Journal of Pharmaceutical and Biomedical Analysis 47 (2008) 892–898

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Pharmacokinetic study of matrine, oxymatrine and oxysophocarpine in rat plasma after oral administration of *Sophora flavescens* Ait. extract by liquid chromatography tandem mass spectrometry

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ARTICLE INFO

Article history:
Received 16 January 2008
Received in revised form 23 February 2008
Accepted 13 March 2008
Available online 27 March 2008

Keywords:
Sophora flavescens Ait.
Matrine
Oxymatrine
Oxysophocarpine
LC-MS
Pharmacokinetics

ABSTRACT

A rapid, sensitive and selective high-performance liquid chromatography tandem mass spectrometric method (HPLC-MS) has been developed and validated for the simultaneous determination of matrine (MT), oxymatrine (OMT) and oxysophocarpine (OSP) in rat plasma after oral administration of *Sophora flavescens* Ait. extract using pseudoephedrine hydrochloride as an internal standard (I.S.). The three analytes were extracted from the plasma samples by liquid–liquid extraction with chloroform. The chromatographic separation was accomplished on a Kromasil C_{18} column (150 mm × 4.6 mm). Detection was performed on a single quadrupole mass spectrometer by selected ion monitoring (SIM) mode via electrospray ionization (ESI) source. The total run time was 12 min between injections. The assay had a lower limit of quantification of 1.0 ng/ml for MT, 2.0 ng/ml for OMT and 2.0 ng/ml for OSP using 200 μl of plasma. The calibration curves were linear in the measured range. The overall precision and accuracy for all concentrations of quality controls and standards was better than 15%. The proposed method enables unambiguous identification and quantification of MT, OMT and OSP in vivo. This was the first report on determination of the major quinolizidine alkaloids in rat plasma after oral administration of *Sophora flavescens* Ait. extract. The results provided a meaningful basis for evaluating the clinical applications of the herbal medicine.

1. Introduction

Sophorae Radix, the dried root of *Sophora flavescens* Ait., is a well-known traditional Chinese medicine (TCM) with the function of relieving heat, depriving the evil wetness, puring fire for removing toxin and killing parasites to relieve itching. It originated from *Shennong Materia Medica,* the earliest Pharmacopoeia of China in Eastern Han (24–220 AD) [1]. As a widely used medicinal herb it is usually prescribed for the treatment of diarrhea, gastrointestinal hemorrhage and eczema [2]. Sophorae Radix is known to be rich in quinolizidine alkaloids [3]. Matrine, oxymatrine and oxysophocarpine (structures in Fig. 1), as the major ones had been investigated intensively, and were found to have anti-inflammatory, antitumor, antipyretic and hepatoprotective [4–7] effects. Recently, much attention has been paid to the absorption and metabolism of quinolizidine alkaloids, and a few papers dealing with their pharmacokinetics and pharmacodynamics have been published [8–16] employing mainly high-performance liquid chromatography tandem mass spectrometry (HPLC-MS) [8–10]. It ensures high sensitivity for quantification with high degree of specificity at relatively short analytical time without a need for complete chromatographic resolution of analytes. However, as far as we are aware, previous researches only aimed directly at administration of single substances, and no paper was reported on the pharmacokinetic studies of matrine, oxymatrine and oxysophocarpine simultaneously. Because the therapeutic effects of TCMs are based on the complex interactions of multiple ingredients, investigation of the metabolism of multi quinolizidine alkaloids after administration of *Sophora flavescens* extracts is essential to understand their role in human health and evaluate the clinical efficacy of this medicinal herb.

In the present study, an HPLC-MS method was firstly developed and validated for simultaneous quantification of matrine, oxymatrine and oxysophocarpine in rat plasma, suitable for the

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0731-7085/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.jpba.2008.03.019

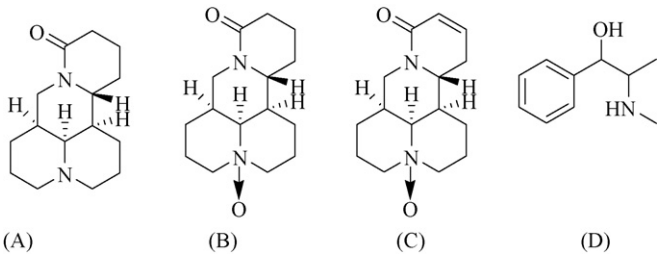

Fig. 1. Chemical structures of matrine (A), oxymatrine (B), oxysophocarpine (C) and internal standard of pseudoephedrine (D).

investigation of their pharmacokinetic profile after oral administration of *Sophora flavescens* extracts.

