSTRACT

Letosteine has been found to be effective in treating patients with chronic bronchopneumopathies in clinical practice. To provide robust support for its pharmacokinetic and clinical studies, a rapid and sensitive method based on liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) was developed and validated for the analysis of letosteine in plasma samples. After protein precipitation, the plasma samples were separated on a reversed-phase C_{18} column in less than 1.5 min. The LC–MS/MS system was performed in the positive ion multiple-reaction-monitoring (MRM) mode to produce intensive product ions of m/z 280.1 → 160.0 for letosteine and m/z 248.1 → 121.1 for the internal standard, tinidazole. The method was found to have excellent linearity ($r \geq 0.9974$), precision (RSD ≤ 5.83%), extraction recovery (71.8–73.0%) and stability (RE, −8.45% to 9.03%) over a concentration range of 0.1140–152.0 μg L^{-1}. Compared to the previous published radioactive method, LC–MS/MS method showed many advantages including shorter analysis time, simpler preparation procedure, increased sensitivity as well as lower safety risks. In addition, this method was successfully applied to study the pharmacokinetics of letosteine following a single and multiple dose oral administration in Chinese healthy volunteers.

1. Introduction

Letosteine, a cyclic cysteine derivative as mucolytic agent, has proved to be highly effective in the treatment of upper respiratory tract infections and chronic bronchopneumopathies, such as chronic bronchitis, pulmonary emphysema and asthma [1–6]. Letosteine may also have a therapeutic effect in preventing oxidative tissue damage induced by the respiratory burst; this can be attributed to its antioxidant activity by scavenging the reactive oxygen species [7]. What's more, letosteine was reported to be effective in children suffering from acute bronchitis [8].

However, no analytical methods using LC–MS/MS for determination of letosteine in biological samples have been published up to now, except for traditional radioactive approaches using letosteine labeled either with ^{14}C or ^{35}S [9,10]. Unfortunately, the methods of radioactivity measurements have many inherent drawbacks, including expensive radioactive sources and measuring devices [11], complicated radioactive labeling techniques [12], long exposure time for autoradiography, especially the ethical and safety issues associated with the use and disposal of radioactive materials [13], which highly limit their applications in handling of large numbers of samples from pharmacokinetic researches or clinical use.

To provide adequate support for the pre-clinical and clinical studies of letosteine, a sensitive and reliable bioanalytical method is highly required for the determination of letosteine in human plasma. So, in this research work, an LC–MS/MS method with high speed and satisfactory sensitivity was developed. A lower limit of quantification (LLOQ) of 0.1140 μg L^{-1} was attained for letosteine in human plasma with retention time of 1.1 min. What's more, the present LC–MS/MS method was successfully applied to study the pharmacokinetics of letosteine in Chinese healthy volunteers after single (25, 50, and 100 mg) and multiple (50 mg, three times daily) dose administration.

2. Experimental

2.1. Reagents and materials

The letosteine tablets containing 25 mg of letosteine per tablet (batch No. 0812002) and the reference standard of letosteine (batch No. 0905001, 99%, Fig. 1a) were both supplied by Xi' an BoHua

* Corresponding author. Tel.: +86 27 83692628; fax: +86 27 83692628.
E-mail address: chenhuitjmu@163.com (H. Chen).

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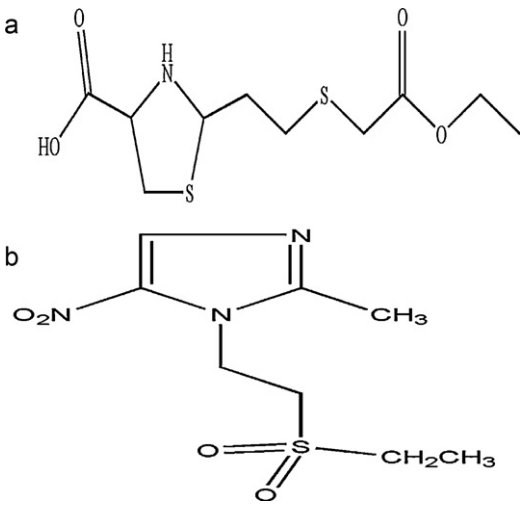

Fig. 1. Chemical structure of letosteine (a) and tinidazole (b).

