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Determination of the active metabolite of moguisteine in human plasma and urine by LC–ESI-MS method and its application in pharmacokinetic study☆

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ABSTRACT

In this study, a sensitive and reproducible electro-spray ionization liquid chromatography–mass spectrometry (LC–ESI-MS) method was established to determine the concentration of M1, the main active metabolite of moguisteine in human plasma and urine. The analysis was performed on a Diamonsil® $C_{18}(2)$ column (150 mm × 4.6 mm, 5 μm) with the mobile phase consisting of 0.1% formic acid–acetonitrile (57:43, v/v, pH = 3.0) at a flow rate of 0.8 mL min^{-1}. The pseudo-molecular ions $[M+H]^+$ (*m/z* 312.2 for M1 and 446.3 for glipizide) were selected as the target ions for quantification in the selected ion monitoring (SIM) mode. Plasma samples were analyzed after being processed by acidification with formic acid and protein precipitation with acetonitrile. Urine samples were appropriately diluted with blank urine for analysis. Calibration curve was ranged from 0.02 to 8 μg mL^{-1}. The extraction recovery in plasma was over 90%. Both the inter- and intra-day precision values were less than 7.5%, and the accuracy was in the range from −6.0% to 6.0%. This is the first reported LC–ESI-MS method for analyzing M1 in human plasma and urine. The method was successfully applied to the pharmacokinetic study after oral administration of single-dose and multiple-dose of moguisteine tablets in healthy Chinese subjects.

1. Introduction

Moguisteine, (*R*,*S*)-2-(2-methoxyphenoxy)-methyl-3-ethoxycarbonyl-acetyl-1,3-thiazolidine) (Fig. 1), is a novel peripheral non-narcotic antitussive agent by having inhibitory effect on the airway [1,2]. The antitussive function is similar to that of codeine, but no interaction with central opioid receptors is observed [3]. Moguisteine is effective against experimentally induced cough in a dose related manner. It was also well tolerated after oral administration of healthy subjects [4–6]. The ATP-sensitive K^+ channels in tracheobronchial tract may play an important role in the antitussive effect of moguisteine inhaled [7,8].

Moguisteine is a prodrug. Previous studies showed that moguisteine underwent a prompt and complete presystemic hydrolysis to produce its principal active metabolite, the free carboxylic acid, named as M1. M1 is partly metabolited to M2, the sulfoxidation derivative. The pharmacological activity of M2 is unknown, and there is no M2 standard from the market. Thus, M1 as the target compound is determined for studying the pharmacokinetics of moguisteine. So far, there is no study on the metabolism of moguisteine. The potential interaction of moguisteine with other drugs is still expected to be researched in future.

An HPLC method for the analysis of M1 in plasma and urine samples has been reported previously [1], however, its disadvantages with the complex sample preparation and the long injection time were presented. In the present study, we aim to develop a simple and sensitive LC–ESI-MS method for the determination of M1 in plasma and urine samples. It was also successfully applied to evaluate the pharmacokinetic characteristics of moguisteine after single-dose and multi-dose of moguisteine tablets in healthy Chinese subjects, which provides guidance for clinical application.

2. Materials and methods

2.1. Chemicals and instrumentation

Moguisteine tablet (batch 091224) and M1 standard (purity 99.5%) were obtained from Shanchuan Pharmaceutical Co., Ltd. (Shandong, China). Glipizide standard (purity 99.1%), used as the internal standard (I.S.), was obtained from Yunmen Pharmaceutical Co., Ltd. (Shandong, China). Methanol and acetonitrile of HPLC grade were from J.T. Baker. Formic acid, HPLC grade, was from Acros