2. Experimental

2.1. Materials, chemicals and reagents

The dried roots of *Sophora flavescens* Ait. were purchased form the Medical Material Co. of Liaoning province (Shenyang, China). Compounds matrine (MT), oxymatrine (OMT), oxysophocarpine (OSP) and pseudoephedrine hydrochloride (PPD, used as internal standard) with purity of greater than 99% were received from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Acetonitrile of chromatographic grade were supplied by Caledon Lab. Ltd. (Georgetown, Canada). Deionized water was purified using an Alpha-Q water purification system (Millipore, Bedford, MA, USA) and was filtered using 0.20 μm membranes. All other chemicals were of analytical reagent grade.

2.2. Instruments and conditions

Shimadzu 2010 series HPLC tandem mass spectrometer equipped with a LC-10AD*vp* binary pump, an on-line degasser, an auto-sampler and a column temperature controller were used for all analyses. The data were processed using Shimadzu software (version 3.0). Chromatographic separations were performed on a Kromasil C_{18} column (150 mm × 4.6 mm, 5 μm particle size) protected by a C_{18} guard column (12.5 mm × 4.6 mm, 5 μm) at room temperature. The mobile phase consisted of acetonitrile–0.1% formic acid (10: 90, v/v). The flow rate was set at 0.8 ml/min with 30% of the eluent being splitted into the inlet of the mass spectrometer. Aliquots of 10 μl were injected into HPLC system for analysis. Mass spectra were acquired using a mono-quadrupole mass spectrometer coupled with an electrospray ionization source (ESI). Nitrogen was used as the sheath and auxiliary gas to assist nebulisation with the flow rate settled at 1.5 L/min. All mass spectra were acquired in the positive ion mode with capillary voltage at 1.5 kV, curved desolvation line (CDL) temperature at 250 °C and block temperature at 200 °C. Target ions were monitored at *m*/*z* 249.10 for MT, 265.10 for OMT, 263.10 for OSP and 166.00 for PPD using selected ion monitoring (SIM) mode.

2.3. Preparation of Kushen extract

The crude drug (5.6 g) was extracted twice by refluxing with boiling water (1:20, w/v), each time for 1.5 h. The solution obtained was concentrated under reduced pressure to give an extract of concentration equivalent to 0.07 g/ml Kushen, and stored at 4 °C until administration to rat. To calculate the administered dose of MT, OMT and OSP, their content in Sophora flavescens extract were quantitatively analyzed by HPLC-UV external standard method using Hypersil–ODS2, 4.6 mm × 200 mm, 5 μm column with a mobile phase of methanol–acetonitrile–water–phosphoric acid 10:30:65:0.05 (containing 33 mmol L^{-1} SDS); UV detector at 210 nm. The content of the three alkaloids in the extract was 3.35, 25.66 and 12.97 mg/g, respectively.

2.4. Animals and blood sampling

Nine-week-old male Wister rats weighing 220–250 g were obtained from the Experimental Animal Center of Shenyang Pharmaceutical University, and kept in an environmentally controlled breeding room (temperature: 24 ± 2 °C, humidity: 60 ± 5%, 12 h dark–light cycle) for 3 days before starting the experiments. They were fed with a standard laboratory food and water ad libitum and fasted for 12 h with free access to water prior to the experiments. All procedures involving animals were in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of People's Republic of China. The *Sophora flavescens* decoction was orally administered to twelve rats (six rats were used for blood collecting at eight time points ahead and the other six rats were used at the remained seven time points to make up the blood loss) at a dose of 0.56 g/kg. Blood samples (0.5 ml) were collected from the abdominal vein according to the specific schedule at times of 0, 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 24.0, 36.0 and 48.0 h after dosing. The blood samples were immediately transferred to heparinized tubes and centrifuged at 4000 × *g* for 10 min. The plasma samples obtained were stored at −20 °C until analysis.

2.5. Pretreatment of plasma sample

The plasma (200 μl) was spiked with 50 μl of I.S. (PPD, 5.0 μg/ml). The mixture was then extracted with 3.0 ml of chloroform by mechanical shaking for 5 min. After centrifugation at 4000 × *g* for 5 min, the lower (organic) layer was transferred to a test tube and evaporated to dryness under a stream of nitrogen. The residue was reconstituted in 100 μl mobile phase and stored at 4 °C until use.

2.6. Preparation of standard solutions, calibration standards and quality control samples

The stock solutions of MT, OMT, OSP and PPD (I.S.) were prepared by dissolving a proper amount of MT, OMT, OS and PPD in methanol to furnish nominal concentrations of 100 μg/ml each. A series of standard mixture working solutions with concentrations 40.0–20000 ng/ml for MT, 8.0–4000 ng/ml for OMT and 8.0–4000 ng/ml for OSP were obtained by diluting the mixture of the stock standard solutions with methanol. The working solution of I.S. (5 μg/ml) was prepared by diluting I.S. stock solution with methanol. All solutions were stored at 4 °C.