Pharmaceutical Co. Ltd. (Shanxi, China). Tinidazole (99.5% purity, Fig. 1b), used as an internal standard (IS), was supplied by Hubei Institute for Food and Dug Control (Hubei, China). Acetonitrile and methanol for HPLC use were from Fisher Scientific (Fair Lawn, NJ, USA). Formic acid and ammonium acetate, both analytical grades, were purchased from the Tianjin HengXing Chemical Reagent Preparation Co. Ltd. (China). Ultra-pure water was prepared by a Milli-Q system (Millipore, Molsheim, France). Heparinized drug-free plasma was obtained from Tongji Hospital (Hubei, China).

2.2. Instrument

An Agilent 1200 HPLC system (Agilent Technologies, Santa Clara, USA) consisted of an on-line degasser, a quaternary pump, an auto-sampler and a thermostated column compartment. The system was coupled on-line to an API 4000 triple quadrupole mass spectrometer (Applied Biosystems, USA) equipped with Turbo Ion Spray source (electrospray) for mass analysis and detection. The system operation and data processing were performed on Analyst 1.5 software package.

2.3. Chromatographic conditions

The mobile phase, acetonitrile–0.5 mM ammonium acetate containing 0.25% formic acid (22:78, v/v), was run at a flow rate of 0.3 mL min^{-1}. Isocratic chromatographic separation was performed on a Phenomenex Luna C_{18} column (50 mm × 2.0 mm i.d., particle size 3 μm) and a 4 mm × 3.0 mm i.d. C_{18} guard column (Phenomenex, Torrance, CA, USA), with the column temperature at 35 °C. The injection volume was 5.0 μL.

2.4. Mass spectrometric conditions

The ESI source was operated in positive ion mode at 3500 V and 650 °C. Quantization was performed using multiple reaction monitoring (MRM) of the transitions m/z 280.1 → 160.0 for letosteine and m/z 248.1 → 121.1 for IS, respectively. The value of DP (declustering potential) was set at 55 V for letosteine and 73 V for IS. The collision energy (CE) of 18.5 eV was used for the analyte and 23 eV for IS. The entrance potential was set at 4 V for letosteine and 10 V for IS. The collision exit potential was adjusted to 12 V for letosteine and 10 V for IS, respectively. High purity nitrogen served as collision-activated dissociation (CAD) gas (simplified setting at 7 psi) and curtain gas (setting at 10 psi). The nebulizer gas (GAS1) and auxiliary heater gas (GAS2) was set at 60 and 70 psi, respectively.

2.5. Preparation of calibration standards and QC samples

The stock solution of letosteine was obtained by dissolving the appropriate amount of drug pure substance in methanol. Then it was serially diluted with methanol to obtain working solutions with the concentrations of letosteine ranging from 1.140 to 1520 μg L^{-1}. The quality control (QC) solutions were prepared at concentrations of 1.140, 3.055, 45.60, and 1216 μg L^{-1} in a similar manner. A solution of IS (114.0 μg L^{-1}) was prepared by dissolving tinidazole with methanol. All solutions were stored at −20 °C when not in use.

The calibration standards (0.114, 0.410, 1.50, 4.56, 15.2, 50.2 and 152 μg L^{-1}) in plasma samples were prepared by spiking 20 μL of each working solutions into 200 μL of drug-free plasma. The QC samples at three different levels (0.3055, 4.560, and 121.6 μg L^{-1}) were prepared in the similar way of the calibration standards. The calibration curve was prepared and assayed along with appropriate QC samples during the process of each analytical batch of unknown clinical samples.

2.6. Preparation of sample

An aliquot of 200 μL of plasma sample was pipetted out into a 1.5 mL eppendorf tube, and 20 μL of IS (tinidazole, 114.0 μg L^{-1}) in methanol was added. After vortex-mixing, the sample mixture was deproteinized with 600 μL of methanol and the precipitate was removed by centrifugation at 12000 rpm for 10 min. Then 200 μL of the supernatant was transferred to an auto-sampler vial. A 5 μL aliquot of mixture was injected into the LC–MS/MS system for analysis.

2.7. Method validation

2.7.1. Selectivity

The specificity of the method was assessed by analyzing blank plasma from six different subjects, blank plasma spiked with letosteine at the LLOQ level (0.1140 μg L^{-1}) and IS, as well as plasma sample in clinical study to ensure that no interference occurred at the retention times of analyte and IS.

2.7.2. Linearity and lower limit of quantification

To evaluate linearity, five sets of calibration standards were prepared and analyzed each day in three continuous days. Calibration curves were constructed by linear least-squares regression analysis employing $1/x^2$ as the weighting factor. LLOQ was defined as the lowest concentration on the calibration curve. The deviations of the back-calculated concentrations of calibration standards from their nominal values should be within ±20% for the LLOQ and ±15% for all other calibration levels. The LLOQ was evaluated by analyzing six replicates of spiked samples prepared with blank plasma from different subjects.