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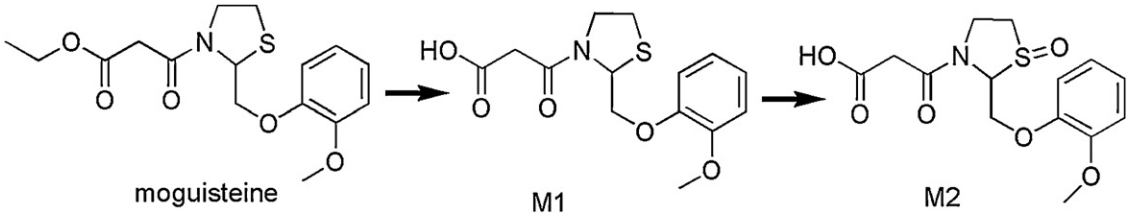

Fig. 1. Metabolic pathway of moguisteine in man.

Organics. Blank human plasma was provided by Shandong Blood Center of China.

Agilent 1100 series LC system (equipped with a quadruple pump, a vacuum degasser, a thermostated column compartment and an autosampler) and an Agilent 1946D single quadrupole mass spectrometric detector equipped with ESI source were used for mass detection (Agilent Technologies, USA). Data analysis was accomplished by using Agilent ChemStation software (Version A.10.01).

2.2. LC–ESI-MS conditions

The separation was performed on a Diamonsil® $C_{18}(2)$ column (150 mm × 4.6 mm, 5 μm), and eluted by the mobile phase of 0.1% formic acid–acetonitrile (57:43, v/v) at a flow rate of 0.8 mL min^{-1}. The column temperature was maintained at 25 °C by using a thermostated column compartment.

The MS conditions were as follows: positive ion mode; capillary voltage, 4000 V; source temperature, 300 °C; drying gas flow, 12 L min^{-1}; nebulizer gas, 50 psi; fragment electric voltage, 70 V for M1 and 100 V for glipizide. The pseudo-molecular ions $[M+H]^+$ (m/z 312.2 for M1 and 446.3 for glipizide) were selected as the target ions for quantification in the selected ion monitoring (SIM) mode.

2.3. Standard solutions and sample preparation

M1 and glipizide (I.S.) standards were accurately weighted and dissolved in methanol for obtaining 1.0 mg mL^{-1} of stock solution. Stock solution was further diluted with methanol for 100, 10 and 1 μg mL^{-1} working solutions of M1, and 10.0 μg mL^{-1} of I.S. All were stored at 4 °C, and equilibrated to room temperature before use (approximately 15 min).

Two hundred microliters of plasma sample was mixed with 20 μL of glipizide (I.S.) working solution, 20 μL of 10% formic acid and 500 μL of acetonitrile (protein precipitation reagent), vortex-mixed for 2 min, and then centrifuged at 10,800 rpm for 10 min. The supernatant was transferred to the vial for analysis. 10 μL of urine sample was appropriately diluted to 1 mL with blank urine, added with 50 μL of I.S. working solution, 50 μL of 10% formic acid, vortex-mixed for 1 min, and centrifuged at 10,800 rpm for 5 min, and then injected for analysis.

2.4. Method validation

The specificity of the method was evaluated by comparing chromatograms of the standard solutions of M1 and I.S., blank plasma, blank urine, blank plasma or blank urine spiked with M1 and I.S., plasma or urine from a subject after oral administration of moguisteine tablets. At least six blank plasma or urine samples from different individuals were examined for the specificity of the method. Calibration curves were made by analyzing spiked calibration samples at 0.02, 0.1, 0.5, 1, 2, 4 and 8 μg mL^{-1} on each analysis day. The linearity of calibration curves was assessed by linear regression with a weighting factor of the reciprocal of the concentration squared ($1/x^2$).

The matrix effects were evaluated by extracting fifteen different lots of blank plasma or urine and then spiking with M1 at three concentration levels (0.05 μg mL^{-1}, 1 μg mL^{-1} and 7 μg mL^{-1}) and I.S. in five replicates at each concentration level, respectively. The corresponding peak areas were compared with those of standard solutions, and peak area ratio was used to evaluate the matrix effect. The matrix suppression was assessed by post-column infusion of an analyte with a syringe pump post column into the MS detector. The extracted blank matrix was injected by an autosampler onto the analytical column. The MS monitored the signal level from post-column infused analyte solution when injecting blank plasma or urine sample.