Calibration standards of MT (10, 20, 100, 500, 2000 and 5000 ng/ml), OMT (2, 4, 20, 100, 400 and 1000 ng/ml) and OSP (2, 4, 20, 100, 400 and 1000 ng/ml) were prepared by spiking the appropriate amount of the standard mixture working solutions (50 μl) and I.S. working solution (50 μl) into 200 μl drug-free rat plasma. Quality control samples (QC samples) were prepared at low, medium, and high concentrations (at 20, 500, 4000 ng/ml for MT, 4, 100, 800 ng/ml for OMT and 4, 100, 800 ng/ml OSP) in the same manner as the calibration standards, and used to assess accuracy and precision of the assay method. The samples were extracted following the procedure described above.

2.7. Method validation [17,18]

2.7.1. Calibration curve and LLOQ

Calibration standards were prepared according to the procedure described in Section 2.5 in triplicate and analyzed on three consequent days. The calibration curves were constructed by plotting the peak area ratio of MT, OMT and OSP to internal standard versus their respective concentrations in rat plasma. Weighted ($1/x^2$) linear least-squares regression method was used to determine the slope, intercept and correlation coefficient. Unknown sample concentrations of MT, OMT and OSP in plasma were calculated from the linear regression equation for the calibration plot of peak area ratio against concentration. The lower limit of quantification (LLOQ) was determined in accordance to the base line noise, considering a signal-to-noise ratio of 10:1.

2.7.2. Precision and accuracy

The accuracy and precision of the established method were evaluated by QC samples at low (20 ng/ml for MT, 4 ng/ml for OMT and 4 ng/ml for OSP), medium (500 ng/ml for MT, 100 ng/ml for OMT and 100 ng/ml for OSP) and high concentrations (4000 ng/ml for MT, 800 ng/ml for OMT and 800 ng/ml for OSP). The concentration of each QC sample was calculated using calibration curves prepared each day. Accuracy was defined as the relative deviation in the calculated value (E) of a standard from that of its true value (T), expressed as relative error (R.E.). Precision was evaluated as the relative standard deviation (R.S.D.). The intra-day accuracy was determined by assaying six replicates at each concentration level on 1 day, and inter-day accuracy was determined by analyzing QC samples in duplicates during three separate and successive days.

2.7.3. Extraction recovery and ionization

The extraction efficiency of MT, OMT and OSP from human plasma matrix using liquid–liquid extraction procedure was evaluated by six processed QC samples at low, medium and high concentrations. It was determined by comparing the mean peak areas ($n = 6$, at each concentration) obtained from plasma sample spiked before extraction with those from plasma samples spiked after extraction. Similarly, the recovery of internal standard was evaluated in the same way. Ion suppression of ionization was assessed by comparing the absolute peak area of control plasma extracted and then spiked with a known amount of drug, to that of neat standard injected in the same reconstitution solvent.

2.7.4. Stability

Stability of MT, OMT and OSP in plasma was evaluated after sample extraction process (QC samples were processed and stored under autosampler condition for 24 h), three freeze–thaw cycles and long-term freezing at −20 °C (14 days) by QC samples in six replicates at each concentration. Freeze and thaw stability was determined after three freeze and thaw cycles. In each cycle the QC samples in six duplicates at low, medium and high concentrations for target compounds were stored at −20 °C for 24 h and thawed at room temperature. When completely thawed the sample was refrozen for 24 h. The cycle was repeated twice and the samples were analyzed after the third cycle. The concentration was calculated from the daily calibration curve. Stability was assessed by comparing the mean concentration of the stored QC samples with the mean concentration of freshly prepared QC samples. Stability samples were to be concluded stable if bias of them were within ± 15% of the actual value.

