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Simultaneous determination of six alkaloids and one monoterpene in rat plasma by liquid chromatography–tandem mass spectrometry and pharmacokinetic study after oral administration of a Chinese medicine Wuji Pill

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ABSTRACT

A simple and sensitive method for simultaneous determination of seven active constituents, jatrorrhizine, berberine, coptisine, palmatine, evodiamine, rutacarpine and paeoniflorin, from a Chinese medicine Wuji Pill in rat plasma was developed based on a liquid chromatography and tandem mass spectrometry method. The separation of these seven compounds was carried out on a Shiseido CAPCELL PAK C_{18} column using a mobile phase consisting of acetonitrile (containing 0.1% formic acid and water (containing 0.1% formic acid and 10 mmol/L ammonium acetate) and carbamazepine as an internal standard. Electrospray ionization in positive-ion mode and multiple reaction monitoring was used to identify and quantitate active components. All calibration curves gave good linearity ($r > 0.993$) over the concentration range from 0.42–208.0 ng/mL to 4.18–418.0 ng/mL for all components. The precision of the *in vivo* study was evaluated by intra- and inter-day assays and the percentages of relative standard deviation were all within 15%. The method was successfully applied to pharmacokinetic study of all six alkaloids and one monoterpene in rat plasma after oral administration of the Wuji Pill.

1. Introduction

Wuji Pill is a prescription of a traditional Chinese medicine (TCM) consisting of three herbs: *Rhizoma Coptidis* (*Ranunculaceae*), *Fructus Evodiae Rutaecarpae* (*Rutaceae*) and *Radix Paeoniae Alba* (*Paeoniaceae*), and its formula is in the ratio of 6:1:6, respectively, as described in the Pharmacopoeia of People's Republic of China [1]. Wuji Pill is commonly used to treat gastro-intestinal disorders [1]. *Rhizoma Coptidis* and its major component, berberine, have been extensively studied for their antineoplastic effects [2,3]. In TCM *Rhizoma Coptidis* is used to treat dysentery [4], bacterial [5], fungal [6] and viral infection [7]. Apart from berberine, three other bioactive alkaloids, palmatine, jatrorrhizine and coptisine, are also present in *Rhizoma Coptidis* [8–12]. In addition to *Rhizoma Coptidis, Fructus Evodiae Rutaecarpae* is prescribed at various combinations to treat gastro-intestinal disorders in TCM. Evodiamine and rutacarpine are bioactive alkaloids of *Fructus Evodiae Rutaecarpae* [13], both have an inhibitory effect on aldose reductase activity [14], and on corticosterone production in rat zona fasciculata-reticularis cells [15]. Evodiamine could improve cognitive abilities in the transgenic models of Alzheimer's disease [16]. Rutacarpine has been shown to ameliorate obesity by inhibiting expression of the orexigenic neuropeptides NPY and AgRP [17]. A bioactive monoterpene glycoside, paeoniflorin, in *Radix Paeoniae Alba* [18], has been reported to show neuroprotective [19,20] and anti-inflammatory effects [21–23]. So far, very few reports have been published on pharmacological studies of Wuji Pill. It is therefore hypothesized that the six bioactive alkaloids, berberine, palmatine, jatrorrhizine, coptisine, Evodiamine and rutacarpine, and the monoterpene glycoside, paeoniflorin, from the three herbs in Wuji Pill are contributing to its pharmacological effects (Fig. 1).

Several analytical methods have been reported to quantify alkaloids simultaneously. Deng et al. have developed a LC–MS/MS method for the determination of berberine, palmatine and jatrorrhizine in rat plasma after oral administration of Coptis and Evodia herbs with a LLOQ of 1 ng/mL for all the three alkaloids [24]. Recently, a similar LC–MS/MS method was reported for simultaneous determination of berberine, palmatine and jatrorrhizine alkaloids in Coptis along with baicalin, baicalein and wogonin

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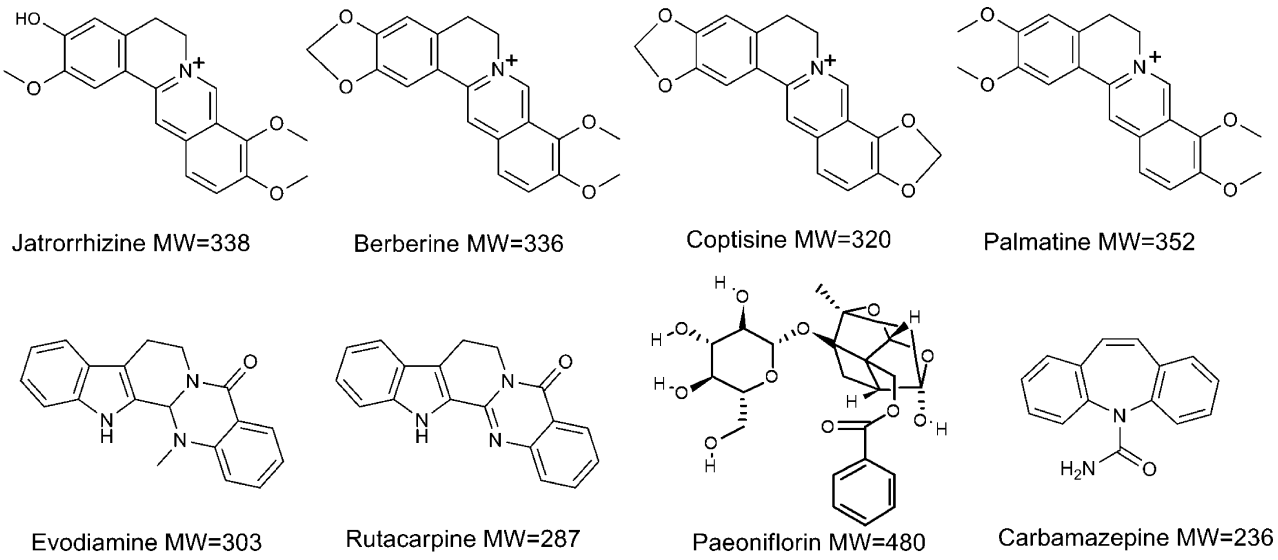