The extraction recoveries of M1 were evaluated by comparing the areas of M1 extracted from plasma or urine with those of standard solutions at equivalent concentrations, and five replicates at each concentration level were disposed with the established extraction procedure. The extraction recovery of I.S. was evaluated in a similar way at the working concentration. The lowest limit of quantification (LLOQ) was evaluated by analyzing five replicates of spiked samples at the concentration of 0.02 μg mL^{-1} with acceptable precision.

Accuracy and precision were assessed by analyzing spiked samples at three concentration levels (0.05 μg mL^{-1}, 1 μg mL^{-1} and 7 μg mL^{-1}) for three consecutive days, and five replicates at each concentration level were applied. The mean value of RE for the accuracy should be within 15% of the actual value except at LLOQ, where it should not deviate by more than 20%. The relative standard deviation (RSD) for the precision determined at each concentration level should not exceed 15% except at LLOQ, where it should not exceed 20%.

The stability of M1 in plasma or urine was evaluated by analyzing samples with five replicates at each concentration level, after storage at −20 °C for 1, 7 or 28 days respectively, or after one or two freeze–thaw cycles (−20 °C to 25 °C).

2.5. Pharmacokinetic studies

The study was approved by the Ethics Committee of the College of Medicine, Shandong University and conformed to the principles of the Declaration of Helsinki. Subjects signed informed consent before any screening item being performed.

2.5.1. Single-dose study

Thirty healthy Chinese subjects, half male and half female, aged of 20–28 years, were randomly assigned to three groups and receive a single oral dose of 200 mg, 400 mg and 600 mg moguisteine tablets, respectively, with 200 mL of warm water after an overnight fast (10 h). Blood samples were collected before and 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0 h after administration. Samples were immediately centrifuged at 5000 rpm for 5 min, separated and transported into two EP tubes, labeled and stored at −20 °C for pending analysis. Urine samples of subjects with 400-mg dose were collected at time zero (blank), 0–1, 1–2, 2–3, 3–4, 4–6, 6–8, 8–10, 10–12, 12–24 h after administration. The volume of urine was recorded and stored at −20 °C until assayed.

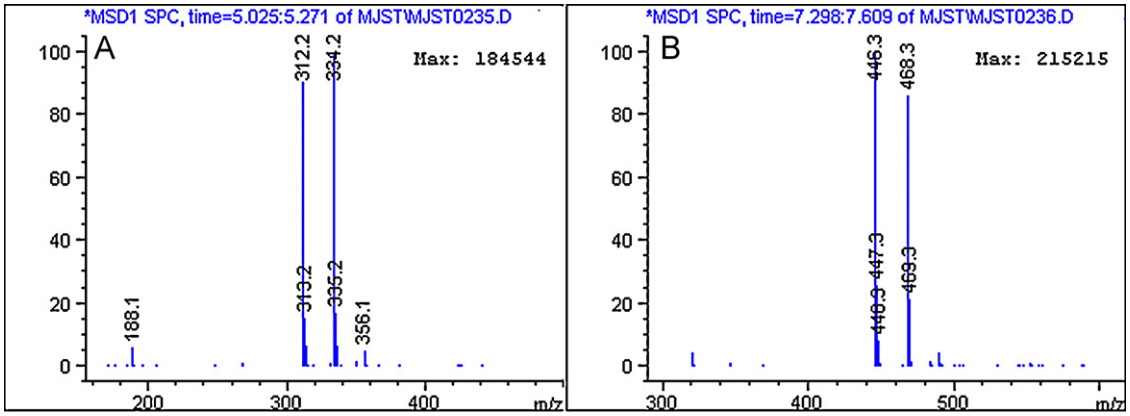

Fig. 2. Mass spectrum of M1 (312.2, A) and glipizide (446.3, B).