2.7.3. Precision and accuracy

To assess the accuracy and precision of the method, five replicates of samples at three concentration levels of QC (0.3055, 4.560, and 121.6 μg L^{-1}) and at LLOQ (0.1140 μg L^{-1}) were analyzed each day in three consecutive days. The precision (relative standard deviations, RSD) was determined by expressing the standard deviation of the measurements as a percentage of the average value. The accuracy is estimated by the relative error (RE) between the measured concentration and the actual concentration. The intra-and inter-

day precision were required to be below 15% (20% for the LLOQ), the accuracy to be within ±15% (±20% for the LLOQ).

2.7.4. Absolute recovery

The absolute recovery of letosteine following the protein precipitation procedure was determined by comparing the mean peak areas obtained from five replicates of QC samples at three concentrations (0.3055, 4.560, and 121.6 μg L^{-1}), with that from spike-after-extraction samples at the same concentrations. Similarly, recovery of IS was also evaluated by comparing the mean peak areas of regularly prepared samples to that of pretreated drug free plasma samples spiked with IS at the concentration of 11.4 μg L^{-1}.

2.7.5. Stability studies

The stability of letosteine in human plasma was evaluated by preparing three replicates of spiked plasma samples at the concentrations of 0.3055, 4.560, and 121.6 μg L^{-1}, which were treated and analyzed after exposed to the following different conditions: in auto-sampler for 8 h at room temperature after protein precipitation; at ambient temperature for 8 h, at −80 °C for 40 days and after three freeze–thaw cycles (24 h for a cycle) from −80 °C to room temperature. The measured concentrations were then compared to those of the same QC samples that had been analyzed immediately after processing. The analyte was considered stable in the biological matrix when 85–115% of the initial concentration was found.

2.7.6. Matrix effect

To evaluate the matrix effect, five replicates of post-extraction blank plasma were spiked with the analyte at 0.3055, 4.560, and 121.6 μg L^{-1}, respectively. The blank plasma samples used in this study were from five different batches of human blank plasma. The corresponding peak areas of letosteine in post-extraction spiked plasma (A) were then compared with peak areas of the solution standards in mobile phase (B) at equivalent concentrations. The ratio (A/B × 100%) was defined as the absolute matrix effect (ME). When the value was between 85% and 115%, it could be considered that there was no significant matrix effect. The same evaluation was performed for IS (11.4 μg L^{-1}).

2.8. Pharmacokinetic application

2.8.1. Drug administration

The validated method was successfully applied to study single- and multiple-dose pharmacokinetics of letosteine in Chinese healthy volunteers. The clinical study protocol was approved by the ethics committee of Tongji Medical College, Huazhong University of Science and Technology. After physical examinations and clinical laboratory tests, eighteen male and eighteen female healthy subjects were screened and enrolled in the study. In the single-dose study, the participants were randomized evenly into three treatment groups according to the different dosage: low dose (25 mg), medium dose (50 mg,) and high dose (100 mg). On the morning of study day 1, at approximately 7:00 am, the medication was given by oral administration. In the multiple-dose study, the volunteers in medium-dose group were further administered with letosteine after 8 h and 16 h of the first dose on day 1, and continued to receive the same treatment at the same time as day 1 for the following 5 consecutive days. On day 7, the subjects received the last dose of letosteine at approximately 7:00 am.

2.8.2. Plasma samples collection

For the evaluation of single-dose pharmacokinetics, blood samples were taken on day 1 just before the first intake of drug and at 0.167, 0.33, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8 h thereafter. For the evaluation of multiple-dose pharmacokinetics, blood samples were collected on day 7 according to the schedule described above for day 1 after drug intake. Additional samples were sampled at the point of 72, 96 and 120 h after the first intake of letosteine to determine the trough plasma concentration level. Blood samples were collected in tubes containing heparin and centrifuged at 3500 rpm for 15 min. The plasma was separated and stored at −80 °C until analysis.

2.8.3. Pharmacokinetic analysis

The pharmacokinetic analysis was performed by means of non-compartmental analysis using the drug analytical system Drug and Statistic software version 2.1.1 program (The Chinese Society of Mathematical Pharmacology, Beijing, China). The individual plasma letosteine concentration–time profiles were used to determine the maximum plasma concentration (C_{max}) and the point (T_{max}). The terminal elimination rate constant (λ_z) was determined by least-squares regression analysis of the log-transformed data in the terminal post-distribution phase, and the elimination half-life ($t_{1/2}$) was calculated through $0.693/\lambda_z$. The area under the plasma concentration–time curve from time zero to the last measurable plasma concentration (AUC_{0-t}) was estimated by the linear trapezoidal method. The area under the plasma concentration–time curve from time zero to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-t} + Ct/\lambda_z$, where Ct was the last measurable plasma concentration. For assessment of multiple-dose study, the following parameters were also calculated: total body clearance (CL/F) and apparent volume of distribution (V_z/F).

