Journal of Chromatography B, 974 (2015) 126–130

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Simultaneous determination of 14-thienyl methylene matrine and matrine in rat plasma by high-performance liquid chromatography–tandem mass spectrometry and its application in a pharmacokinetic study

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ARTICLE INFO

Article history:
Received 27 July 2014
Accepted 29 October 2014
Available online 6 November 2014

Keywords:
LC/MS/MS
14-Thienyl methylene matrine
Matrine
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 14-thienyl methylene matrine (TMM) and matrine (MT) in rat plasma in the present study. The analytes were separated on a C18 column (1.9 μm, 2.1 mm × 100 mm) with a security guard C18 column (5 μm, 2.1 mm × 10 mm) and a triple-quadrupole mass spectrometry equipped with an electrospray ionization (ESI) source was applied for detection. With pseudoephedrine hydrochloride as internal standard, sample pretreatment involved in a one-step protein precipitation with isopropanol:ethyl acetate (v/v, 20:80). The method was linear over the concentration ranges of 5–1000 ng/ml for TMM and 10–2000 ng/ml for MT. The intra-day and inter-day relative standard deviations (RSD) were less than 15% and the relative errors (RE) were all within 15%. The proposed method enables unambiguous identification and quantification of TMM and MT in vivo. This was the first report on determination of the TMM and MT in rat plasma after oral administration of TMM. The results provided a meaningful basis for evaluating the clinical applications of the medicine.

1. Introduction

Matrine (MT, Fig. 1a), a quinolizidine alkaloid isolated from *Sophora alopecuroides*, *Sophora flavescens* or *Sophora subprostrata* in traditional Chinese medicine, has been extensively used in China for the treatment of viral hepatitis, cancer, cardiac and skin diseases [1–3]. But the disadvantages of short in vivo half-life and low oral bioavailability limit its clinical application [4]. To improve its pharmacological properties, 14-thienyl methylene matrine (TMM, Fig. 1b) was prepared, its biological activity is higher than that of MT has been demonstrated in previous study [5]. Therefore, a sensitive and accurate analytical method for the simultaneous determination of TMM and MT is required to support pharmacokinetic (PK) study. Recently, much attention has been paid to the absorption and metabolism of matrine and oxymatrine, and a few papers dealing with their pharmacokinetics and pharmacodynamics have been published [6–14] employing mainly high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS) [6–8]. 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 TMM and MT simultaneously. In the present study, a new HPLC–MS/MS method was developed and validated for simultaneous quantification of TMM and MT in rat plasma, suitable for the investigation of their pharmacokinetic profile.

2. Experimental

2.1. Reagents and chemicals

MT (99.99% pure, batch number 20031010) was supplied by Shanghai Jiagu Pharmaceuticals (Shanghai, China). TMM (99.8% of purity) was synthesized in Department of Chemistry and Chemical Engineering, Guangxi University (Nanning, China).

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http://dx.doi.org/10.1016/j.jchromb.2014.10.041
1570-0232/

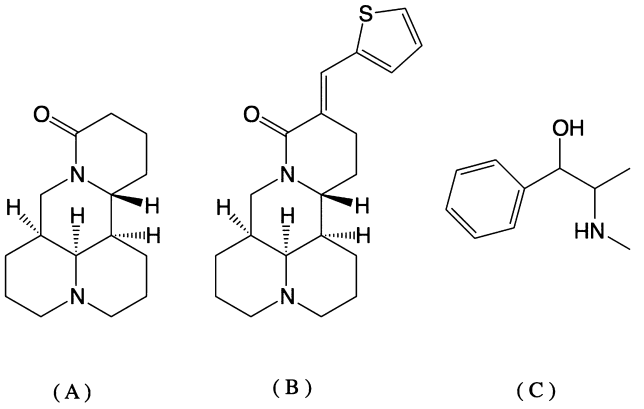

Fig. 1. Chemical structures of MT (A), TMM (B) and PPD (C).

Pseudoephedrine hydrochloride (PPD, Fig. 1c, 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). Isopropanol was purchased from Caledon (Georgetown, Ontario, Canada). Ethyl acetate was the provision of Dikma (Richmond Hill, ON, USA). Methanol and acetonitrile, both HPLC grade, were imported from Sigma–Aldrich Chemicals (St. Louis, MO, USA). Deionized water was purified using an Alpha-Q water purification system (Millipore, Bedford, MA, USA) and was filtered using 0.20 μm membranes.

2.2. Instruments and conditions

HPLC analysis was performed using the Dionex UltiMate 3000 HPLC equipped with a binary pump, an on-line degasser, an auto-sampler and a column temperature controller. Chromatographic separations were performed on a Hypersil GOLD C18 column (100 mm × 2.1 mm, 1.9 μm particle size) protected by a C18 guard column (10 mm × 2.1 mm, 5 μm) at 40 °C. The mobile phase consisted of acetonitrile–0.1% formic acid (65:35, v/v). The flow rate was set at 0.2 ml/min. Aliquots of 2 μl were injected into HPLC system for analysis.