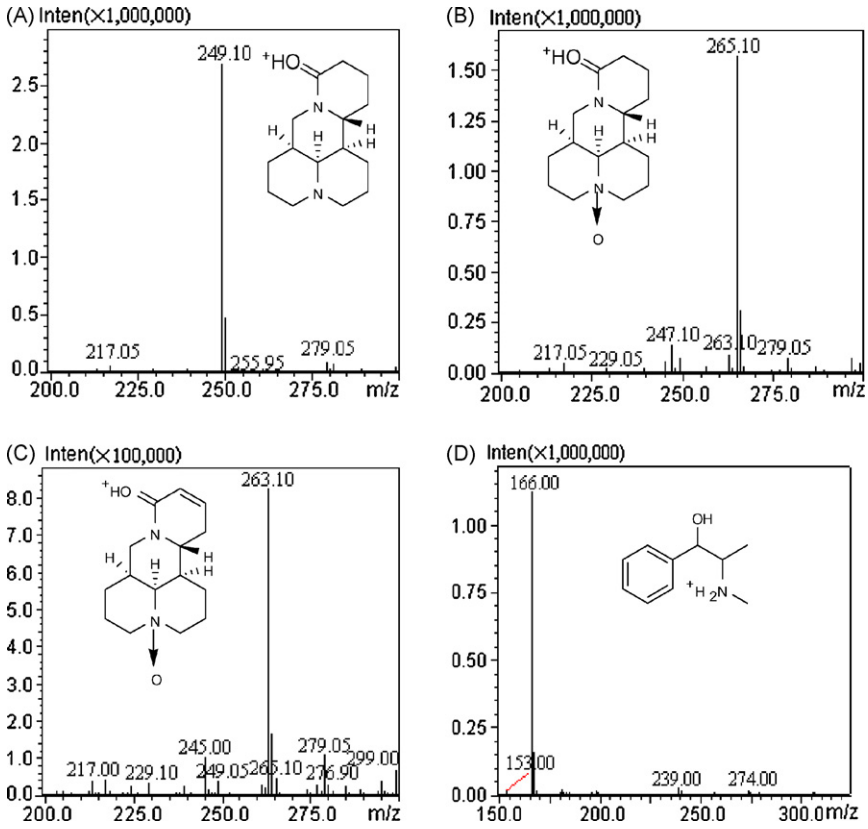

Fig. 2. Positive full scan mass spectra of matrine (A), oxymatrine (B), oxysophocarpine (C) and I.S. (D) at 1.5 kV detector voltage using ESI source.

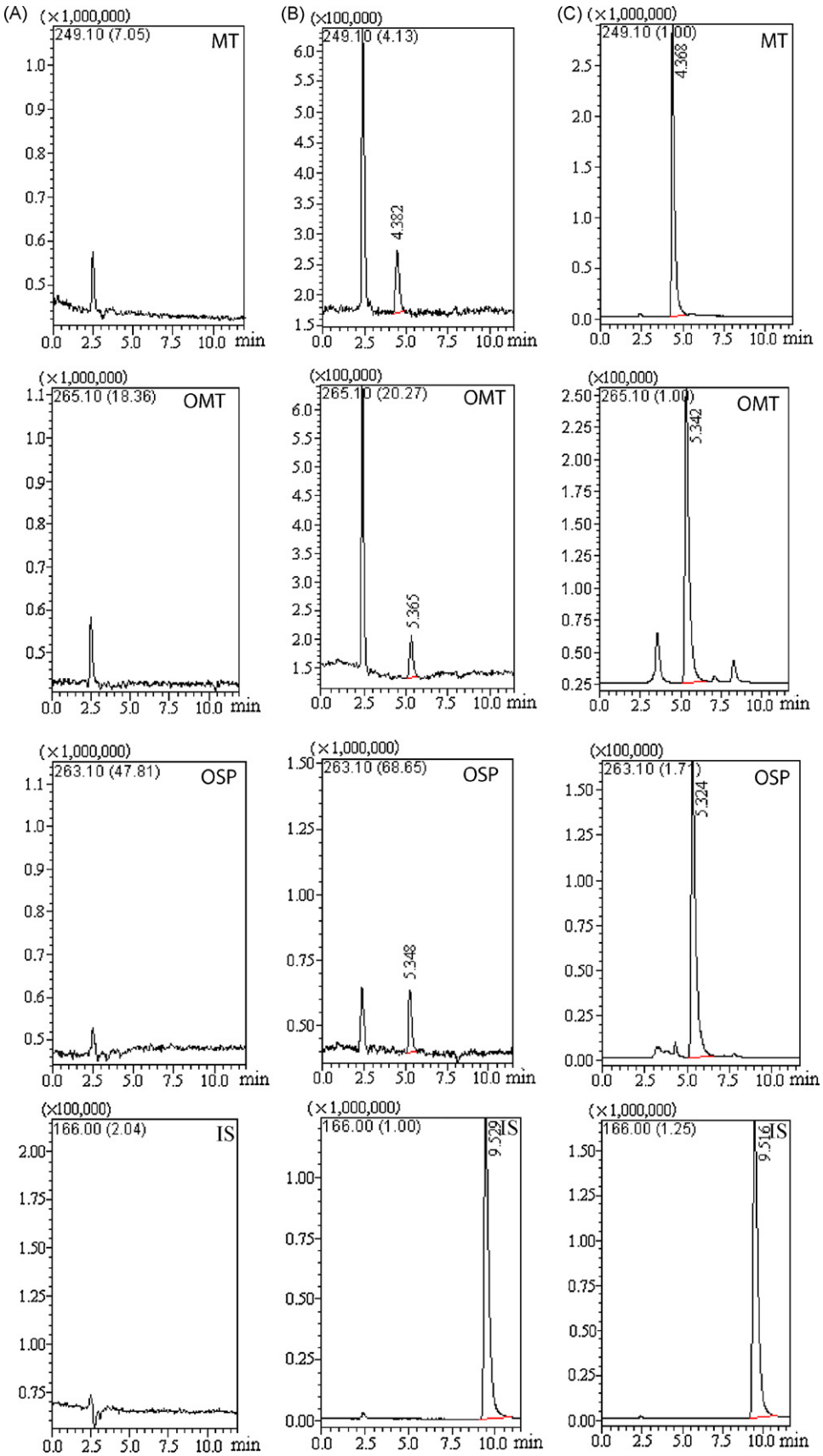

Fig. 3. Typical SIM chromatograms of rat blank plasma (A), blank plasma spiked with MT (10.0 ng/ml), OMT (2.0 ng/ml), OSP (2.0 ng/ml) and I.S. (B), and the rat plasma sample colleted at 30 min after oral administration of *Sophora flavescens* Ait. extract (C).