2.8.4. Statistical analysis

The statistical package SPSS version 11.5 (SPSS, Inc., Chicago, Illinois) was used for the statistical calculation. The difference in pharmacokinetic parameters among each dose level was investigated for significance by one-way analysis of variance (ANOVA) on log-transformed data (factor dose). Prior to analysis, dose-dependent parameters (C_{max} and AUC) were normalized with the dose to assess the parameters among dose levels. The one-way ANOVA method was also applied to investigate whether the pharmacokinetic parameters after multiple dosing were in concordance with those calculated from single-dose study. The effect of gender on the pharmacokinetic parameters of letosteine was also studied by one-way ANOVA method. A P value of ≤ 0.05 was considered statistically significant.

3. Results and discussion

3.1. Method development

Tandem MS spectrometric parameters were first optimized in order to obtain the most specific and sensitive detection. To achieve the optimal sensitivity, direct infusion of standard solution was carried out to optimize the electrospray ion source parameters. The full scan mass spectra were recorded, and the protonated ion was selected as the precursor ion since it provided high sensitivity and a better, more stable fragmentation compared to the deprotonated ion. The precursor ions of letosteine and IS obtained from ESI were m/z 280.1 and m/z 248.1, respectively, which were chosen to provide product ions mass spectrum. The most suitable collision energy was determined by observing the response of the obtained fragment ion peak. After collision-induced dissociation, the most abundant ion in the product ions mass spectra were at m/z 280.1 → 160.0 for letosteine at collision energy of 18.5 eV, and m/z 248.1 → 121.1 for IS at collision energy of 23.0 eV. In the present

