Determination of Oxymatrine in Human Plasma by LC–MS and Study on its Pharmacokinetics

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Abstract

A sensitive and selective liquid chromatographic–mass spectrometric method is built to determine oxymatrine in human plasma. After a liquid–liquid extraction for samples, samples are analyzed on a C_{18} column interfaced with a mass spectrometer. Positive electrospray ionization is employed as the ionization source. The mobile phase is methanol–water containing 10 mmol/L ammonium acetate (60:40) at the flow rate of 0.8 mL/min. The method is linear in the concentration range of 10–1000 ng/mL. The lower limit of quantitation is 10 ng/mL. The intra- and inter-day relative standard deviation across three validation runs over the entire concentration range is less than 14.27%. The accuracy determined at three concentrations (20, 100, and 500 ng/mL for oxymatrine) is within ± 10.0% in terms of relative error. The method herein described is successfully applied to the evaluation of pharmacokinetic profiles of oxymatrine tablets pills in 18 healthy volunteers. The results show AUC, T_{max}, C_{max}, and $T_{1/2}$ between the testing formulation and reference formulation have no significant difference ($P > 0.05$). Relative bioavailability is 104.2 ± 13.8%.

Introduction

Oxymatrine (OMT), a quinolizidine alkaloid, extracted from the Chinese herb *Sophora flavescens* Ait, has been proven to be effective in the treatment of viral hepatitis, cancer, cardiac diseases (such as viral myocarditis), and skin diseases (such as psoriasis and eczema). (1–3)

In PR China, OMT, called kushensu, is frequently used in clinical practice, especially for oral administration. Various analytical methods have been described for the measurement of OMT in biological fluids. These methods include liquid chromatography–mass spectrometry (LC–MS) (4–6), high-performance liquid chromatography–UV detection (7–9), gas chromatography–mass spectrometry (10), and capillary electrophoresis (11–13). However, up to now, the pharmacokinetics of OMT after the oral administration of OMT have seldom been reported (5). To address the pharmacokinetics of OMT, a sensitive method that allows an accurate measurement of low concentrations of OMT in plasma is required. In this work, we established a LC–MS method to determine OMT in human plasma, and the pharmacokinetics of OMT in human plasma was investigated. This article presents that the plasma concentrations of OMT versus time showed double peaks after the administration of a single dose of OMT. This would be used as a suitable reference in clinical applications.

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Experimental

Materials and reagents

OMT (99.0% purity) and oxpentifylline [internal standard (IS) internal standard, 98.0% purity] were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, PR China). Methanol (HPLC-grade) was purchased from Merck Company (Darmstadt, Germany). Ammonium acetate (analytical grade) was from Nanjing Chemical Co. (Nanjing, PR China). Heparinized blank (drug-free) human plasma was obtained from Nanjing Blood Donor Service (Nanjing, PR China). Distilled water, doubly distilled in the laboratory, was used throughout the study.

Instrumentation

The chromatographic system used consisted of an Agilent 1100 series LC–MSD (Agilent, Palo Alto, CA), including a binary pump (Model G1312A), a vacuum degasser (Model G1322A), a mass detector (Model G1946A), an autosampler (Model G1313A), and a column oven (Model G1316A). The HPLC system was coupled to a quadrupole MS with an electrospray ionization (ESI) interface. Data acquisition was performed with Agilent ChemStation A.08.03.

LC–MS conditions

The chromatographic separation was achieved on a Lichrospher C18 column (5 µm, 250 mm × 4.6 mm, Jiangsu Hanbon Science & Technology Co., Ltd, HuaiAn, PR China). The

mobile phase was methanol–water containing 10 mmol/L ammonium acetate (60:40, v/v) at the flow rate of 0.8 mL/min. The column temperature was maintained at 30°C.

In order to optimize all the MS parameters, a standard solution (1 µg/mL) of the analyte and I.S. was infused into the MS at the rate of 20 µL/min. For both OMT and oxpentifylline, the following optimized parameters were obtained. The nebulizer pressure was set at 60 psi, while the drying gas (nitrogen) was delivered at a flow-rate of 10 L/min at a temperature of 350ºC. Capillary voltage was set at 4.0 kV and the fragmentor (collision-induced dissociation cell) was set at 70 V.