Table 1
Validation of the intra-day and inter-day assays

Analytes	Spiked (ng/ml)	Intra-day ($n=6$)			Inter-day ($n=3$)		
		Measured (ng/ml)	Accuracy (R.E., %)	Precision (R.S.D., %)	Measured (ng/ml)	Accuracy (R.E., %)	Precision (R.S.D., %)
MT	20.0	20.86	4.32	5.2	20.57	2.85	8.0
	500.0	534.5	6.91	4.5	545.4	9.09	5.6
	4000	3966	−0.85	3.4	3944.1	−1.40	8.3
OMT	4.0	3.89	−2.75	7.3	3.91	−2.24	2.7
	100.0	101.1	1.10	5.4	97.6	−2.38	7.6
	800.0	760.7	−4.91	4.8	770.8	−3.65	5.0
OSP	4.0	3.70	−7.50	6.1	3.91	−4.24	7.0
	100.0	98.72	−1.28	6.7	102.9	2.96	10.1
	800.0	767.7	−4.03	3.6	787.1	−1.62	7.8

2.8. Application of the method and pharmacokinetic study

The method established was applied to determine the concentration of MT, OMT and OSP in rat plasma after oral administration of *Sophora flavescens* extract at a dose of 8 ml/kg, equivalent to the daily dose of a human. The plasma concentrations of MT, OMT and OSP at different times were calculated from the daily calibration curve, and expressed as means ± S.D. The mean concentration–time curve was plotted. All data were processed by non-compartmental analysis using the TopFit 2.0 software package (Thomae, Germany). Maximum plasma concentration (C_{max}) and the time to reach the concentration (T_{max}) were obtained directly from the observed values. The apparent elimination rate constant (K_e) was calculated by fitting mean data at four terminal points of the plasma concentration profile with a log-linear regression equation using the least-squares method. $T_{1/2\beta}$, the plasma half-life, was calculated as $0.693/K_e$. The area under the plasma concentration–time curve from zero to the time of the final measurable sample (AUC_{0-t}) was calculated by use of the linear-trapezoidal rule up to the last sampling point with detectable levels (C). The area under the plasma concentration–time curve from zero to infinity ($AUC_{0-\infty}$) was calculated by means of the trapezoidal rule with extrapolation to infinity with K_e. The mean residence time (MRT) was calculated as the ratio of the area under the first moment curve (AUM_{0-t}) to (AUC_{0-t}).

3. Results and discussion

3.1. Mass spectra

The full mass spectra of MT, OMT, OSP and internal standard (I.S.) after direct injection are shown in Fig. 2(A)–(D), respectively. The analytes and I.S. formed predominantly protonated molecules $[M+H]^+$ of 249.10 for MT, 265.10 for OMT, 263.10 for OSP and 166.00 for I.S. Because these compounds contained basic nitrogen, the addition of formic acid to the mobile phase improved the sensitivity and the shape of target peaks.

3.2. Optimization of sampling process

For an efficient clean up of the plasma sample, various sample preparation method were tested, such as deproteinization with methanol or acetonitrile, liquid–liquid extraction with different organic solvents including dichloromethane:isopropanol (95:5, v/v), *N*-hexane:dichloromethane:isopropanol (100:50:5, v/v/v) and chloroform. Liquid–liquid extraction with chloroform was chosen for it provided a clean supernatant with high extraction recovery. Additionally, the simple extraction procedure made it feasible for quantitative analysis.

3.3. Method validation

3.3.1. Specificity

The extent of interference of endogenous plasma constituents with MT, OMT, OSP and I.S. was assessed by inspection of chromatograms derived from processed blank plasma samples. Typical chromatograms obtained from blank plasma, blank plasma spiked with target compounds and I.S., and plasma sample after administration of *Sophora flavescens* extract are presented in Fig. 3(A)–(C). The retention times of MT, OMT, OSP and PPD (I.S.) were approximately 4.38, 5.37, 5.35 and 9.53 min, respectively. There was no endogenous interference and matrix effect on ionization.