2.5.2. Multiple-dose study

Ten healthy Chinese subjects, half male and half female, aged of 20–28 years, were assigned to receive multiple dose of 200 mg of moguisteine tablets (200 mg, three times daily [tid]) under fed conditions for 7 days. Blood samples were collected on the morning of 4th, 5th, 6th, and 7th days before and 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0 h after administration on the morning of 7th day, then were processed with the same method as the single-dose study.

2.5.3. Pharmacokinetic analysis

The pharmacokinetic parameters including the maximum plasma concentration (C_{max}), time to maximum plasma concentration (t_{max}) were obtained directly from the measured data. The mean residence time (MRT), terminal elimination half-life ($t_{1/2}z$), apparent volume of distribution (Vz), clearance (CLz), and area under the plasma concentration–time curve from 0 to last measurable time (AUC_{0-8}) or from 0 to infinity ($AUC_{0-\infty}$) were calculated by using *Drug and Statistics Software-Version 2.0* (*DAS 2.0*, PR China).

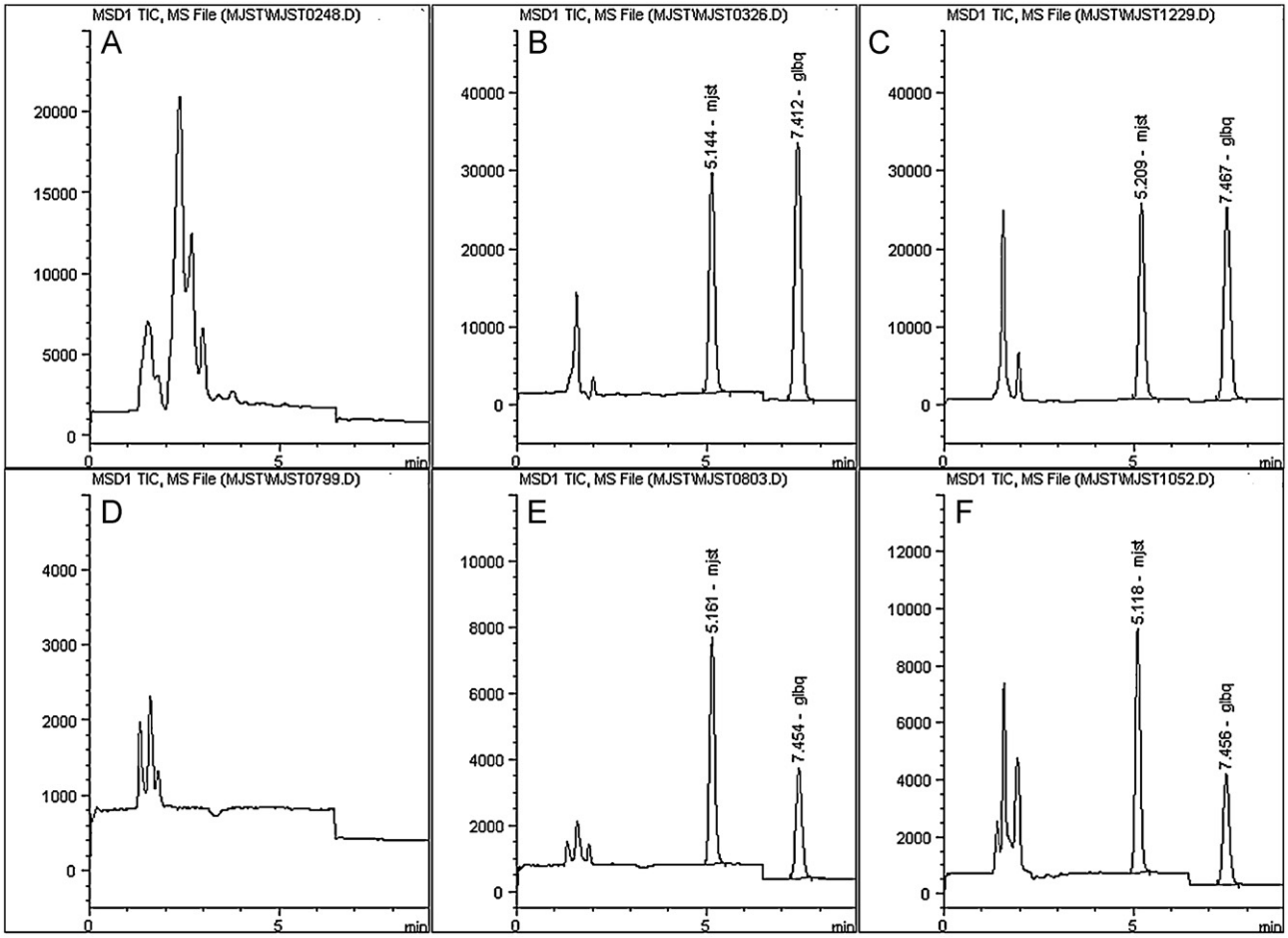

Fig. 3. The typical chromatograms of blank plasma (A), plasma sample spiked with M1 and I.S. (1 μg mL^{-1}) (B), and plasma sample from a subject 0.5 h after oral administration of moguisteine tablets (400-mg dose) spiked with I.S. (C), blank urine (D), urine sample spiked with M1 and I.S. (1 μg mL^{-1}) (E), and urine sample from a subject 0–1 h after oral administration of moguisteine tablets (400-mg dose) spiked with I.S. (F).

3. Results and discussion

3.1. Optimization of chromatographic and mass spectrometric conditions

Formic acid can promote the ionization of target compounds, improve the peak shape and response of M1. Acetonitrile which is volatile and low ionic suppression facilitates MS detection. 0.1% formic acid–acetonitrile was finally selected as the mobile phase.