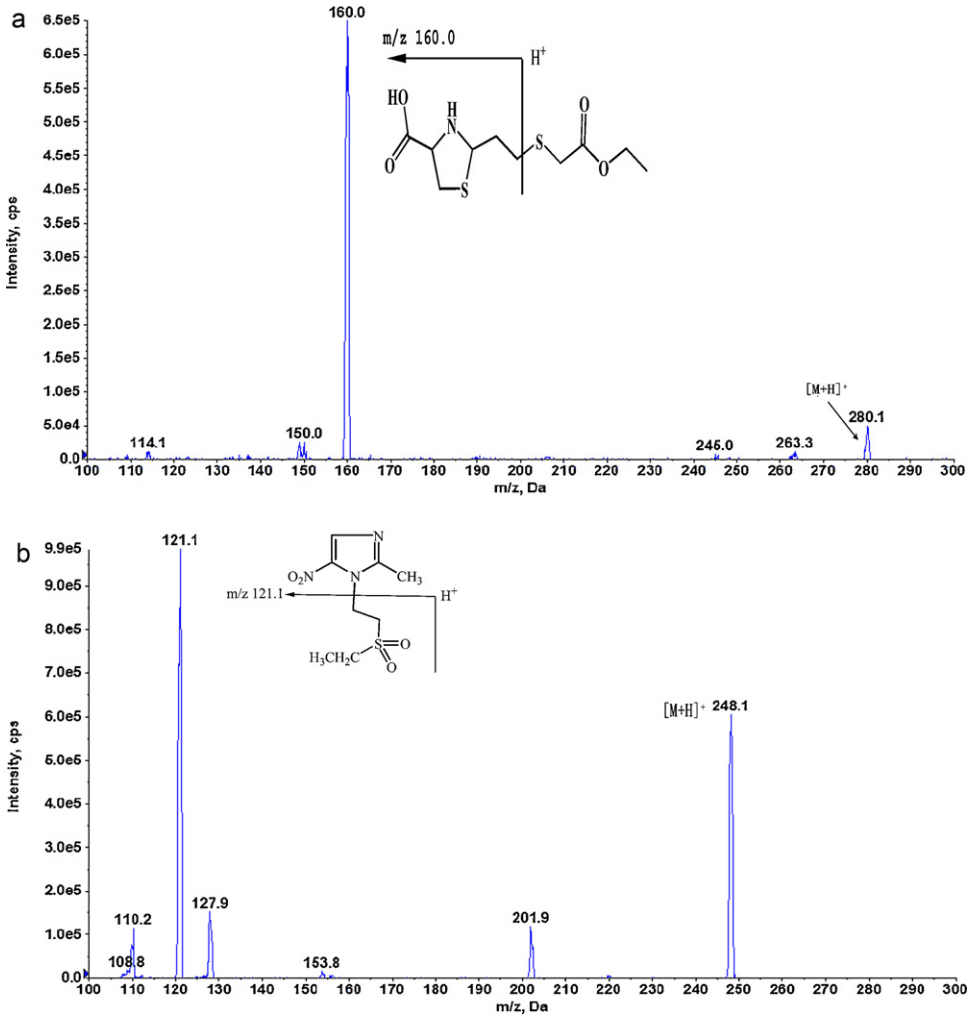

Fig. 2. Product ions scan spectra for the protonated molecular ions of letosteine (m/z 280.1, a) and tinidazole (m/z 248.1, b).

method, LC–MS/MS in multiple reaction monitoring (MRM) mode was performed using the transitions described above to obtain high specificity and low noise. Fig. 2 showed the full-scan product ions MS/MS spectra of letosteine and IS.

The influences of mobile phase composition on chromatographic performance were investigated to achieve the optimum separation of the analytes in the mixture within the minimum time. Both acetonitrile and methanol were tested as mobile phases, which demonstrated that acetonitrile and water were preferable for separation. Ammonium acetate was usually added to mobile phase to improve the peak shape and promote source ionization, and the best results in this study were obtained by adding ammonium acetate at 0.5 mM, because the higher the content of the salt is, the greater the loss of sensitivity during measurements. The best response signal and peak shape for letosteine and tinidazole occurred with a small percentage of formic acid (0.25%) added to the mobile phase. Therefore, acetonitrile–0.5 mM ammonium acetate aqueous solution containing 0.25% formic acid (22:78, v/v) was used as mobile phase to optimize the separation and get the maximal signal of peaks. For appropriate runing time, the flow rate of isocratic elution was set to 0.3 mL min^{-1}. Tinidazole was selected as IS because of its suitable sensitivity and retention time. In preparation of samples, protein precipitation using methanol was adopted in the present study, which could reduce the processing time, simplify the sample preparation and improve the reproducibility.

3.2. Method validation

3.2.1. Specificity and selectivity

Under optimized LC–MS/MS conditions, letosteine and IS were separated with retention times of 1.1 and 1.3 min, respectively. And the total run time was less than 3.0 min. Fig. 3 showed the typical chromatograms of plasma samples, as it can be seen, no significant interference from endogenous components was observed at the retention times of the analyte and IS, and the signals of the LLOQ level were easily distinguishable from blank responses.

3.2.2. Linearity and lower limit of quantification

Good linearity of letosteine in the concentration range (0.1140–152.0 μg L^{-1}) was obtained during the study. All calibration standards were up to the acceptance criteria. A typical regression equation was $y = 1.77x + 0.00501$, $r = 0.9989$, where y was the peak area ratio of letosteine to IS and x was the plasma concentration of letosteine.

The LLOQ was set at 0.1140 μg L^{-1} with RE ranging from −3.8 to 7.7% and an RSD value of 7.3%. Under the present LLOQ, the concentration of letosteine in plasma samples could be determined in a span of 8 h after a single oral dose of 25 mg letosteine, which was sensitive enough to investigate the pharmacokinetic behavior of the drug.

