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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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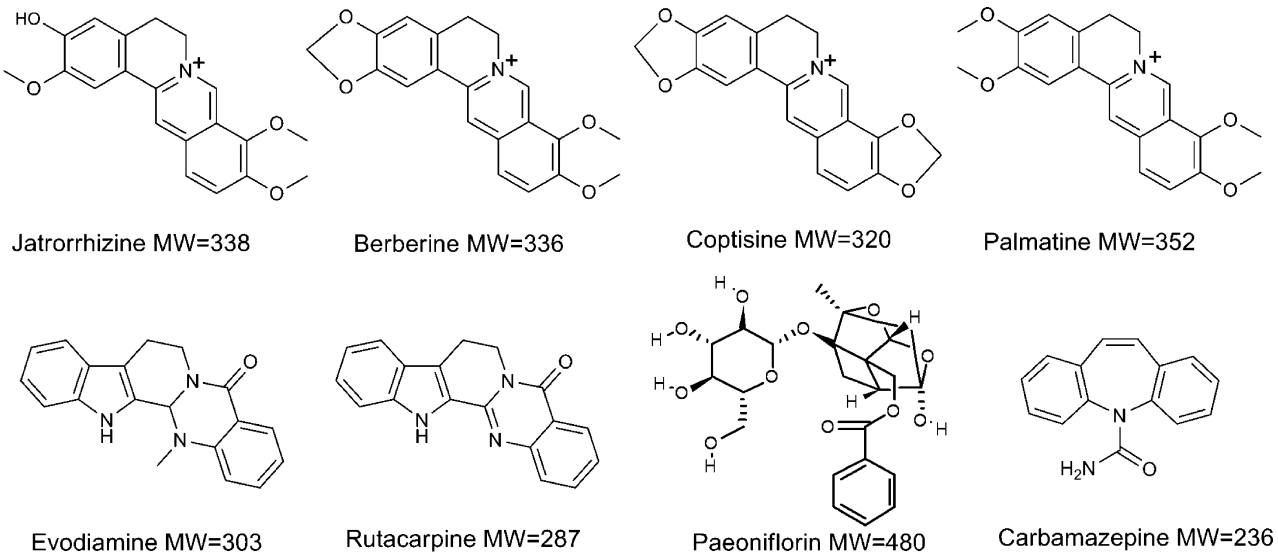

Fig. 1. Chemical structures and molecular weights (MW) of jatrorrhizine, berberine, coptisine, palmatine, evodiamine, rutacarpine, paeoniflorin and carbamazepine (IS).

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

by monitoring the precursor ion to product ion transitions of *m*/*z* 338.4–323.1 for jatrorrhizine, *m*/*z* 336.0–292.1 for berberine, *m*/*z* 320.0–292.2 for coptisine, *m*/*z* 352.2–337.1 for palmatine, *m*/*z* 303.7–134.2 for evodiamine, *m*/*z* 287.9–273.0 for rutacarpine, *m*/*z* 481.2–178.9 for paeoniflorin and *m*/*z* 236.9–193.8 for carbamazepine (IS).

2.3. Preparation of standard and quality control (QC) samples

Stock solutions were separately prepared by dissolving the accurately weighed seven standard reference compounds in methanol. Stock solution I was obtained by mixing all the seven stock solutions above, and giving a final concentration of 1.10 μg/mL for jatrorrhizine, 1.20 μg/mL for berberine, 1.30 μg/mL for coptisine, 1.04 μg/mL for palmatine, 1.19 μg/mL for evodiamine, 1.05 μg/mL for rutacarpine, 2.09 μg/mL for paeoniflorin, respectively. Standard working solutions of chemicals were obtained by serially diluting the stock solution I with acetonitrile. The internal standard solution of carbamazepine was prepared to the concentration of 1.10 μg/mL in methanol.

The analytical standard and quality control (QC) samples were prepared as the following: 10 μL of standard working solution was evaporated to dryness by a gentle stream of nitrogen, and then 100 μL of blank rat plasma was added. The samples were prepared prior to use during validation and pharmacokinetic study. The final calibration concentration ranges were made from 0.42–208 ng/mL to 4.18–418 ng/mL for the seven compounds. The QC samples were prepared at concentrations of 0.44, 2.20, 55.0, 220 ng/mL for jatrorrhizine, 0.48, 2.40, 60.0, 240 ng/mL for berberine, 0.52, 2.60, 65.0, 260 ng/mL for coptisine, 0.42, 2.08, 52.0, 208 ng/mL for palmatine, 2.38, 4.76, 59.5, 238 ng/mL for evodiamine, 2.10, 4.20, 52.5, 210 ng/mL for rutacarpine and 4.18, 8.36, 105, 418 ng/mL for paeoniflorin in drug-free plasma.

