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Determination and pharmacokinetic study of oxymatrine and its metabolite matrine in human plasma by liquid chromatography tandem mass spectrometry

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Abstract

There is little information about the pharmacokinetics of oxymatrine (OMT) and its metabolite matrine (MT) after i.v. administration of OMT in human. Therefore a specific and sensitive liquid chromatography tandem mass spectrometry (LC–MS/MS) method was established for the determination and pharmacokinetic study of OMT and its metabolite MT in human plasma after i.v. infusion administration of 600 mg of OMT in 100 ml of 5% glucose injection in 0.5 h. The analysis was carried out on a Lichrospher-CN column (250 mm × 4.6 mm, i.d., 5 μm, Merck) with mobile phase of methanol–ammonium acetate (20 mmol/l; 85:15, v/v) pumped at a flow rate of 1.0 ml/min. The tandem mass detection was made with electrospray ionization in positive ion selected reaction monitoring mode, with argon collision-induced dissociation ion transitions *m/z* 265.2 to *m/z* 265.2 for OMT at 25 eV, *m/z* 249.2 to *m/z* 249.2 for MT at 25 eV and *m/z* 340.2 to *m/z* 324.0 at 35 eV for the internal standard (papaverine), respectively. The assay was validated to be accurate and precise for the analysis in the concentration range of 1.0–40,000 ng/ml for both OMT and MT with the LOD being 0.5 and 0.2 ng/ml, respectively, when 0.25 ml of human plasma sample was processed with papaverine as internal standard. The pharmacokinetic study was made with 10 healthy male Chinese subjects. The plasma concentration time profiles of OMT and MT obtained were best fitted with two-compartment and one-compartment models, respectively. The main pharmacokinetic parameters found for OMT and MT after i.v. infusion were as follows: C_{max} (20,519 ± 7581) and (247 ± 45) ng/ml, T_{max} (0.5 ± 0.1) and (5.6 ± 1.7) h, AUC_{0-t} (20,360 ± 5205) and (3817 ± 610) ng h/ml, $AUC_{0-\infty}$ (20,436 ± 5188) and (3841 ± 615) ng h/ml, $t_{1/2}$ (2.17 ± 0.49) and (9.43 ± 0.62) h, respectively. The CL/F and V_d/F of OMT were (43.8 ± 10.8) l h^{-1} and (70.1 ± 26.6) l, respectively. Therefore only a small amount of OMT was reduced to MT following i.v. administration of OMT judged by the AUCs.

Keywords: Oxymatrine; Matrine; Metabolism; Human plasma; Pharmacokinetics; LC–tandem MS

1. Introduction

Sophora flavescens Ait having a wide range of pharmacological and toxicological activities is a traditional Chinese medicinal herb commonly used in China. Oxymatrine (OMT, Fig. 1(A)) and matrine (MT, Fig. 1(B)), the major quinolizidine alkaloids from the dried roots of *Sophora flavescens Ait* [1] have long been regarded as the main active components accounting for the pharmacological properties. They are also found in *Sophora subprostrata* (*shandougen*) and in the above-ground portion of *Sophora alopecuroides* [2]. They have been extensively used in China for the treatment of viral hepatitis, cancer, cardiac diseases (such as viral myocarditis) and skin diseases (such as psoriasis and eczema) [3]. OMT is used especially for chronic hepatitis B, while MT is mostly used for cardiac diseases [4]. It is reported that when taken orally, most of OMT can be reduced to the more absorbable MT by intestinal bacteria in the gastrointestinal tract where a large number of bacteria exist [5], which might have pharmacological and toxicological implications in clinical practice [6].

A number of assay methods have been developed for the determination of OMT and/or MT such as fluorescence quenching [7], thin layer chromatography [7], high performance capillary electrophoresis [8], or high performance liquid chromatography [9–12]. But most of them with UV detection at 218 nm for