3.3.2. Linearity and LLOQ

The calibration curves showed good linearity over the concentration range 10–5000 ng/ml for MT, 2–1000 ng/ml for OMT and 2–1000 ng/ml for OSP. The typical calibration plot equations and their correlation coefficients were calculated as follows: MT, $y = 1.05 \times 10^{-3}x + 2.88 \times 10^{-3}$ ($r^2 = 0.9955$); OMT, $y = 8.00 \times 10^{-4}x - 1.50 \times 10^{-4}$ ($r^2 = 0.9974$); OSP, $y = 1.05 \times 10^{-3}x - 3.30 \times 10^{-4}$ ($r = 0.9963$). In the regression equation $y = ax + b$, x referred to the concentration of the analytes in serum (ng/ml); y referred to the peak area of the analytes and I.S. The LLOQ for MT, OMT and OSP were 1.0, 2.0 and 2.0 ng/ml with coefficient of variation 11.31%, 8.25% and 9.63%, respectively.

3.3.3. Precision and accuracy

Intra-day and inter-day precision and accuracy were determined by measuring QC samples at three concentrations as described in Section 2. The intra-day accuracy ranged from −0.85% to 6.91% with R.S.D. less than 5.2% for MT, −4.91% to 1.10% with R.S.D. less than 7.3% for OMT and −7.50% to −1.28% with R.S.D. less than 6.7% for OSP. The inter-day accuracy ranged from −1.40% to 9.09% with R.S.D. less than 8.3% for MT, −3.65% to −2.4% with R.S.D. less than 7.6% for OMT and −4.24% to 2.96% with R.S.D. less than 10.1% for OSP. The results indicated that overall reproducibility of the method was acceptable (Table 1).

3.3.4. Extraction recovery and ionization

The mean extraction recoveries determined using six replicates of QC samples at three concentration levels were found to be 90.65 ± 3.14% (R.S.D., 3.46%), 93.53 ± 8.60% (R.S.D., 9.2%), 89.14 ± 5.44% (R.S.D., 6.20%) for MT, 86.76 ± 5.85% (R.S.D., 6.74%), 83.87 ± 4.96% (R.S.D., 5.91%), 91.27 ± 3.98% (R.S.D., 4.36%) for OMT, 85.38 ± 6.76% (R.S.D., 7.81%), 88.01 ± 4.61% (R.S.D., 10.2%), 86.65 ± 2.43% (R.S.D., 2.90%) for OSP and 95.28 ± 5.36% (R.S.D., 5.60%) for I.S.

As for ionization, the peak area ratios of the three target compounds and I.S. after spiking evaporated plasma samples at three

Table 2
Summary of stability of MT, OMT and OSP in plasma ($n = 6$)

Analyte	Spiked (ng/ml)	Stability I[a]		Stability II[b]		Stability III[c]	
		Measured	R.E. (%)	Measured	R.E. (%)	Measured	R.E. (%)
MT	20.0	19.42 ± 1.71	−2.89	19.07 ± 0.86	−4.65	20.81 ± 1.02	4.06
	500.0	527.1 ± 25.50	5.42	538.2 ± 27.98	7.65	484.4 ± 29.21	−3.13
	4000	3624 ± 128.3	−9.41	3795 ± 225.0	−5.13	3845.6 ± 142.3	−3.86
OMT	4.0	4.16 ± 0.22	3.87	3.69 ± 0.32	−7.70	3.59 ± 0.10	−10.3
	100.0	106.4 ± 3.97	6.46	108.1 ± 6.36	8.15	102.5 ± 2.88	2.51
	800.0	732.5 ± 14.74	−8.43	822.5 ± 54.9	2.81	740.2 ± 29.7	−7.47
OSP	4.0	3.93 ± 0.22	−1.84	4.12 ± 0.16	3.10	3.77 ± 0.28	−5.85
	100.0	99.02 ± 4.14	−0.98	106.27 ± 4.58	6.27	102.2 ± 6.60	2.24
	800.0	771.4 ± 27.11	−3.57	746.6 ± 34.98	−6.68	814.4 ± 63.8	1.79

[a] Refers to the post-preparative stability which was determined by processed samples after storage in auto sampler condition for 24 h.
[b] Refers to the freeze and thaw stability which was determined by plasma samples after three freeze–thaw cycles.
[c] Refers to the long-term cold storage stability which was determined by plasma samples after 14 days storage at −20 °C.

concentration levels compared to neat standard solutions ranged form 98.4% to 105.7% for MT, 96.7% to 100.6% for OMT and 95.4% to 102.7% for OSP, suggesting that the method was free from matrix effect.