Glipizide, a readily available compound, was selected as the I.S. in positive ion mode; its chromatographic behavior and extraction efficiency were similar to M1; glipizide in plasma and urine was stable and reproducible in the LC/MS system; and in addition, there were no interferences from M1 and endogenous substances.

By monitoring M1 before 6.5 min and I.S. after 6.5 min in time-sharing monitoring mode, the ion intensity was increased to 10-fold significantly. The mass spectrums of M1 and glipizide were shown in Fig. 2, and typical chromatograms were shown in Fig. 3.

The column was timely washed at each 15 chromatographic runs in method validation and each one subject samples (about 12 samples) to move strongly retained components of the mixture. Wash the column with methanol–water (5:95, v/v) for 20 min, and then methanol–water (95:5, v/v) for over 40 min.

3.2. Method validation

The calibration curves of M1 were linear within the concentration ranges in plasma ($n = 5$) and urine ($n = 5$). The regression equations (weight = $1/x^2$) were $y = 0.7144x + 0.00108$ ($r^2 = 0.9997$) for plasma, and $y = 1.5728x + 0.00238$ ($r^2 = 0.9990$) for urine. The LLOQ of M1 was 0.02 μg mL^{-1} with signal/noise ratio of 12 and 15, respectively. The matrix effects, values of peak area ratios of M1 at concentrations of 0.05 μg mL^{-1}, 1 μg mL^{-1}, 7 μg mL^{-1} and I.S. at 1 μg mL^{-1} in plasma were ranged from 98.85% to 101.93% and 103.5%, and in urine from 95.21% to 105.59% and 99.15% (Table 1). No matrix suppression from blank plasma or urine was observed during analysis, as shown in Fig. 4. The extraction recoveries of M1 at three concentrations in plasma were all over 90%, and I.S. over 95%. The intra-day and inter-day precision values (RSD) were less than 7.5%, and the accuracies (RE) were ranged from −6.0% to 6.0%. The results obtained were shown in Table 2. M1 was stable after samples stored at −20 °C for 1 day, 7 days, 28 days and two freeze–thaw cycles in plasma or urine (92.86–102.77% of the initial value).