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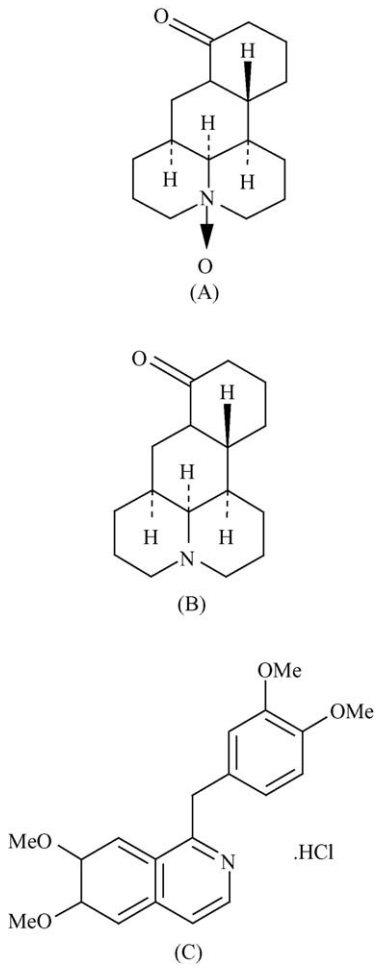

Fig. 1. Chemical structures of: (A) OMT, (B) MT and (C) PPV (internal standard).

OMT and/or MT in plasma with a limit of quantitation of about 0.5 μg/ml are not practical and sensitive enough for detecting the metabolite MT in human plasma after an intravenous dose of 600 mg OMT injection. LC–MS has been successfully applied for qualitative analysis of alkaloids extracted from *Sophora flavescens Ait* [13]. Although there are a few reports about the determination of OMT and/or MT in biological samples with LC–MS [14,15] or GC–MS [16] methods at low ng/ml concentration levels for animal studies after oral administration, they all use liquid–liquid extraction procedure for sample preparation and there is little information about the pharmacokinetics of OMT and its active metabolite MT after i.v. administration of OMT in human up until now. Therefore, a more specific and sensitive liquid chromatography tandem mass spectrometry (LC–MS/MS) method was developed for the simultaneous determination of OMT and its metabolite MT in human plasma and pharmacokinetic study after i.v. administration of OMT.

2. Materials and methods

2.1. Chemicals and materials

The reference substances of OMT, MT and papaverine hydrochloride (internal standard, PPV, Fig. 1(C)) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC grade methanol was obtained from Tedia Company Inc (Fairfield, OH, USA). All other chemicals were of analytical reagent grade. Water was prepared with double distillation.

OMT glucose injection (600 mg of OMT in 100 ml of 5% glucose injection; Lot No. 20040915) was supplied by Jiangsu Chia Tai-Tian Qing Pharmaceutical Co. Ltd. (Lianyungang, China).

2.2. Chromatography and tandem mass spectrometry

The LC–MS/MS system consisted of a Surveyor LC pump, a Surveyor auto-sampler and a TSQ Quantum Ultra AM triple–quadrupole tandem mass spectrometer with an ion max source, Xcalibur 2.1 software for data acquisition and analysis (Thermo Finnigan, San Jose, CA, USA).

HPLC separation was performed on a Lichrospher-CN analytical column (4.6 mm × 150 mm, i.d., 5.0 μm, Merck) maintained at 30 °C with a mobile phase of methanol–ammonium acetate (20 mmol/l; 85:15, v/v) delivered at 1.0 ml/min and 30% of the eluent was split into the inlet of the mass spectrometer using an electrospray ionization (ESI) source.

The mass spectrometer was operated in the positive ion detection mode with the spray voltage set at 5000 V. The heated capillary temperature was 350 °C. The nitrogen sheath gas and the auxiliary gas were set at 35 and 4 psi, respectively. Quantification was performed with multiple selected reaction monitoring (MRM) with argon at a pressure of 1.5 mTorr for collision-induced dissociation (CID) of the following transitions: OMT *m/z* 265.2 to *m/z* 265.2, MT *m/z* 249.2 to *m/z* 249.2, both with the collision energy set at 25 eV, and PPV *m/z* 340.2 to *m/z* 324.0 with collision energy set at 35 eV and a dwell time of 0.20 s per transition. The product ion scans for the analytes and internal standard are shown as Fig. 2(A–E).

2.3. Standard solutions

The standard stock solutions of OMT and MT were prepared by dissolving a proper amount of OMT and MT in methanol each with the concentration of 1250 μg/ml (calculations were corrected for potency). The stock solutions were then diluted with the same solvent to obtain a series of working solutions of 0.025, 0.625, 12.5 and 125 μg/ml, respectively. The internal standard solution of PPV was prepared in the same way with the concentration of 0.5 μg/ml. The stock and working solutions were stored at 4 °C when not in use.