Sample preparation

The plasma was prepared by a liquid–liquid extraction method for detection. In a 10-mL centrifuge tube, an aliquot (0.4 mL) of human plasma was spiked with 20 µL of the IS working solution (40 µg/mL). After being vortexed (30 s), 4 mL chloroform were added to the tube and vortexed for 3 min. After centrifugation at 2130 × *g* for 10 min, the organic phase (3 mL) was transferred to a 10-mL centrifuge tube and evaporated to dryness at 40°C under a gentle stream of nitrogen. The dry residue was redissolved in 200 µL mobile phase, vortexed for 30 s, and then transferred to a 1-mL centrifuge tube. The reconstituted plasma extraction was highly centrifuged at 16880 × *g* for 10 min and 40 µL supernatant fluid was injected into the HPLC–MS system for analysis.

Preparation of standard and quality control samples

A stock solution of OMT was prepared in water at the concentration of 100 µg/mL. A stock solution of I.S. was prepared in water at the concentration of 400 µg/mL and diluted to 40 µg/mL with water. Calibration curves were prepared by spiking the appropriate standard solution to 0.4 mL of blank plasma. Effective concentrations in plasma samples were 10, 20, 50, 100, 200, 500, and 1000 ng/mL for OMT. The quality control (QC) samples were separately prepared in blank plasma at the concentrations of 20, 100, and 500 ng/mL, respectively. The spiked plasma samples (standards and quality controls) were then treated following the "Sample preparation" procedure on each analytical batch along with the unknown samples.

Method validation

Plasma samples were quantitated using the ratio of the peak area of OMT to that of I.S. as the assay parameter. Peak area ratios were plotted against OMT concentrations and standard curves were in the form of $y = A + Bx$.

To evaluate linearity, plasma calibration curves were prepared and assayed in duplicate on separate five days. The accuracy and precision were also assessed by determining QC samples at three concentration levels on three different validation days. The accuracy was expressed by (mean observed concentration)/(spiked concentration) × 100% and the precision by relative standard deviation (14–16).

Absolute recoveries of OMT at three QC levels were determined by assaying the samples as described above and comparing the peak areas of both OMT and I.S. with those obtained from direct injection of the compounds dissolved in the supernatant of the processed blank plasma.

OMT stability in plasma was assessed by analyzing QC samples at concentrations of 20, 100, and 500 ng/mL, respectively, that had been exposed to different time and temperature conditions. The long-term stability was assessed after storage of the test samples at –20°C for 5 days. The freeze-thaw stability was determined after five freeze-thaw cycles (–20°C to 20°C) on consecutive days. The extraction storage stability was assessed by placing QC samples being separate at –20°C for five days and analyzed. The results were compared with those QC samples freshly prepared, and the percentage concentration deviation was calculated.

Pharmacokinetic study

To demonstrate the reliability of this method for the study of the pharmacokinetics of OMT, it was used to determine OMT concentrations in plasma samples 0–13 h after the administration, in random order, of a single oral dose of 300 mg of OMT tablet to 18 healthy volunteers in a pharmacokinetic study approved by the Ethics Committee of the Institute of Dermatology, Chinese Academy of Medical Science. All volunteers gave written informed consent to participate in the study according to the principles of the Declaration of Helsinki (1964) in the revised version of 1996 (Somerset West). Serial blood samples (3 mL) from a suitable antecubital vein were collected into sodium heparin-containing tubes before and 0.25, 0.5, 1, 1.25, 1.5, 2, 2.5, 3, 3.5, 4 , 5, 6, 9, and 13 h after the administration of OMT. Plasma was separated by centrifugation at 2130 × *g* for 10 min and stored frozen at –20°C until analysis.

Pharmacokinetic parameters were determined from the plasma concentration–time data. To compare the two formulations, an analysis of variance was performed on the appropriate log-transformed and non-transformed PK parameters using BAPP2.0 software. (Center of Drug Metabolism & Pharmacokinetics, China Pharmaceutical University, Nanjing, China).

Results and Discussion

Chromatography and mass spectrum

Because OMT and IS have basic groups in their structures (Figure 1), all mass spectra were collected in the positive ion mode. The electrospray mass scan spectra of OMT and I.S. are shown in Figure 2. The ions of $[M+H]^+$, m/z = 265.2 for OMT and $[M+ Na]^+$, m/z =301.1 for oxpentifylline were selected for the SIM (+) due to their high stability and intensity, and no significant solvent adduct ions and fragments ions were observed.