3.3. Pharmacokinetics

The validated method was applied to the pharmacokinetic study in human plasma after single-dose and multi-dose of oral moguisteine tablets in healthy Chinese subjects. The mean plasma concentration–time profile was shown in Fig. 5. No accumulation of M1 was observed with the proposed multiple-dose regimen of moguisteine (200 mg, tid) for seven days. The results showed that t_{max} and $t_{1/2}$ of M1 in plasma after oral administration of 200 mg moguisteine (Table 3) were similar to previous study carried out in Italy [9]. But C_{max} and AUC were lower that might be due to different ethnic populations.

The mean C_{max} and AUC_{0-8} were well dose-dependent. The dose-related curve is typically described by equation $y = ax + b$. The linearity was assessed by linear regression with a weighting factor of the reciprocal of the concentration squared ($1/x^2$). The equations were $y = 0.009x + 0.160$ ($r^2 = 0.992$) and $y = 0.018x - 0.030$ ($r^2 = 0.999$), respectively. It is demonstrated that C_{max} and AUC_{0-8} of M1 had a good linear dose-dependent relationship.

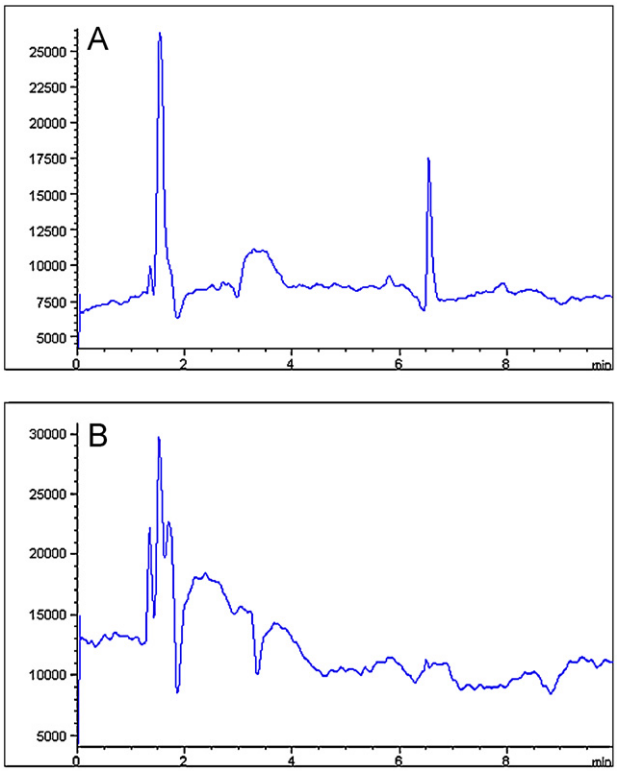

Fig. 4. Evaluation of matrix suppression using extracted blank plasma (A) and urine (B) in parallel with continuous post-column infusion of M1 and I.S. (100 ng/mL) in the ESI source.