2.4. Sample preparation

A plasma sample of 0.25 ml in 1.5 ml Eppendorf tube added with 50 μl of the internal standard working solution (0.5 μg/ml) was protein precipitated by the addition of 0.75 ml methanol and vortex-mixed for 60 s. The mixture was centrifuged at 16,000 rpm for 10 min at 4 °C. The top limpid layer was transferred to an auto-sampler vial for LC–MS/MS analysis.

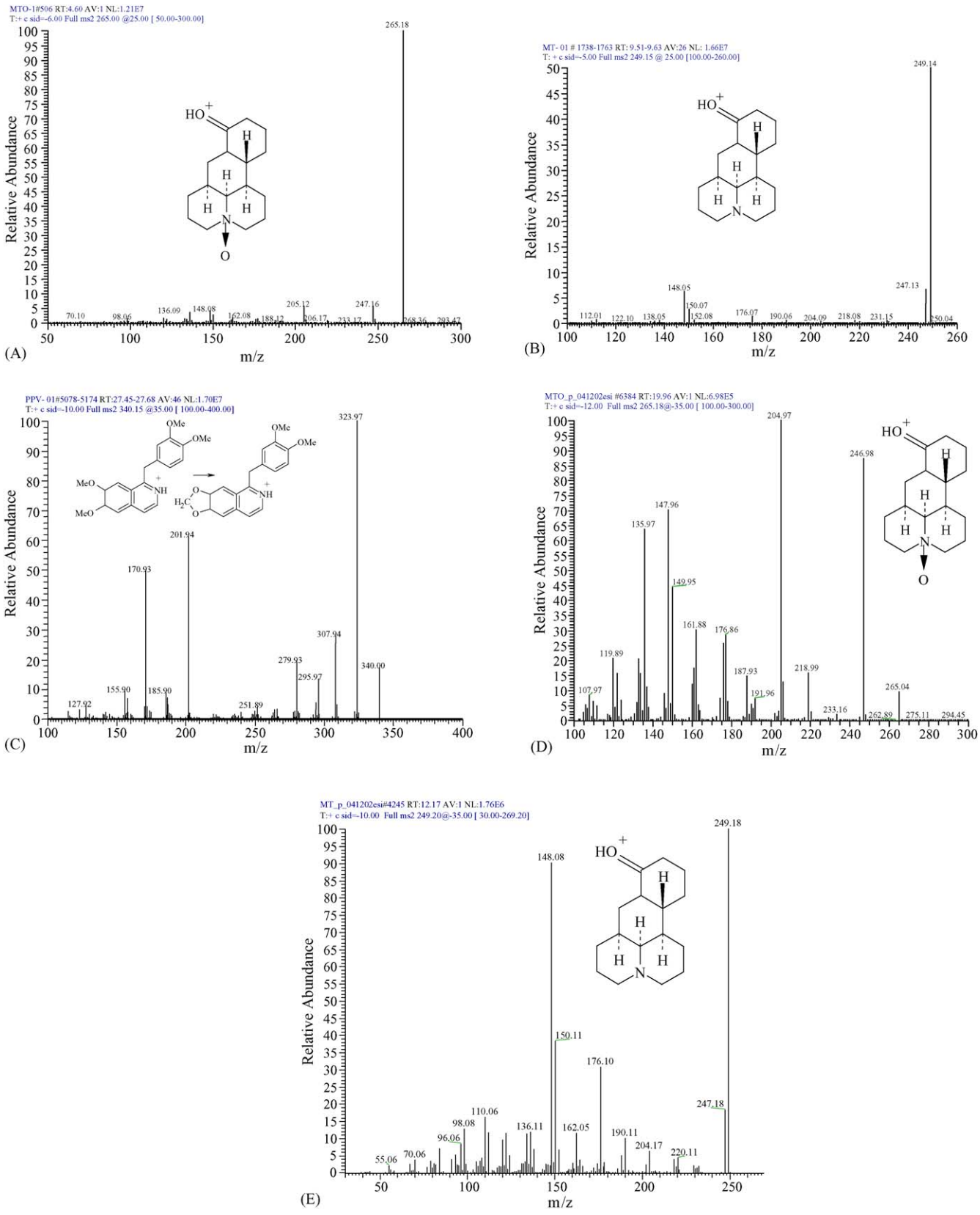

Fig. 2. The product ion scan spectra of $[M+H]^+$ ions of: (A) OMT with argon CID at 25 eV, (B) MT with argon CID at 25 eV, (C) PPV with argon CID at 35 eV, (D) OMT with argon CID at 35 eV and (E) MT with argon CID at 35 eV, respectively.