The polarity of OMT was large. Thus it was more difficult to retain on the C_{18} column than on the CN column. When we added 10 mmol/L ammonium acetate into the mobile phase, the retention time of the OMT was delayed remarkably. At last, the C_{18} column was selected instead of CN column (5) because the C_{18} column has low cost and general applicability. As shown in Figure 3, the retention times of OMT and IS were approximately 3.9 and 4.9 min, respectively. There were no endogenous plasma components interfering with them. The overall chromatographic run time was finished within 6 min. Ion suppression was investigated and not detected in the assay.

Method validation

Selectivity

Selectivity was assessed by comparing the chromatograms of six different batches of blank human plasma with the corresponding spiked plasma. Figure 3 shows the typical chromatograms of a spiked plasma sample with OMT (100 ng/mL) and IS and a plasma sample from a healthy volunteer 2 h after an oral administration. There was no significant interference or ion suppression from endogenous substances observed at the retention times of the analytes. Typical retention times for OMT and IS were about 3.9 and 4.9 min.

Linearity of calibration curves and lower limits of quantitation

The results of the plotted duplicate calibration curves and correlation coefficients > 0.999 confirmed that the calibration curves were linear over the concentration ranges 10–1000 ng/mL for the analyte. The typical standard curve was $y = 0.00521x + 0.00109$ (weigh = 1/c), where y represents the ratios of OMT peak area to that of I.S. and x represents the plasma concentrations of OMT.

The lower limit of quantitation was defined as the lowest concentration on the calibration curve for which an acceptable accuracy of ± 15% and a precision below 15% were obtained. The present LC–MS method offered a lower limit of quantitation of 10 ng/mL in 0.4 mL plasma sample. It is sensitive enough to investigate the pharmacokinetic behaviors of OMT, to establish the relationship between the dose and the pharmacological effects in humans.

Precision and accuracy

Table I summarizes the intra- and inter-day precision and accuracy for OMT evaluated by assaying the QC samples. The precision was calculated by one-way ANOVA (17). In this assay, the intra-run precision was 14.27% or less, and the inter-run precision was 12.52% or less for each QC level of OMT. The accuracy was within ± 10.0%. The results above demonstrated that the values were within the acceptable range and the method was accurate and precise. This conclusion is similar to previous research (4–5).

Recovery and stability

The recovery of OMT, determined at three concentrations (20, 100, 500 ng/mL), was 87.79 ± 6.58%, 85.33 ± 4.88%, and 89.47 ± 3.65% (n = 5), respectively.

The results of stability experiments showed that no significant degradation occurred at –20°C for 5 days and after five freeze-thaw cycles for OMT plasma samples. The accuracy values of low (20 ng/mL), medium (100 ng/mL), and high (500 ng/mL) concentrations of OMT in human plasma

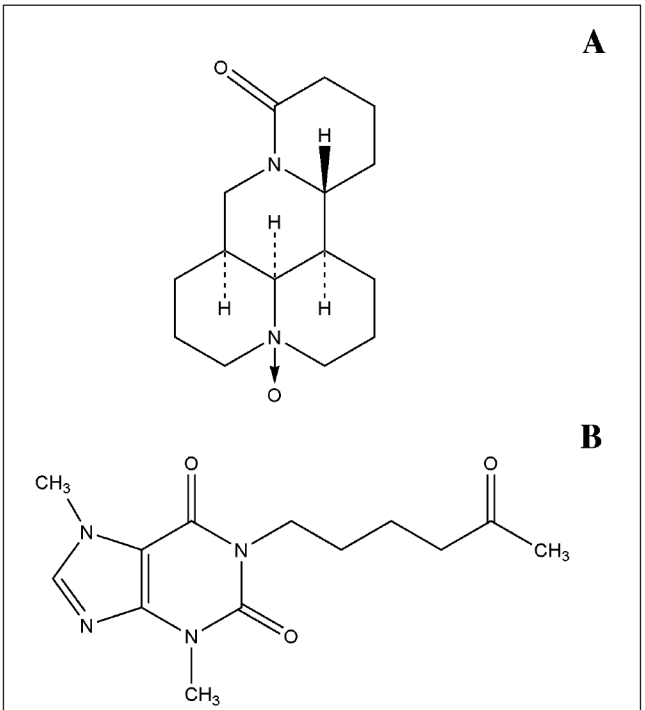

Figure 1. Chemical structures of OMT (A) and I.S. (B).

