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# Determination of oxymatrine and its active metabolite matrine in human plasma after administration of oxymatrine oral solution by high-performance liquid chromatography coupled with mass spectrometry

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ABSTRACT

A rapid, sensitive and selective high-performance liquid chromatography mass spectrometric method has been developed and validated for the simultaneous determination of oxymatrine and its active metabolite matrine in human plasma after administration of oxymatrine oral solution. Analytes were extracted from the plasma by liquid-liquid extraction with chloroform. The chromatographic separation was accomplished on a Venusil $C_{18}$ column (150 mm × 4.6 mm, 5 μm) protected by a $C_{18}$ guard column (4.0 mm × 2.0 nm; Phenomenex, Torrance, CA, USA). Analytes were detected on a single quadruple mass spectrometer by selected ion monitoring mode via electrospray ionization source. The assay had a lower limit of quantification of 1.5 ng · $mL^{-1}$ for oxymatrine and 3 ng·$mL^{-1}$ for matrine in plasma. The calibration curves were linear in the measured range. The overall precision and accuracy for all concentrations of quality controls and standards were within ±15%. The proposed method enabled unambiguous identification and quantification of oxymatrine and its active metabolite matrine in vivo. The results provided a meaningful basis for evaluating the clinical applications of the oxymatrine oral solution.



## 1. Introduction

*Sophora flavescens Ait*, with a wide range of pharmacological and toxicological activities is a traditional Chinese medicinal herb commonly used in China. Oxymatrine (OMT), also known as oxymatrine extract, and its active metabolite matrine (MT) have anti-inflammatory modulation of immune antiviral role, mainly for clinical treatment of chronic hepatitis B and neutropenia induced by radiation and chemotherapy [1–5] (MT, Fig. 1(A); OMT, Fig. 1(B)). It was reported that when taken orally, most of OMT can participate in reduction reaction and change to matrine (MT) in the gastrointestinal tract [6]. Besides, MT could adjust the cardiovascular system [7].

Recently, much attention has been related to the absorption and metabolism of OMT, and the pharmacokinetics of OMT had been previously reported [8–21], however there is little information about the pharmacokinetics of OMT and its active metabolite MT after administration of oxymatrine oral solution in human. Therefore, it is necessary to investigate the pharmacokinetic profiles of the oxymatrine oral administration combined with the distribution of OMT and MT in vivo.

In the present study, a more sensitive high-performance liquid chromatography mass spectrometry (HPLC-MS) method has been developed and validated for simultaneous quantification of OMT and MT in human plasma. The samples were submitted to the simple instrument operation and easy pretreatment process to achieve high throughput HPLC-MS assay. It was suitable for the investigation of their pharmacokinetic profiles after administration of oxymatrine oral solution.

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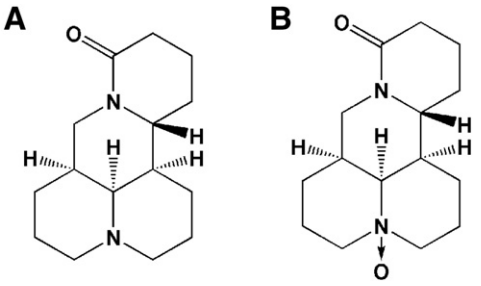


**Fig. 1.** Chemical structures of matrine (A), and oxymatrine (B).

## 2. Experimental

### 2.1. Chemicals and reagents

Oxymatrine oral solution (10 mL of specifications: 0.2 g); the oxymatrine reference standard (batch number: 110780-201007, China Pharmaceutical and Biological products); matrine reference standard (batch number: 110805-200508, China Pharmaceutical and Biological products); aminopyrine (batch number: 100503-200301, China pharmaceutical and Biological Products); blank plasma (People's Liberation Army Hospital 208); distilled water prepared with demineralized water was used throughout the study. Methanol of HPLC grade was from Fisher Scientific (Fair Lawn, NJ, USA). HPLC grade reagents such as formic acid and chloroform, analytical grade reagents such as ammonium acetate and sodium hydrate were provided by Shandong Yuwang Industrial (Yucheng, China).