The same sample handling process was used for linearity, recovery, precision and accuracy determination.

2.5. Subjects and pharmacokinetic study protocol

Ten healthy male Chinese volunteers (aged 20–24 years, body weight 65 ± 5 kg) were selected as subjects. All subjects gave written consent to their participation after having been informed by the medical supervisor about the aim, course and possible risks of the study. The study protocols were approved by the relevant Ethical Review Committee, in accordance with the principles of the Declaration of Helsinki and the recommendations of the State Food and Drug Administration of China. The volunteers participated in a single dose pharmacokinetic study. Subjects fasted 10 h before and 4 h after drug administration. Venous blood samples about 3.5 ml were collected in heparinized tubes at pre-dose (0 h) and 0.17, 0.33, 0.5, 0.75, 1.0, 2.0, 3.0, 5.0, 7.0, 9.0, 12, 15, 24, 36, 48, 72 h after i.v. infusion administration of 600 mg OMT in 100 ml of 5% glucose injection in 0.5 h. The plasma samples were separated by centrifugation at $3000 \times g$ force for 10 min and stored at $-20\,^{\circ}C$ until analysis.

2.6. Validation of the assay

Calibration standards were prepared with plasma concentrations of 1.00, 5.00, 25.0, 100.0, 500, 1000, 2500, 5000, 10,000, 25,000 and 40,000 ng/ml for both OMT and MT, each containing 100.0 ng/ml of PPV as internal standard, with an appropriate volumes of the working solutions added into the Eppendorf tubes and then dried before mixed with 0.25 ml of blank human plasma. The OMT and MT calibration curves were constructed by plotting the peak area ratios of the corresponding analyte to PPV versus concentrations.

The intra-day precision and accuracy of the method were determined by analyzing five replicates at 5.00, 100.0, 2500 and 40,000 ng/ml levels of OMT and MT carried out in a single day, while using five replicates at these four concentrations over 3 days of the validation period to demonstrate the inter-day precision and accuracy, respectively. The concentration of the quality control (QC) samples was determined using calibration standards prepared on the same day. The accuracy of the method was expressed as the relative error (RE%), obtained by calculating the percentage difference between the measured and spiked concentration over that of the spiked value. The precision was denoted by the relative standard deviation (R.S.D.%).

The absolute recovery of the method was determined by comparing the peak areas obtained from the plasma samples with peak areas obtained by the direct injection of the corresponding reference standards in water solution of the same volume at four different concentration levels of 5.00, 100, 2500 and 40,000 ng/ml.

Long-term storage stability of OMT and MT in human plasma was established by analyzing triplicate spiked quality control samples at 5.00, 100 and 2500 ng/ml levels for both OMT and MT which had been stored at $-20\,^{\circ}C$ for a different period of time. The effect of three freeze–thaw cycles on the compound stability in plasma was evaluated by repeated analyses ($n = 3$) of quality control samples of 100 ng/ml for both OMT and MT with reference to the freshly prepared calibration curve and quality control samples.

2.7. Pharmacokinetics

The maximum plasma concentrations (C_{max}) and their time of occurrence (T_{max}) of OMT or MT were obtained directly from the observed data. The area under the plasma concentration–time curve (AUC) from the time zero to the last measured concentration (AUC_{0-t}) was calculated according to the linear trapezoidal rule. The terminal elimination rate constant (λ_Z) was calculated by least-squares regression of the plot of logarithms of concentration against time for the last five measurable points, the terminal half-life was calculated with $t_{1/2} = 0.693/\lambda_Z$ accordingly, and the $AUC_{0-\infty}$ was the corresponding area extrapolated to infinity by $AUC_{0-t} + C_t/\lambda_Z$, where C_t was the last measurable drug concentration. The clearance (CL/F, $l\,h^{-1}$) and the volume of distribution (V/F, l) were calculated through the model fitting of the concentration time curves for each subject.