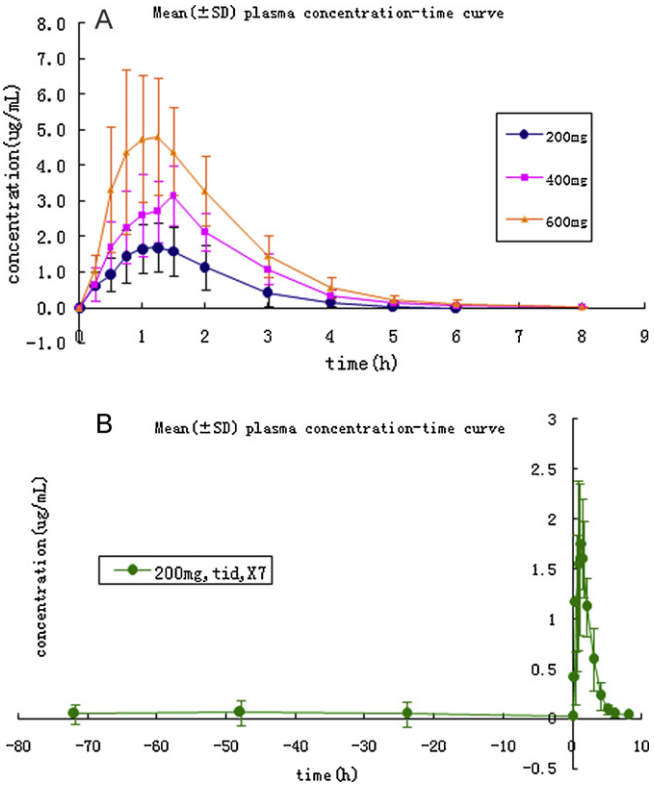

Fig. 5. Mean (±SD) plasma concentration–time curves of M1 after single-dose (A) and multi-dose (B) of oral moguisteine tablets in healthy Chinese subjects.

Table 1
The matrix effects and recoveries of M1 and I.S. ($n = 5$, %).

Sample	0.05 μg mL^{-1}		1 μg mL^{-1}		7 μg mL^{-1}		I.S.	
	Matrix effect (%)	Recovery (%)	Matrix effect (%)	Recovery (%)	Matrix effect (%)	Recovery (%)	Matrix effect (%)	Recovery (%)
Plasma	101.9	92.86	98.85	94.23	101.4	95.31	103.5	97.58
Urine	105.6		102.9		95.21		99.15	

Table 2
Accuracy and precision of the proposed method in plasma and urine sample ($n = 5$).

Sample	Concentration (μg mL^{-1})	Intra-day		Inter-day	
		Accuracy (RE%)	Precision (RSD%)	Accuracy (RE%)	Precision (RSD%)
Plasma	0.05	6.0	6.0	4.0	5.3
	1	−5.3	1.8	−2.1	5.0
	7	−3.0	3.3	0.4	3.5
Urine	0.05	3.0	1.4	3.0	7.2
	1	5.7	0.2	4.2	2.5
	7	2.4	1.0	−1.8	4.4

Table 3
Main pharmacokinetic parameters of M1 after single-dose and multi-dose of oral moguisteine tablets.

Parameter	Single-dose (200 mg)	Multi-dose (200 mg, tid, 7×)	Precious study [9] (200 mg)
$t_{1/2}z$	0.65 ± 0.09	0.82 ± 0.21	0.65 ± 0.13
t_{max} (h)	1.30 ± 0.44	1.25 ± 0.35	1.30 ± 0.40
Vz/F (L)	54.62 ± 17.77	67.38 ± 26.82	
CLz/F (L h^{-1})	58.87 ± 21.12	50.46 ± 8.93	
AUC_{0-8} (μg mL^{-1} h)	3.73 ± 1.20	4.02 ± 0.63	4.46 ± 0.62
$AUC_{0-\infty}$ (μg mL^{-1} h)	3.76 ± 1.21	4.06 ± 0.63	4.59 ± 0.60
AUC_{ss} (μg mL^{-1} h)		4.02 ± 0.63	
MRT_{0-8} (h)	1.67 ± 0.37		1.62 ± 0.26
$MRT_{0-\infty}$ (h)	1.71 ± 0.38		
C_{max} (μg mL^{-1})	2.11 ± 0.70	2.05 ± 0.49	2.83 ± 0.55

$t_{1/2}z$: terminal elimination half-life; t_{max}: time of peak; Vz/F: apparent volume of distribution; CLz/F: clearance; AUC: area under the concentration–time curve; MRT: mean residence time; C_{max}: peak concentration.