Fig. 1. Chemical structures and molecular weights (MW) of jatrorrhizine, berberine, coptisine, palmatine, evodiamine, rutacarpine, paeoniflorin and carbamazepine (IS).

flavonoids in Scutellaria in rat plasma [25]. The LLOQ for all the three alkaloids was 0.6 ng/mL. For the quantitative determination of paeoniflorin in rat plasma after oral administration of *Radix Paeoniae Alba* extract, a LC–MS method was developed with a LLOQ of 2.5 g/mL for paeoniflorin [26]. Another LC–MS/MS assay was reported for determining paeoniflorin in rat plasma when co-administrated *Radix Angelicae Sinensis* and *Radix Paeoniae* Rubra and the LLOQ for paeoniflorin was reduced to 1.0 ng/mL [27].

In this study we aim to develop an assay for simultaneous quantification of the seven active constituents, jatrorrhizine, berberine, coptisine, palmatine, evodiamine, rutacarpine and paeoniflorin, in Wuji Pill. The chemical structures of four alkaloids (jatrorrhizine, berberine, coptisine, palmatine) are very similar (Fig. 1) and it is therefore challenging to determine these four alkaloids simultaneously. A simple to use and sensitive method for simultaneous quantification of the seven active constituents is needed because these compounds can be used as quality chemical markers of the medicine (Wuji Pill) due to their biological significance. In addition, such a method can be used to extract pharmacokinetic data of these compounds in biological fluids. In this paper we present a validated LC–MS/MS method using a one-step liquid–liquid extraction procedure prior to the determination of these constituents in rat plasma with a relatively short run-time.

2. Experimental

2.1. Materials

The reference standards of jatrorrhizine, berberine, palmatine and paeoniflorin, and internal standard of carbamazepine with a purity of over 98% were all obtained from the Chinese National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). Reference standards coptisine (98%) was supplied by Jingke Chemical Technologies Co. Ltd. (Shanghai, China), and evodiamine (98%) and rutacarpine (98%) were purchased from Shanghai Sunny Biotech Co. Ltd. (Shanghai, China). HPLC-grade acetonitrile, methanol, and formic acid were purchased from Tedia Company Inc (Beijing, China). Ultra-pure water was prepared using a Milli Q-plus system (Billerica, MA, USA). All other reagents were of analytical grade.

Rhizome coptidis (Batch No.: 090720), *Fructus Evodiae Rutaecarpae* (Batch No.: 091217) and *Radix Paeoniae Alba* (Batch No.: 100531) were all from Kangqiao Medicinal Materials Electuary Co., Ltd (Shanghai, PR China). Wuji Pill was prepared according to the Chinese Pharmacopoeia 2010 [1]. *Rhizoma Coptidis* (300 g), *Fructus Evodiae Rutaecarpae* (50 g) and *Radix Paeoniae Alba* (300 g) were ground into a fine powder and extracted three times by refluxing the mixture for an hour using 80% ethanol. The extracts were combined and evaporated to dryness under reduced pressure producing a Wuji Pill for this study.

The blank rat plasma was obtained by drawing blood from vein of rat (Batch No.: scxk 2003-0002, supplier, Slac Laboratory Animal Co., Ltd.) to a heparinized tube. Plasma was separated by centrifugation at 6000 rpm for 5 min and stored at −20 °C.

2.2. Instrument and chromatographic conditions

The LC–MS/MS analyses were carried out with a Shimadzu liquid chromatography system (Shimadzu Corporation, Kyoto, Japan), equipped with two LC-20AD pumps, a SIL-HTC autosampler and an online DGU-20A3 vacuum degasser, and a triple quadrupole tandem mass spectrometer API 3200 (Applied Biosystems/MDS Sciex, Toronto, Canada) equipped with a turbo ion spray source operated in the positive-ion mode detection. The ion spray voltage was set at 5 kV and the source temperature was maintained at 450 °C. The collision energy for jatrorrhizine, berberine, coptisine, palmatine, evodiamine, rutacarpine, paeoniflorin and carbamazepine was set at 32, 41, 40, 32, 35, 45, 27 and 29 V, respectively. Nitrogen was used as the collision gas. The flow rates of the curtain gas, nebulizer gas1 and gas2 were set at 25, 60, 70 L/min, respectively. The operation of the LC–MS/MS and data analysis were performed using the analyst 1.4 software (Applied Biosystems/MDS Sciex, Toronto, Canada).

Liquid chromatography analyses were performed in a gradient elution mode using Shiseido CAPCELL PAK C_{18} column (100 mm × 2.0 mm i.d., 5 μm) coupled with a Phenomenex C_{18} (4.0 mm × 3.0 mm i.d., 5 μm) guard column at room temperature. The mobile phase consisted of water (containing 0.1% formic acid and 10 mmol/L ammonium acetate) and acetonitrile (containing 0.1% formic acid). A linear gradient at a flow rate of 0.3 mL/min consisted of (acetonitrile containing 0.1% formic acid) was run at 5–50% over 0–3 min, 50–80% over 3–4 min, and the composition was maintained at 80% for 1 min and then returned to initial condition. The samples were kept at 4 °C in the auto-sampler, and a volume of 10 μL was injected onto the HPLC system.

Multiple reaction monitoring (MRM) was used to perform mass spectrometric quantification. The MRM analysis was conducted