3. Results and discussion

3.1. Chromatography and mass spectrum

Fig. 2(A–C) shows the tandem mass product spectra of OMT, MT and PPV obtained in positive ion ESI mode, respectively. The transition m/z 340.2 to m/z 324.0 for PPV arises from the loss of CH_4 in SRM mode. The product ions of $[M+H]^+$ ions for neither OMT nor MT are produced selectively and efficiently (Fig. 2(D and E)). Therefore, the $[M+H]^+$ ions of OMT and MT were monitored essentially in SIM mode, respectively, while the CID energy of 25 eV was important for the elimination of background noise and enhancement of sensitivity. All analytes gave excellent responses under these conditions. No sodium or other solvent adducts or dimers were observed.

Typical chromatograms are shown in Fig. 3(A–C). The retention times of OMT, MT and PPV were approximately 3.6, 3.3 and 2.6 min, respectively. The overall chromatographic run time was 6 min. Since the analytes were all well separated from the major endogenous materials in the HPLC column, there was no obvious endogenous interference and matrix effect on ionization. PPV was selected as the internal standard for its similarity in the retention and ESI ionization conditions to those of OMT and MT. Other candidate compounds (e.g. sophocarpine that are much closer in structure to the analytes) may also be used as IS. But they are easily concomitant because of the same botanical origin and/or produced by bio-transformation.

3.2. Linearity, recovery, precision and accuracy

Linear responses were obtained for both OMT and MT ranging from 1 to 1000 ng/ml with correlation coefficient values >0.999, and quadratic responses were obtained in the range from 1000 to 40,000 ng/ml with correlation coefficient values >0.999. The limit of detection (LOD) for OMT and MT were