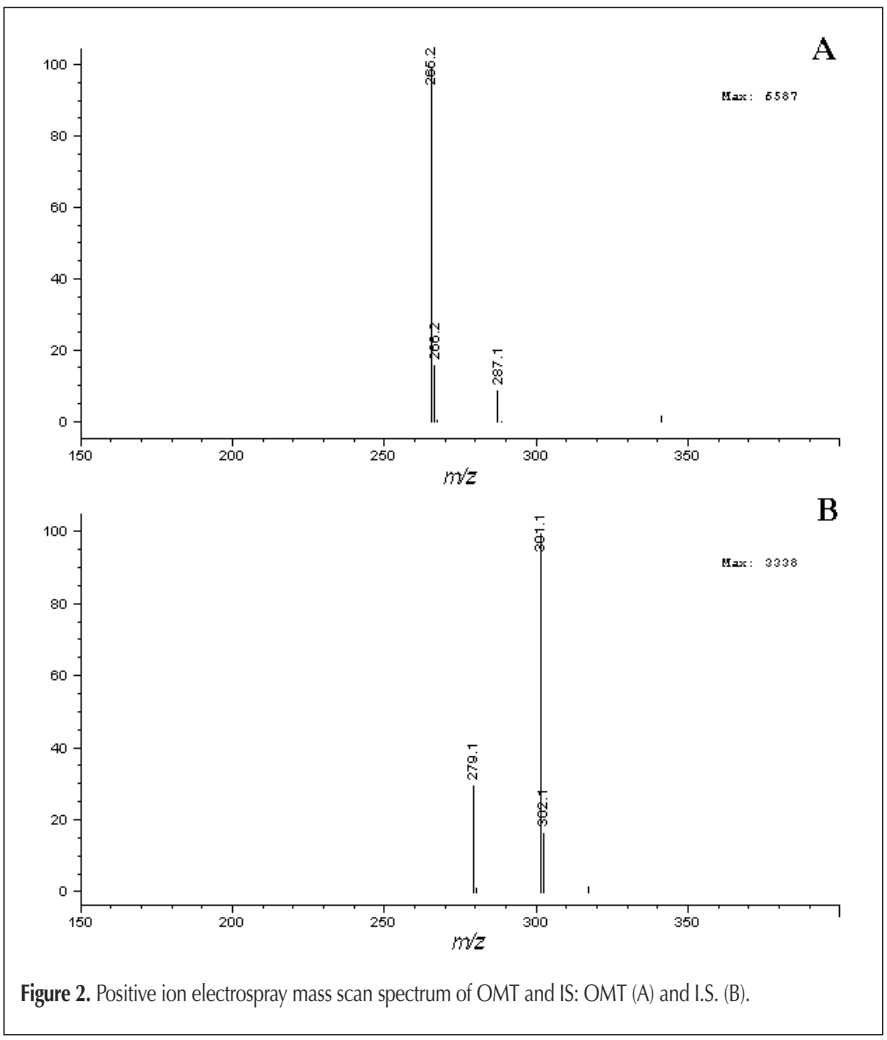

Figure 2. Positive ion electrospray mass scan spectrum of OMT and IS: OMT (A) and I.S. (B).

were 104.6%, 94.25%, and 87.36% after five freeze-thaw cycles, and 90.80%, 88.96%, and 91.91% at –20°C for 5 days. The standard solutions of OMT in water were allowed to stand at 4°C for a week. The solution of IS (400 µg/mL) was proved stable at room temperature for more than 8 h and at 4°C for a week.

Application of the method to a pharmacokinetic study on healthy volunteers

The method was applied to determine the plasma concentration of OMT after an oral administration of reference tablets and test tablets (containing 300 mg OMT) to 18 volunteers. The mean plasma concentration–time curve of OMT is shown in Figure 4. It was reported that there was a biotransform pathway between OMT and its main metabolite matrine, which could be affected by the biological status of volunteers. So the concentration of individuals at the time point between 1–5 h and the profile of Figure 4 were significantly different from previous research (4–5). The main pharmacokinetic parameters of OMT in 18 volunteers are shown in Table II. The relative bioavailability of the test formulation is (104.2 ± 13.8%), and there are no remarkable differences between the test formulation and the reference formulation. The main pharmacokinetic parameters in Table II were significantly different from the case following i.v. administration of OMT (5).