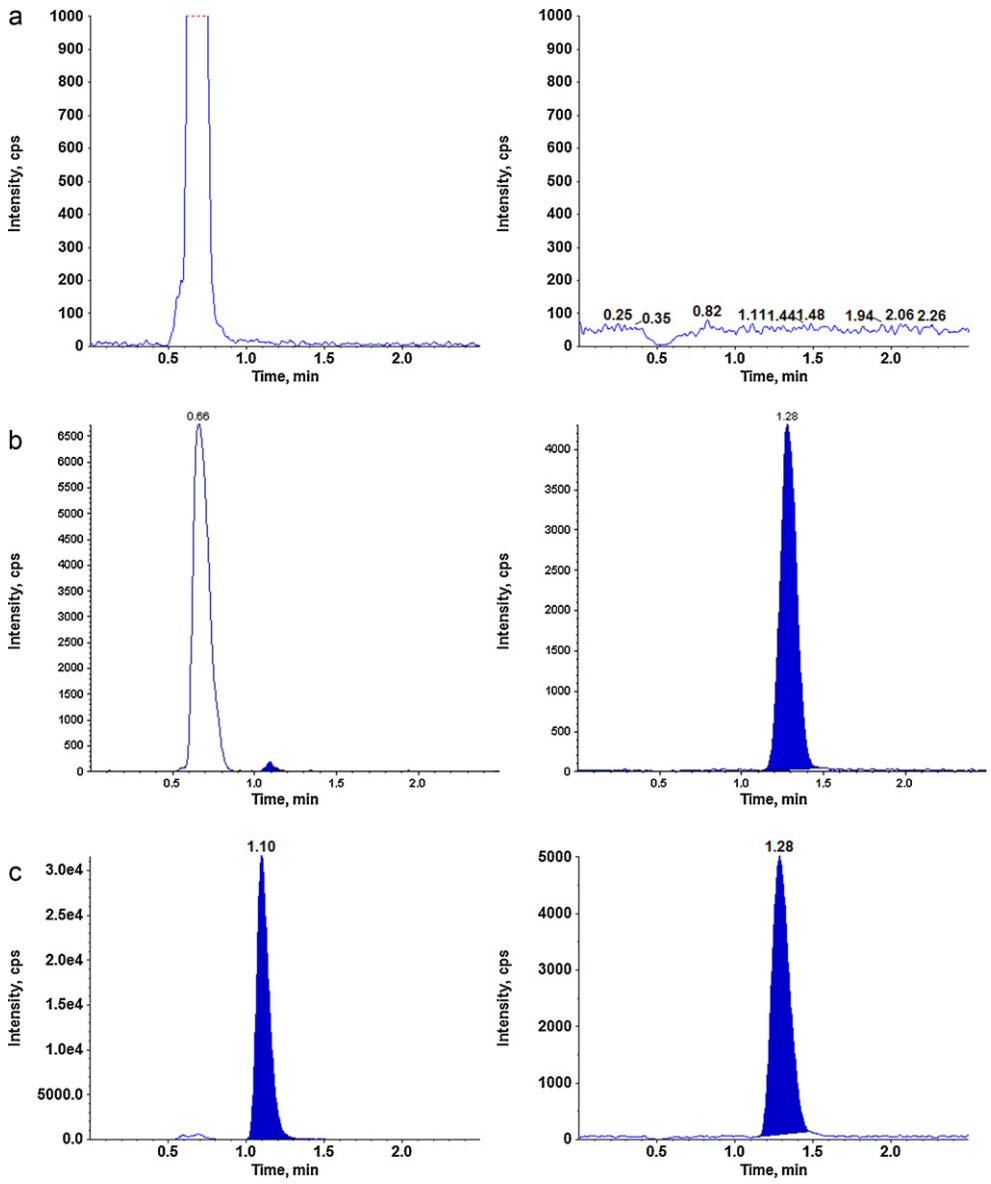

Fig. 3. Representative chromatograms of letosteine (left) and tinidazole (right) in human plasma: (a) blank plasma sample; (b) spiked plasma at LLOQ (0.1140 μg L^{-1}) concentration with IS (11.4 μg L^{-1}) and (c) plasma sample collected at 0.5 h after an single dose of letosteine to NO. 2 volunteer in medium dose group.

3.2.3. Precision and accuracy

The data about precision and accuracy of letosteine are shown in Table 1. For all the concentration levels of letosteine, the result was within the requirements of the guidelines for bioanalytical methods [14].

3.2.4. Absolute recovery

The recovery of letosteine using the described procedure was steady at all the three concentrations studied. The data obtained for recoveries of the analyte and IS were summarized in Table 2, all recoveries were acceptable for validation of the assay.

Table 2
The absolute recovery of letosteine and tinidazole in human plasma ($n = 5$).

Samples	Concentration (μg L^{-1})	Extraction recovery (mean ± SD)	RSD (%)
letosteine	0.3055	71.8 ± 6.6	9.27
	4.560	72.2 ± 2.0	2.76
	121.6	73.0 ± 3.8	5.16
IS	11.4	90.2 ± 3.2	3.59

Table 1
Intra-day and inter-day precision and accuracy for assay of letosteine in human plasma.

Nominal conc. (μg L^{-1})	Intra-day ($n = 5$)			Inter-day ($n = 3$)		
	Measured conc. (mean ± SD)	RSD (%)	RE (%)	Measured conc. (mean ± SD)	RSD (%)	RE (%)
0.1140	0.1156 ± 0.0042	3.67	1.37	0.1162 ± 0.0016	1.36	1.91
0.3055	0.333 ± 0.012	3.69	9.00	0.322 ± 0.018	5.69	5.47
4.560	4.38 ± 0.21	4.83	−4.06	4.45 ± 0.25	5.51	−2.41
121.6	129 ± 4	2.86	5.92	121 ± 7	5.83	−0.66

Table 3
Stability of letosteine in human plasma and processed samples ($n = 3$).