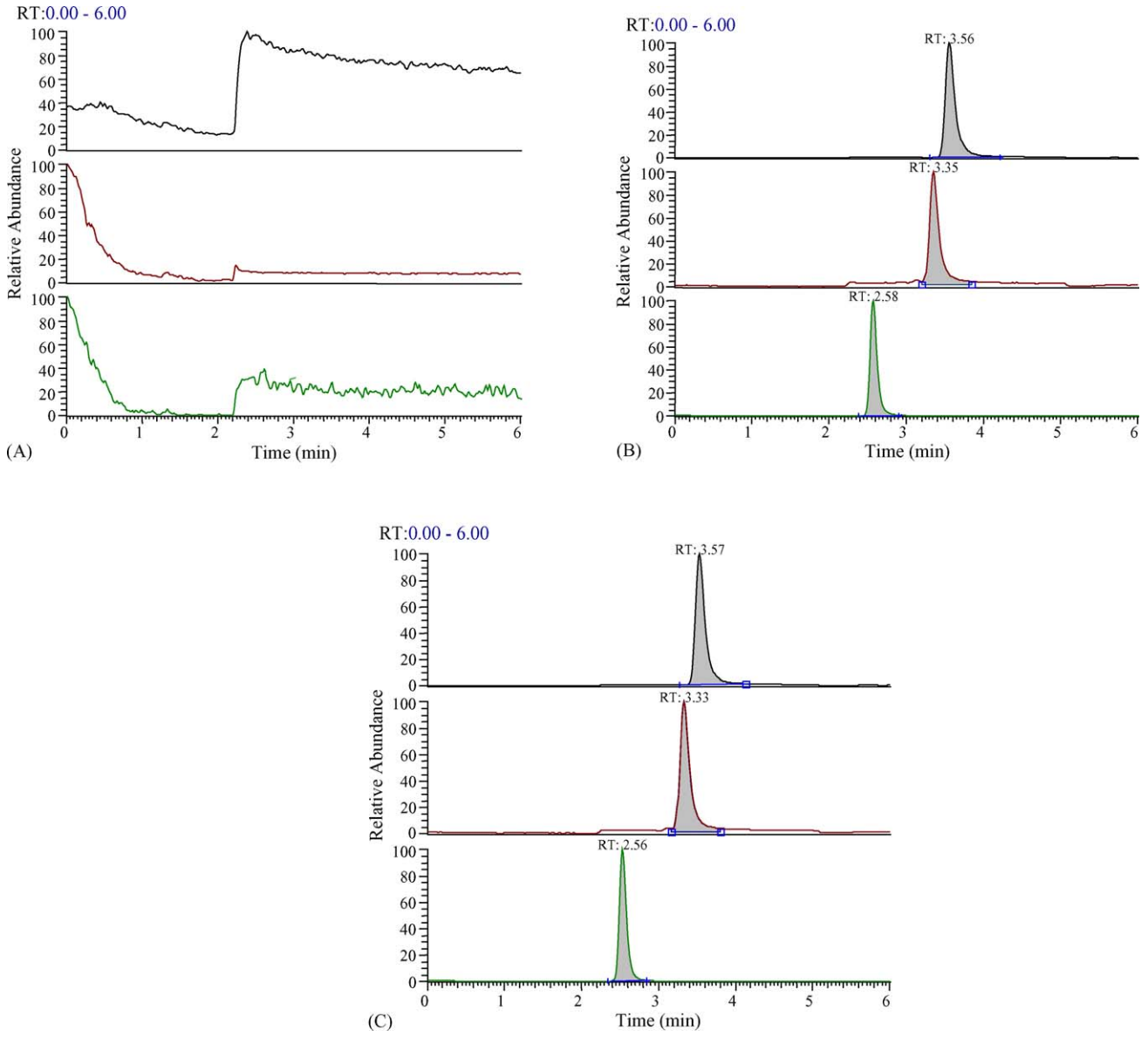

Fig. 3. Representative chromatograms for OMT and the metabolite MT in: (A) human blank plasma, (B) plasma spiked with 100 ng/ml OMT (t_R = 3.35 min), 100 ng/ml MT (t_R = 3.56 min) and 100 ng/ml PPV (t_R = 2.58 min), (C) plasma sample from a subject 5 h after administration of 600 mg OMT injection, the concentrations of OMT and MT were found to be 576.4 ng/ml and 189.2 ng/ml, respectively.

0.5 and 0.2 ng/ml, respectively. While the lower limit of quantitation used in the construction of the calibration curves was set at 1.0 ng/ml for both.

Both OMT and MT were easily recovered from human plasma (Table 1) by protein precipitation with methanol.

The intra- and inter-day precision for OMT analysis in human plasma ranged from 2.9 to 16.4% at levels of 5.00, 100, 2500 and 40,000 ng/ml, while the intra- and inter-day accuracy ranged from −15.1 to 6.7%. The corresponding intra- and inter-day precision for the determination of MT was between 2.2 and 14.5%, and the accuracy ranged from −10.7 to 7.3% (Table 2).

Table 1
Recoveries of OMT and MT in human plasma (n = 5)

Constituent	Spiked concentration (ng/ml)	Measured concentration (ng/ml)	Recovery (%)	R.S.D. (%)
OMT	5.00	4.84	96.8	6.1
	100.0	101	101.4	4.2
	2500	2630	105.2	2.8
	40000	37080	92.7	2.4
MT	5.00	5.24	104.8	6.8
	100.0	94.2	94.0	3.6
	2500	2427	97.1	2.0
	40000	37440	93.6	3.1

3.3. Stability

The stability of OMT and MT in plasma was evaluated by analyzing quality control samples containing 5.00, 100 and

Table 2
Intra-day ($n = 5$) and inter-day ($n = 3 \times 5$) precision and accuracy of the assay

Constituent	Concentration (ng/ml)	Intra-day ($n = 5$)		Inter-day ($n = 3 \times 5$)	
		Precision (R.S.D.%)	Accuracy (RE%)	Precision (R.S.D.%)	Accuracy (RE%)
OMT	5.00	4.3	5.7	16.4	−15.1
	100.0	2.9	6.7	9.7	−11.4
	2500	3.8	−3.8	4.2	4.9
	40000	3.4	−4.5	3.8	−5.3
MT	5.00	5.6	7.3	14.5	−10.7
	100.0	2.2	−3.1	7.6	6.6
	2500	2.7	−7.4	5.1	4.2
	40000	3.1	−4.6	3.8	−5.8

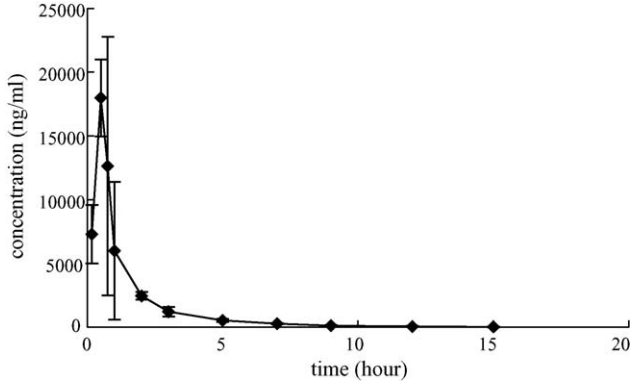

Fig. 4. Mean plasma concentration–time profile of oxymatrine following i.v. infusion of 600 mg OMT in 100 ml 5% glucose injection to 10 healthy Chinese male volunteers. Each point represents the mean ± S.D. of 10 subjects.