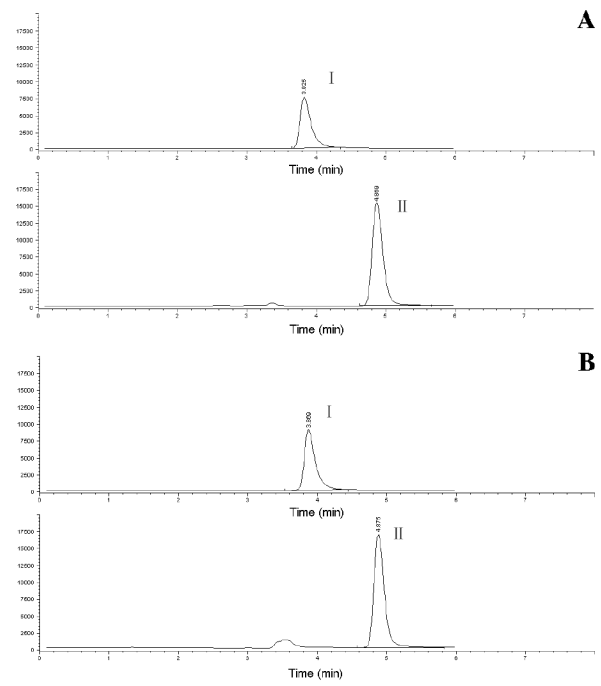

Figure 3. Representative SRM chromatograms of OMT (I) and IS (II) in human plasma samples. Blank plasma sample spiked with OMT at 100 ng/mL and IS (A); plasma sample from a volunteer 2.0 h after oral administration of OMT (B).

Table I. Accuracy and Precision for the Analysis of Oxymatrine in Human Plasma (in Prestudy Validation, *n* = 3 Days, Six Replicates Per Day)

Added C (ng/mL)	Found C (ng/mL)	Intra-run RSD (%)	Inter-run RSD (%)
20	21.81	14.27	12.52
100	94.87	8.95	8.56
500	479.91	5.38	6.36

Conclusions

Liquid chromatography–mass spectrometry (LC–MS) combines the temporal resolution of HPLC with the mass resolution and sensitivity of mass spectrometry and permits the analysis of small amounts of non-volatile drugs or polar metabolites in complex biological matrices. The LC–MS method described in the present study was simple, rapid, sensitive, accurate, and precise for the determination of OMT It was then successfully applied to the evaluation of pharmacokinetic profiles of OMT in 18 healthy volunteers. The plasma concentrations of OMT versus time showed double peaks after administration of a single dose of OMT. Although there have been a number of oral drugs reported to have this phenomenon (18), there have been no similar reports about OMT. This can be used as a suitable reference in clinical applications.

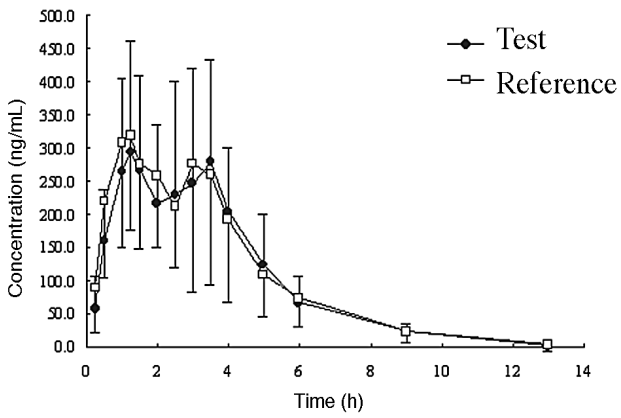

Figure 4. Mean plasma concentration-time curve of OMT in 18 volunteers after a single oral dose of OMT.

Table II. Pharmacokinetic Parameters for 18 Volunteers after Administration of a Single Dose of Oxymatrine

Parameter	Test formulation	Reference formulation
$T_{1/2/h}$	2.00 ± 0.62	1.79 ± 0.41
C_{max}/ng/mL	392.33 ± 182.65	392.04 ± 184.52
T_{max}/h	1.8 ± 0.9	2.1 ± 1.1
$AUC_{0\rightarrow 13h}$/ng/h/mL	1367.62 ± 683.84	1300.72 ± 582.02
$AUC_{0\rightarrow \infty}$/ng/h/mL	1431.53 ± 688.69	1354.17 ± 588.64

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Manuscript received October 11, 2006;
revision received February 28, 2007.