Storage condition	Concentration ($\mu g\,L^{-1}$)		RE (%)
	Nominal	Measured (mean ± SD)	
Processed sample at room temperature for 8 h	0.3055	0.30 ± 0.03	−1.34
	4.560	4.58 ± 0.10	0.44
	121.6	119 ± 3	−2.14
Ambient temperature for 8 h	0.3055	0.333 ± 0.025	9.03
	4.560	4.48 ± 0.14	−1.75
	121.6	127 ± 12	4.44
−80 °C for 40 days	0.3055	0.280 ± 0.021	−8.45
	4.560	4.4 ± 0.4	−3.95
	121.6	114 ± 5	−5.84
Three freeze–thaw cycles	0.3055	0.32 ± 0.03	4.88
	4.560	4.43 ± 0.25	−2.85
	121.6	119.64 ± 0.09	−1.64

3.2.5. Stability studies

The results of stability are presented in Table 3, which indicated that no significant degradation occurred under the storage conditions described above. The stability of letosteine in human plasma may simplify the analytical procedures.

3.2.6. Matrix effect

The absolute matrix effects for letosteine at concentrations of 0.3055, 4.560, and 121.6 $\mu g\,L^{-1}$ and IS (11.4 $\mu g\,L^{-1}$) were 99.10%, 108.35%, 91.81% and 102.29%, respectively. The results obtained were well within the acceptable limit, which indicated that ion suppression or enhancement from plasma matrix could be negligible in this study.

3.3. Pharmacokinetic study

The described method was applied to study pharmacokinetics of letosteine in human. Fig. 4 depicts the mean plasma concentration–time curve after single oral administration of letosteine 25, 50, and 100 mg, respectively. The main pharmacokinetics parameters of single-dose study are given in Table 4. Letosteine was rapidly absorbed, with median T_{max} values increasing from 0.403 to 0.534 h. The C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of letosteine appeared to exhibit approximately dose-proportional increase over the dose range studied. And there were no significant differences among these three dosage groups in T_{max}, $t_{1/2z}$, V_z/F and CL/F. In addition, between-gender comparisons of above-mentioned main pharmacokinetic parameters suggested that there were no clinically relevant pharmacokinetic differences.

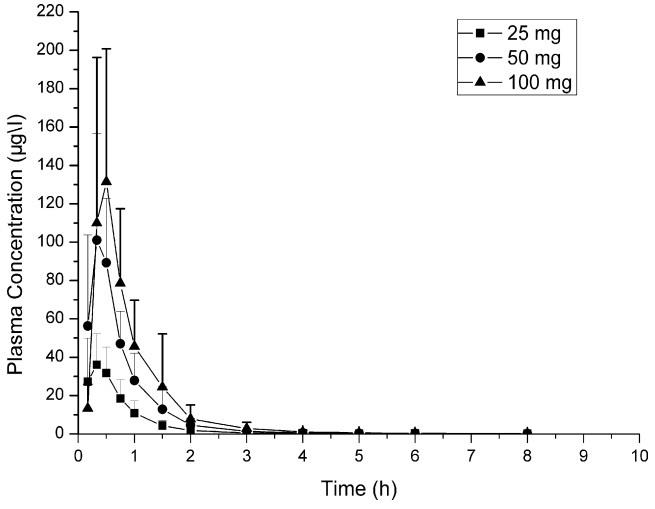

Fig. 4. Mean plasma concentration–time profiles of letosteine in Chinese healthy participants after single-dose oral administration. Data represent mean ± SD ($n = 12$ per dose).

Table 4
The pharmacokinetic parameters of letosteine in plasma following single dose administration of letosteine in healthy subjects (mean ± SD, $n = 12$).