3.3.5. Stability

Stability of MT, OMT and OSP in processed samples, after freeze–thaw cycle and long-term cold storage (−20 °C, 14 days) were evaluated and summarized in Table 2. The results suggested that the three analytes was stable for 24 h in autosampler condition after preparation, for 14 days under cold storage and within three freeze–thaw cycles in plasma samples, since there was not any obvious change in the concentrations of MT, OMT and OSP in plasma tested within the time period under the indicated storage conditions

3.4. Pharmacokinetic studies

The validated analytical method was employed to study the pharmacokinetic profiles of MT, OMT and OSP in rat plasma after oral administration of *Sophora flavescens* extract simultaneously. The mean plasma concentration–time profiles of the three analytes are presented in Fig. 4(A)–(C). MT, OMT and OSP concentration time profile conformed to a two-compartment pharmacokinetic model. The estimated pharmacokinetic parameters are shown in Table 3. These three analytes exhibited inconsistent tendency in plasma concentration–time profiles although they had similarity in structure features. OMT was rapidly absorbed with peak concentrations occurring at around 1.58 h, and rapidly eliminated from plasma with $T_{1/2\beta}$ of only about 3.44 h. However, the pharmacokinetic behavior for MT was on the contrary. MT was absorbed with T_{max} being 2.08 h, and slowly eliminated as the plasma concentration was still much higher than LLOQ after 36 h. In previous studies [9], The AUC_{0-t} value for MT following oral administration of OMT capsules to beagle dogs was only about 0.2 times larger than that of OMT. Nevertheless, the AUC_{0-t} value for MT in this paper were about 20 times as large as that of OMT following oral administration of *Sophora flavescens* extract containing MT only 3.35 mg/g, while OMT 25.66 mg/g. According to the literature [9–10,16], OMT could be reduced to MT by bacteria. The reasons that caused so conspicuous difference between AUC_{0-t} for MT and OMT were probably

Table 3
Mean pharmacokinetic parameters for MT, OMT and OSP in rat plasma ($n = 6$) after oral administration of *Sophora flavescens* extract

Parameter	MT	OMT	OSP
C_{max} (μg/l)	2529 ± 1029	408 ± 156.7	207.2 ± 161.6
T_{max} (h)	2.08 ± 0.49	1.58 ± 0.20	1.42 ± 0.86
AUC_{0-t} (μg h/l)	21858 ± 8217	795.7 ± 239.1	432.0 ± 154.5
$AUC_{0-\infty}$ (μg h/L)	22688 ± 8493	908.7 ± 215.8	453.7 ± 134.8
MRT_{0-t} (h)	11.8 ± 1.48	3.32 ± 0.55	4.09 ± 2.59
$T_{1/2\beta}$ (h)	9.75 ± 2.76	3.44 ± 2.51	2.68 ± 0.96
K_e (1/h)	0.062 ± 0.015	0.273 ± 0.136	0.294 ± 0.124
V_β (l)	0.389 ± 0.145	14.99 ± 3.561	13.93 ± 4.14
CL (l/h)	0.024 ± 0.009	4.198 ± 0.997	4.388 ± 1.304

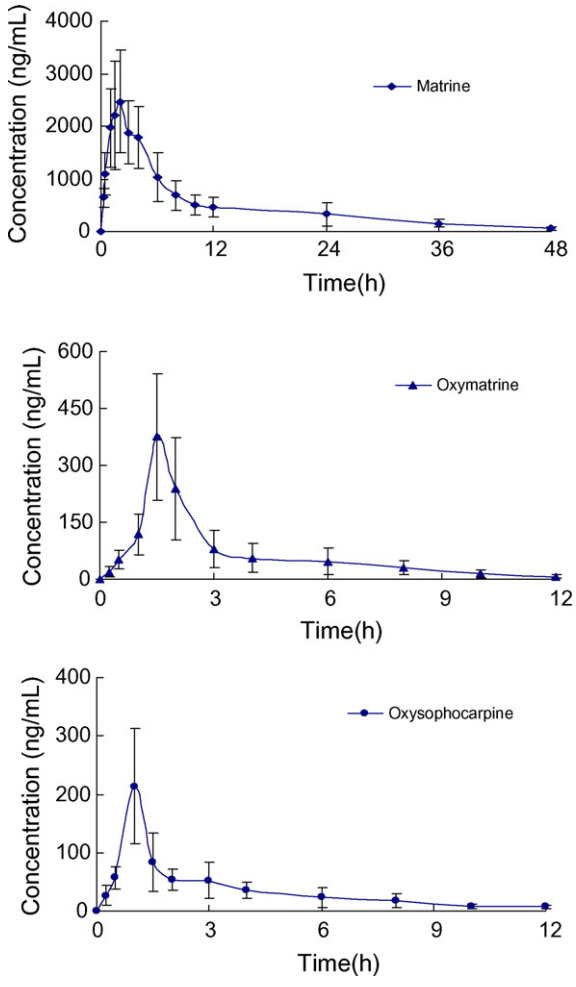

Fig. 4. Plasma concentration–time profiles of matrine (A), oxymatrine (B) and oxysophocarpine (C) following oral administration of *Sophora flavescens* Ait. extract to rats. Each point with bar represents mean ± S.D. ($n = 6$) as the percentage of the administered dose.