MS analysis was carried out on a Thermo Scientific TSQ Quantum Access MAX triple stage quadrupole mass spectrometer with an electrospray ionization (ESI) source running in a positive-ionization mode. The typical ion source parameters were: spray voltage: 3500 V; sheath gas pressure (N_2): 20 units; auxiliary gas pressure (N_2): 5 units; ion transfer tube temperature: 350 °C; collision gas (Ar): 1.5 mTorr; Q1/Q3 peak resolution: 0.7 Da; scan width: 0.002 Da; samples were analyzed via selective-reaction monitoring (SRM) with monitoring ion pairs at m/z 343 → 123 for TMM, m/z 249 → 148 for MT, and m/z 166 → 133 for IS. The scan dwell time was set at 0.1 s for every channel. All data collected in centroid mode were acquired and processed using Xcalibur 2.2 software (Thermo Fisher Scientific Inc., USA).

2.3. Preparation of standards and quality control samples

Stock standard solutions of TMM and MT were prepared by dissolving approximate 10 mg of accurately weighted substance in 100 ml of methanol. And the solutions were then serially diluted with methanol to provide working standard solutions of desired concentrations. The PPD (10.0 mg) was dissolved and diluted with methanol to yield a stock solution with a concentration of 1.0 mg/ml, which was further diluted with methanol yielding an IS working solution at concentration of 5.0 μg/ml. All the solutions were stored at 4 °C and brought to room temperature before use. Calibration standards were prepared daily by spiking appropriate working standard solutions (50 μl of TMM and MT) to 100 μl of blank plasma giving TMM concentrations of 5, 25, 50, 100, 200, 500 and 1000 ng/ml and MT concentrations of 10, 25, 50, 100, 500, 1000 and 2000 ng/ml. The quality control (QC) samples were prepared at low, middle and high concentrations in the same way.

2.4. Plasma sample preparation

100 μl of plasma sample in 1.5 ml labeled microcentrifuge tubes was mixed with 20 μl of the working internal standard solution and vortexed for 5 s. Then 800 μl of isopropanol:ethyl acetate (v/v, 20:80) was added and vortexed for 1 min. The tubes were subsequently centrifuged at 12,000 × g for 10 min. The organic layer was transferred to another tube and evaporated to dryness at ambient temperature under a gentle stream of nitrogen. The residue was reconstituted in 100 μl of mobile phase and 2 μl was injected into the HPLC–MS/MS system for analysis.

2.5. Method validation

Selectivity was assessed by comparing chromatograms of six different batches of blank rat plasma with the corresponding spiked rat plasma. Linearity was assessed by weighted ($1/x^2$) analysis of six different calibration curves. Intra- and inter-day precision (the relative standard deviation, RSD) and accuracy (the relative error, RE) were determined by analysis of low, medium, and high QC samples (n = 6) on three different days. The matrix effect was investigated by comparing the peak areas of analytes in the post-extraction spiked blank plasma at low and high concentrations with those of the corresponding standard solutions. The extraction recovery was determined by comparing the mean peak areas of six extracted samples at low, medium, and high QC concentrations with the mean peak areas of spike-after-extraction samples. The stability was assessed by analyzing replicates (n = 6) of low and high QC samples during the sample storage and processing procedures. The freeze–thaw stability was determined after three freeze–thaw cycles. Post-preparation stability was estimated by analyzing QC samples at 24 h at 4 °C. Six aliquots of QC samples were stored at −20 °C for 14 days and at ambient temperature for 4 h to determine long-term and short-term stability, respectively.

2.6. Pharmacokinetic (PK) study in rats

Male Sprague–Dawley rats weighing from 250 to 300 g were used for PK study. All animal experiments were performed in accordance with institutional guidelines and were approved by the University Committee on Use and Care of Animals, Guangxi Medical University. The aqueous solutions of TMM and MT were separately administrated to 12 rats by gavage at 10 mg/kg. Serial blood samples (0.5 ml) were obtained at 0, 0.25, 0.5, 0.75, 1, 2, 3, 5, 7, 10, 24, 36 h after oral administration separately. All samples were placed into heparinized tubes. After centrifugation at 12,000 rpm and 4 °C for 10 min, plasma was collected and frozen at −20 °C until analysis. Pharmacokinetic parameters were estimated by non-compartmental model using TopFit 2.0 software package (Thomae, Germany). The elimination half-life ($t_{1/2}$) was $0.693/k_e$, where k_e, the elimination rate constant, 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. The maximum drug plasma concentrations (C_{max}) and time to C_{max} (T_{max}) were read directly from the observed data. 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.