Parameter	Single dose (mg)		
	25	50	100
T_{max} (h)	0.40 ± 0.23	0.42 ± 0.15	0.53 ± 0.15
C_{max} ($\mu g\,L^{-1}$)	45.91 ± 14.80	118.70 ± 50.30	157.28 ± 91.28
AUC_{0-t} ($\mu g\,h\,L^{-1}$)	30.21 ± 9.11	79.22 ± 20.01	107.74 ± 22.03
$AUC_{0-\infty}$ ($\mu g\,h\,L^{-1}$)	30.36 ± 9.14	79.43 ± 20.14	108.08 ± 21.97
$t_{1/2z}$(h)	0.75 ± 0.40	0.90 ± 0.55	1.10 ± 0.49
V_z/F (L)	911.95 ± 413.73	819.66 ± 421.71	1534.143 ± 773.24
CL/F ($L\,h^{-1}$)	891.91 ± 255.78	668.93 ± 172.94	962.96 ± 207.65

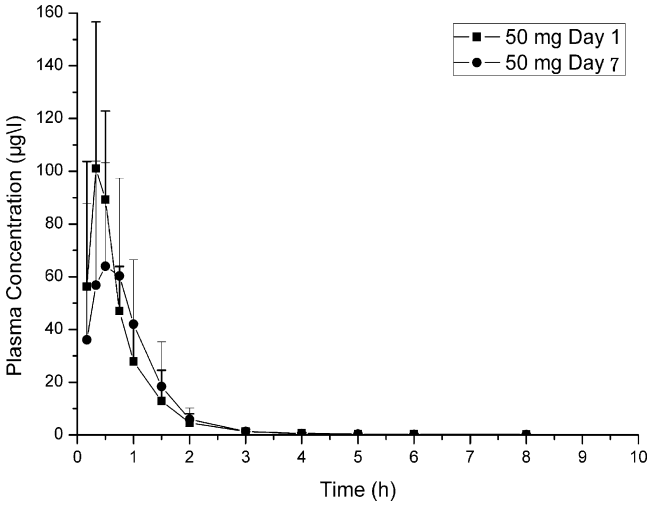

Fig. 5. Mean plasma concentration–time curves in Chinese healthy participants of multiple-dose group. Data represent mean ± SD ($n = 12$).

Table 5
The pharmacokinetic parameters in 12 healthy volunteers after a single dose and multiple dose of 50 mg letosteine (mean ± SD, $n = 12$).

Parameter	Single dose (day 1)	Multiple dose (day 7)
T_{max} (h)	0.42 ± 0.15	0.70 ± 0.38
C_{max} ($\mu g\,L^{-1}$)	118.70 ± 50.30	94.883 ± 30.97
AUC_{0-t} ($\mu g\,h\,L^{-1}$)	79.22 ± 20.01	72.72 ± 18.25
$AUC_{0-\infty}$ ($\mu g\,h\,L^{-1}$)	79.43 ± 20.14	72.88 ± 18.27
$t_{1/2z}$ (h)	0.90 ± 0.55	0.56 ± 0.11
V_z/F (L)	819.66 ± 421.71	597.83 ± 220.17
CL/F ($L\,h^{-1}$)	668.93 ± 172.94	723.46 ± 166.42

The mean plasma concentration versus time profiles of letosteine following first-dose administration on day 1 and last-dose administration on day 7 in multiple-dose study are presented in Fig. 5, and the main pharmacokinetics parameters are given in Table 5. No significant differences were observed by ANOVA analysis ($P > 0.05$) between the derived pharmacokinetic parameters summarized in Table 5, indicating that there was no accumulation of letosteine following repeated drug administrations. And gender did not appear to affect the multiple-dose pharmacokinetics of letosteine ($P > 0.05$).

4. Conclusions

In this paper, a rapid and sensitive LC–MS/MS method for the determination of letosteine in human plasma is described for the first time. The method consists of simple sample preparation

process by protein precipitation, chromatographic separation and tandem MS detection. No interfering peaks were observed at the elution times of letosteine and IS. Adequate precision, accuracy, stability and suitability of the proposed method were demonstrated over the concentration range of 0.1140–152.0 $\mu g\,L^{-1}$ ($r \geq 0.9974$). Unlike radioactive measurements, LC–MS/MS method is out of ethical dilemma, and does not require complicated radioactive labeling techniques or special detector such as liquid scintillation counter. The present method was proved to be superior for small sampling volume (200 μL), high-speed analysis (t_R = 1.1 min), much better sensitivity (LLOQ at 0.114 $\mu g\,L^{-1}$), simple and single step extraction procedure using inexpensive chemicals. The established method provides a safe and sensitive bioanalytical methodology for carrying out the pharmacokinetics of letosteine in human.

Furthermore, as shown in this paper, the method was successfully applied for the evaluation of the pharmacokinetic profiles of letosteine in Chinese healthy volunteers, which was found to be reasonably reliable. Letosteine underwent rapid and extensive elimination after being almost quickly absorbed, and dose-proportionality was observed. The in vivo data described in this manuscript provide a valuable application to the developed method and a clear insight into the pharmacokinetics of letosteine.

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