due to the following aspects: (1) different animals possessed different transformation capability; (2) other constituents in the *Sophora flavescens* extract had an influence on the transformation reaction, and on the absorption, distribution, metabolism and excretion of the two analytes; (3) MT rather than OMT present in the extracts was more easier absorbed by rat. To draw the conclusion, further studies about the interaction of drugs are demanded. The T_{max} (1.42 h) and $T_{1/2\beta}$ (2.68 h) of OSP were similar to those of OMT. Comparing the structures of the two compounds, we tentatively deduced that the OSP might have the similar pharmacokinetic procedure to that of OMT in vivo. The values of clearance (CL) for OSP (4.388 L/h) and OMT (4.198 L/h) were much higher than that of MT (0.024 L/h), indicating that OSP and OMT were more easily cleared from the rat body than compound MT. The apparent distribution volumes (V_d) of the three analytes were much larger than the blood volume of rat, hence it could be speculated that they were widely distributed in the organs. The present studies suggested that MT seemed to play an important role in the pharmacological action of orally administered *Sophora flavescens* extract. OSP might be mostly reduced to SP in rats, and exerts its effect mainly in the form of this metabolite. Furthermore, additional studies are needed to clarify the deduction that we proposed.

4. Conclusion

The analytical method for the simultaneous determination of MT, OMT and OSP in rat plasma after oral administration of *Sophora flavescens* extract, based on liquid–liquid extraction of analytes from basified biological matrix with high extraction efficiency, was developed and validated. The assay was rapid, sensitive, specific, selective and reproducible. In addition, the method has advantages of the use of a small sample volume and non-tedious sample preparation. Therefore, the method proposed is useful for simultaneous pharmacokinetic studies of quinolizidine alkaloids in biological samples.

References

[1] The State Pharmacopoeia Commission of P.R. China, Pharmacopoeia of the People's Republic of China, vol. 1, Chemical Industry Publishing House, Beijing, 2005, p. 141.
[2] Jiangsu New Medical College, Dictionary of Chinese Traditional Medicine, Shanghai Science and Technology Press, Shanghai, 1977, p. 1283.
[3] Y.P. Zhu, Chinese Materia Medica: Chemistry, Pharmacology and Applications, Harwood Academic Publishers, Amsterdam, 1998, p. 149.
[4] C.Y. Chuang, J.G. Xiao, G.C. Chiou, J. Ocul. Pharmacol. 3 (1987) 129–134.
[5] X.S. Liu, J. Jiang, X.Y. Jiao, Y.E. Wu, J.H. Lin, Planta Med. 72 (2006) 501–506.
[6] C.H. Cho, C.Y. Chuang, C.F. Chen, Planta Med. 5 (1986) 343–345.
[7] Y. Long, X.T. Lin, K.L. Zeng, L. Zhang, Hepatobiliary Pancreat Dis. Int. 3 (2004) 69–72.
[8] Y.Q. Wang, Y.Y. Ma, X.H. Li, F. Qin, X.M. Lu, F.M. Li, Biomed. Chromatogr. 21 (2007) 876–882.
[9] S.J. Wang, G.J. Wang, X.T. Li, J.G. Sun, R.L. Ma, L.S. Sheng, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 817 (2005) 319–325.
[10] X.L. Wu, T.J. Hang, J.P. Shen, Y.D. Zhang, J. Pharm. Biomed. Anal. 41 (2006) 918–924.
[11] Y.H. Wei, X.A. Wu, X.F. Liu, J.Y. Luo, J. Chromatogr. B Analyt Technol. Biomed. Life Sci. 843 (2006) 10–14.
[12] X. Wang, W. Zhang, L.Y. Fan, B. Hao, A.N. Ma, C.X. Cao, Y.X. Wang, Anal. Chim. Acta 594 (2007) 290–296.
[13] X.H. Liu, L. Li, J. Sun, Y.H. Sun, T.H. Zhang, D.W. Chen, Z.G. He, Chromatographia 63 (2006) 483.
[14] Y. Li, G. Min, Q. Xue, L. Chen, W. Liu, H. Chen, Biomed. Chromatogr. 18 (2004) 619–624.
[15] D.S. Sit, G. Gao, F.C. Law, P.C. Li, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 808 (2004) 209–214.
[16] Y. Chen, H.X. Chen, P. Du, F.M. Han, Acta Pharm. Sin. 40 (2005) 740–745.
[17] V.P. Shah, K.K. Midha, J.W.A. Findlay, H.M. Hill, J.D. Hulse, I.J. McGilveray, K.J. Miller, R.N. Patnaik, M.L. Powell, A. Tonelli, C.T. Viswanathan, A. Yacobi, Pharm. Res. 17 (2000) 1551–1557.
[18] Guidance for Industry, Bioanalytical Method Validation, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), May 2001.