3.4. Urinary excretion

The accumulated amount of M1 in urine was monitored over a 24-h interval after administration of 400 mg moguisteine tablets in ten subjects. The cumulative excretion rate-time curve was shown in Fig. 6. The results demonstrated that a large amount of administered dose was excreted in urine within 6 h, accounted for 32.42%. The individual differences of the cumulative urinary excretion rate of M1 were greatly ranged between 11.83% and 57.09%.

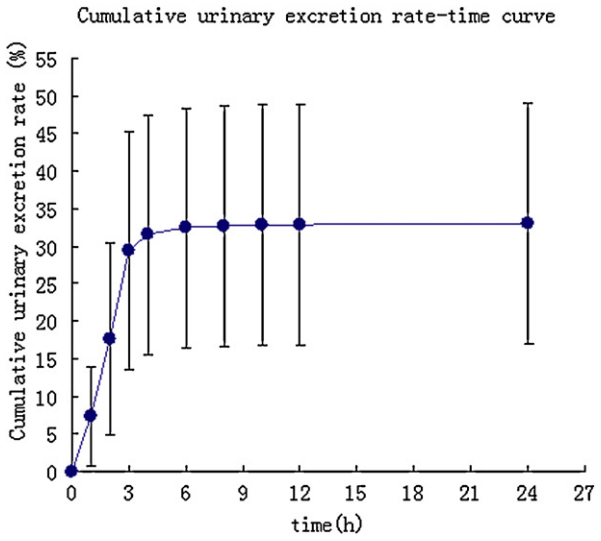

Fig. 6. The curve of cumulative urinary excretion rate-time of M1.

4. Conclusion

A sensitive and reproducible LC–ESI-MS method with simple protein precipitation was developed and validated for the quantification of M1, the principal active metabolite of moguisteine, in human plasma and urine. The method showed good precision and accuracy (RSD less than 7.5%, RE within ±6.0%), high extraction recovery in plasma (over 90%), wide linear range of 0.02–8 μg·mL^{-1}, as well as low consumption of sample. This method was successfully applied for evaluating the pharmacokinetic characteristics of moguisteine by determining M1 after single-dose and multi-dose of moguisteine tablets in healthy Chinese subjects.

References

[1] D. Castoldi, A. Oggioni, M.I. Renoldi, E. Ratti, S. Di Giovine, A. Bernareggi, J. Chromatogr. B: Biomed. Appl. 655 (1994) 243.
[2] T. Morikawa, L. Gallico, J. Widdicombe, Pharmacol. Res. 35 (1997) 113.
[3] L. Gallico, A. Borghi, C. Dalla Rosa, R. Ceserani, S. Tognella, Pharmacol. Br. J. 112 (1994) 795.
[4] G. Fasciolo, A. Nicolini, N. Vacca, P. Viglierchio, Curr. Ther. Res. Clin. E 55 (1994) 251.
[5] J. Kamei, K. Morita, T. Kashiwazaki, M. Ohsawa, Pharmacol. Eur. J. 347 (1998) 253.
[6] R. Ishii, M. Furuta, M. Hashimoto, T. Naruse, L. Gallico, R. Ceserani, Pharmacol. Eur. J. 362 (1998) 207.
[7] K. Morita, K. Onodera, J. Kamei, Jpn. J. Pharmacol. 89 (2002) 171.
[8] K. Morita, J. Kamei, Pharmacol. Eur. J. 395 (2000) 161.
[9] A. Bernareggi, A. Crema, R.M. Carlesi, D. Castoldi, E. Ratti, M.I. Renoldi, D. Ratti, R. Ceserani, S. Tognella, Eur J. Drug Metab. Pharmacokinet. 20 (1995) 263.