### 2.2. Instruments and conditions

Shimadzu 2010 series HPLC mass spectrometer equipped with a LC-10ADvp binary pump, an online degasser, an autosampler and a column temperature controller were used for all analyses. Chromatographic separations were achieved on a Venusil $C_{18}$ column (150 mm × 4.6 mm, 5 μm) protected by a $C_{18}$ guard column (4.0 mm × 2.0 nm; Phenomenex, Torrance, CA, USA) at 40 °C. The column was eluted with a gradient mixture of methanol (phase B) and ammonium acetate solution (10 mmol, containing 0.125% formic acid, phase A). The gradient program was as follows: 23%–43% A from 0 to 7.5 min. The flow rate was set at 0.8 $min \cdot mL^{-1}$ and 30% of the eluent was split into the inlet of the mass spectrometer. Aliquots of 4 μL were injected into HPLC system for analysis. Mass spectra were acquired using a mono quadruple mass spectrometer coupled with an electrospray ionization source (ESI). Nitrogen was used as the sheath and auxiliary gas to assist nebulization with the flow rate settled at 1.5 $L \cdot min^{-1}$. All mass spectra were acquired in the positive ion mode with capillary voltage at 1.6 kV, curved desolvation line (CDL) temperature at 250 °C and block temperature at 200 °C. Target ions were monitored at $m/z$ = 265.20 for OMT, 249.20 for MT and 232.20 for aminopyrine (internal standard) using selected ion monitoring (SIM) mode.

### 2.3. Pretreatment of plasma sample

Aliquots (1 mL) of plasma were spiked with 50 μL of I.S. (1.6 $\mu g \cdot mL^{-1}$), 100 μL methanol-water (23:77) and 50 μL of 1 $mol \cdot mL^{-1}$ NaOH solution by vortexing for 30 s, then the mixture was extracted with 3 mL of chloroform by vortex-mixing for 5 min. After centrifugation at 3200 rpm for 5 min, the lower (organic) layer was transferred to a clean centrifuge tube and evaporated to dryness under a gentle stream of nitrogen at 35 °C. Then the residue was reconstituted with 100 μL of methanol-water (23:77) and vortex-mixed for 3 min and sonicated for 3 min, followed by being centrifuged at 12,000 rpm for 5 min. At last, 4 μL of the supernatant was injected into the LC-MS system for analysis.

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

The stock solutions of OMT, MT and I.S. were prepared in methanol at the concentration of 100 $\mu g \cdot mL^{-1}$, 50 $\mu g \cdot mL^{-1}$ and 50 $\mu g \cdot mL^{-1}$, respectively. A series of standard mixture working solutions with concentrations of 15–15,000 $ng \cdot mL^{-1}$ for OMT and 30–30000 $ng \cdot mL^{-1}$ for MT were obtained by diluting the mixture of the stock standard solutions with methanol-water (23:77). The working solution of the I.S. was diluted by the stock solution with methanol-water (23:77), and the stock and working solution of I.S. were 50 $\mu g \cdot mL^{-1}$ and 1.6 $\mu g \cdot mL^{-1}$, respectively. All solutions were stored at 4 °C. Stabilities of OMT, MT and I.S. in methanol-water (23:77) at 4 °C for 15 days were satisfied with determination.

Calibration standards of OMT (1.5, 3, 6, 30, 150, 750 and 1500 $ng \cdot mL^{-1}$) and MT (3, 6, 12, 60, 300, 1500 and 3000 $ng \cdot mL^{-1}$) were prepared by spiking the appropriate amount of the standard mixture working solutions (100 μL) and I.S. working solution (50 μL) into 1 mL drug-free human plasma. Quality control samples (QC samples) were prepared at low, medium, and high concentrations (3.75, 67.5, 1200 $ng \cdot mL^{-1}$ for OMT and 7.5, 135, 2400 $ng \cdot mL^{-1}$ for MT) 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.

### 2.5. Method validation

#### 2.5.1. Calibration curve and LLOQ

Calibration standards were prepared according to the procedure described in Section 2.4 in triplicate and analyzed on three consequent days. The calibration curves were constructed by plotting the peak area ratio of OMT and MT to I.S. versus their respective concentrations in human plasma. Weighted ($1/x^2$) linear least-squares regression method was used to determine the slope, intercept and correlation coefficient. Unknown sample concentrations of OMT and MT 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.

#### 2.5.2. Precision and accuracy

The accuracy and precision of the established method were evaluated by QC samples at low (3.75 $ng \cdot mL^{-1}$ for OMT and 7.5 $ng \cdot mL^{-1}$ for MT), medium (67.5 $ng \cdot mL^{-1}$ for OMT and 135 $ng \cdot mL^{-1}$ for MT) and high concentrations (1200 $ng \cdot mL^{-1}$ for OMT and 2400 $ng \cdot mL^{-1}$ for MT). The