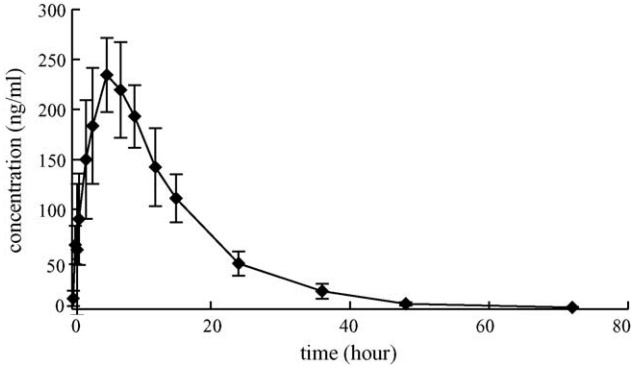

Fig. 5. Mean plasma concentration–time profile of the active metabolite MT following i.v. infusion of 600 mg OMT in 100 ml 5% glucose injection to 10 healthy Chinese male volunteers. Each point represents the mean ± S.D. of 10 subjects.

2500 ng/ml of both OMT and MT stored at −20 °C for 2 months and following three freeze–thaw cycles, respectively. OMT and MT were found to be stable in plasma at −20 °C for at least 2 months and for up to three freeze–thaw cycles, since there was not any obvious change in the concentrations of OMT and MT in plasma tested within the time period under the indicated storage conditions.

3.4. Pharmacokinetic studies

The mean plasma concentration–time curves of OMT and the metabolite MT were presented in Figs. 4 and 5, respectively. OMT concentration time profile conformed to a two-compartment pharmacokinetic model after i.v. administration. The estimated pharmacokinetic parameters are shown in Table 3.

The $AUC_{0-\infty}$ value for MT (3841 ng h/ml) was only about 19.9% of that of the OMT (20,436 ng h/ml), indicating that only a small amount of OMT was reduced to MT following i.v. administration of OMT. The T_{max} of MT was about 5.6 h, which was much longer than that of the OMT. This was very likely due to the time required for the delivery of OMT to the intestine where the reduction of OMT by bacteria takes place. All of these were significantly different from the case following oral administration of OMT [16].

Table 3
Pharmacokinetic parameters of OMT and its metabolite MT after i.v. infusion of 600 mg OMT to 10 healthy volunteers (mean ± S.D.)

Parameter	OMT	MT
C_{max} (ng/ml)	20519 ± 7.6	247 ± 45
T_{max} (h)	0.5 ± 0.1	5.6 ± 1.7
MRT (h)	1.77 ± 0.26	13.91 ± 1.25
$t_{1/2}$ (h)	2.17 ± 0.49	9.43 ± 0.62
AUC_{0-t} (ng h/ml)	20360 ± 5205	3817 ± 610
$AUC_{0-\infty}$ (ng h/ml)	20436 ± 5188	3841 ± 615
$(AUC_{0-\infty})_{MT}/(AUC_{0-\infty})_{OMT}$	19.9 ± 5.4%	–
CL/F ($l\,h^{-1}$)	43.8 ± 10.8	–
V_d/F (l)	70.1 ± 26.6	–
K_{21} (h^{-1})	0.43 ± 0.17	–
K_{10} (h^{-1})	0.68 ± 0.14	–
K_{12} (h^{-1})	0.023 ± 0.008	–

4. Conclusions

The pharmacokinetics of OMT had been previously reported [9–11]. However the pharmacokinetics of its metabolite MT was not simultaneously studied.

The LC–tandem mass spectrometry method established for the simultaneous determination of OMT and its active metabolite MT in human plasma following i.v. administration of 600 mg OMT in 100 ml of 5% glucose injection is more selective and sensitive than the previously reported LC single quadrupole

MS detection method [15]. The pharmacokinetic study of OMT injection in healthy male Chinese volunteers indicates that only a small portion of the OMT administered is reduced to MT after i.v. administration ($(AUC_{0-\infty})_{MT}/(AUC_{0-\infty})_{OMT} = 19.9 \pm 5.4\%$).

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