2.4. Sample preparation

Rat plasma (100 μL) and 5 μL of IS solution (carbamazepine, 1.10 μg/mL) were placed in an eppendorf tube, and 20 μL of sodium hydroxide (0.5 mol/L) followed by ethyl acetate (1 mL) were added. Extraction was performed by vortex mixing for 3 min at 2500 rpm, and centrifugation for 15,000 rpm for 5 min. The supernatant was transferred into another Eppendorf tube and dried under a flow of nitrogen gas at 37 °C. The residue was re-constituted in 100 μL of mobile phase, and centrifuged (15,000 rpm for 5 min). The supernatant was transferred to an auto-sampler vial with a vial insert (LVI, 150 μL, Waters) and a 10 μL aliquot was injected onto the LC–MS/MS system for analysis.

2.5. Method validation

The method was validated in terms of specificity, calibration curve, sensitivity, matrix effect, accuracy, precision and stability according to the USA Food and Drug Administration (FDA) bioanalytical method validation guidance [28].

2.5.1. Specificity and selectivity

The specificity of the method was tested by comparing the chromatograms of six individual blank rat plasma samples, plasma samples spiked with the analytes and IS, and plasma samples after an oral dose. Blank rat plasma samples were analysed for endogenous interference, followed by spiking with IS for the interference of IS.

2.5.2. Linearity and lower limits of quantification (LLOQ)

Calibration curves were obtained by plotting the measured peak area ratios of analytes to IS. Standard curves representing peak area ratios *versus* analyte concentrations were described in the form of $y = a + bx$ (weighing factor $1/x$). The LLOQ for the analytes were the lowest concentrations with signal-to-noise ≥10, which could be quantitatively determined with precision and accuracy were less than or equal to 20%, evaluated by analyzing samples in six replicates. The lower limit of detection (LLOD) was defined as the amount that could be detected with a signal-to-noise ratio ≥3.

2.5.3. Precision and accuracy

The intra-day and inter-day precision and accuracy were carried out through quantifying four concentration levels of QC samples (six samples for each concentration level) on the same day and on three consecutive validation days, respectively. The precision was evaluated by relative standard deviation (RSD%) and accuracy by (mean measured concentration/spiked concentration) × 100%.

2.5.4. Recovery and matrix effect

The extraction recoveries of analytes at three QC levels were determined by comparing the response obtained from six extracted QC samples with those obtained from pure reference standards spiked in post-extracted blank rat plasma at the same concentrations. The matrix effects were evaluated by comparing the peak areas obtained from samples where the extracted matrix was spiked with standard solutions to those obtained from the pure reference standards solutions at the same concentration.

2.5.5. Stability experiments

The post-preparation stability was tested by determining the extracted QC samples stored in the auto-sampler (4 °C) for 24 h. The freeze and thaw stability was determined after three freeze-thaw cycles (−70 to 20 °C). Long-term stability in rat plasma stored at −70 °C was studied for a period of one month employing QC samples at three different levels.

2.6. Method application: animals, drug administration and plasma collection

Sprague-Dawley male rats ($n = 6$), weighting 180–200 g, were obtained from Laboratory Animal Centre of Shanghai University of TCM. They were kept in a controlled environment at 23 °C and 55% relative humidity under a 12 h dark–light cycle, with free access to the standard laboratory food and water. All studies on animals were in accordance with the guidelines of the Committee on the Care and Use of Laboratory Animals in China. After a 12 h fast prior to the experiment, the rats were orally administrated with Wuji Pill (in which the concentrations of jatrorrhizine, berberine, coptisine, palmatine, evodiamine, rutacarpine and paeoniflorin were 4.00, 8.30, 6.70, 6.31, 0.17, 0.11, 2.02 mg/g, respectively) at a dose of 15 g/kg. Blood samples were collected from the suborbital veniplex before intragastric gavage and at 0.083, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24 h after dosing. The rats had free access to water during the experiment. The blood samples were immediately transferred to heparinized tubes, and centrifuged at 6000 rpm for 5 min, and the supernatant was transferred into 1.5 mL Eppendorf tubes and stored at −20 °C prior to analysis.

3. Results and discussion

3.1. Optimization of the mass spectrometry conditions

The chemical structures and molecular weights of jatrorrhizine, berberine, coptisine, palmatine, evodiamine, rutacarpine, paeoniflorin and carbamazepine (internal standard) were shown in Fig. 1. From the positive ESI spectra, the peak attributed to the protonated molecule $[M+H]^+$ was observed for all of the analytes. By manual optimization using infusion with a syringe pump, the most suitable

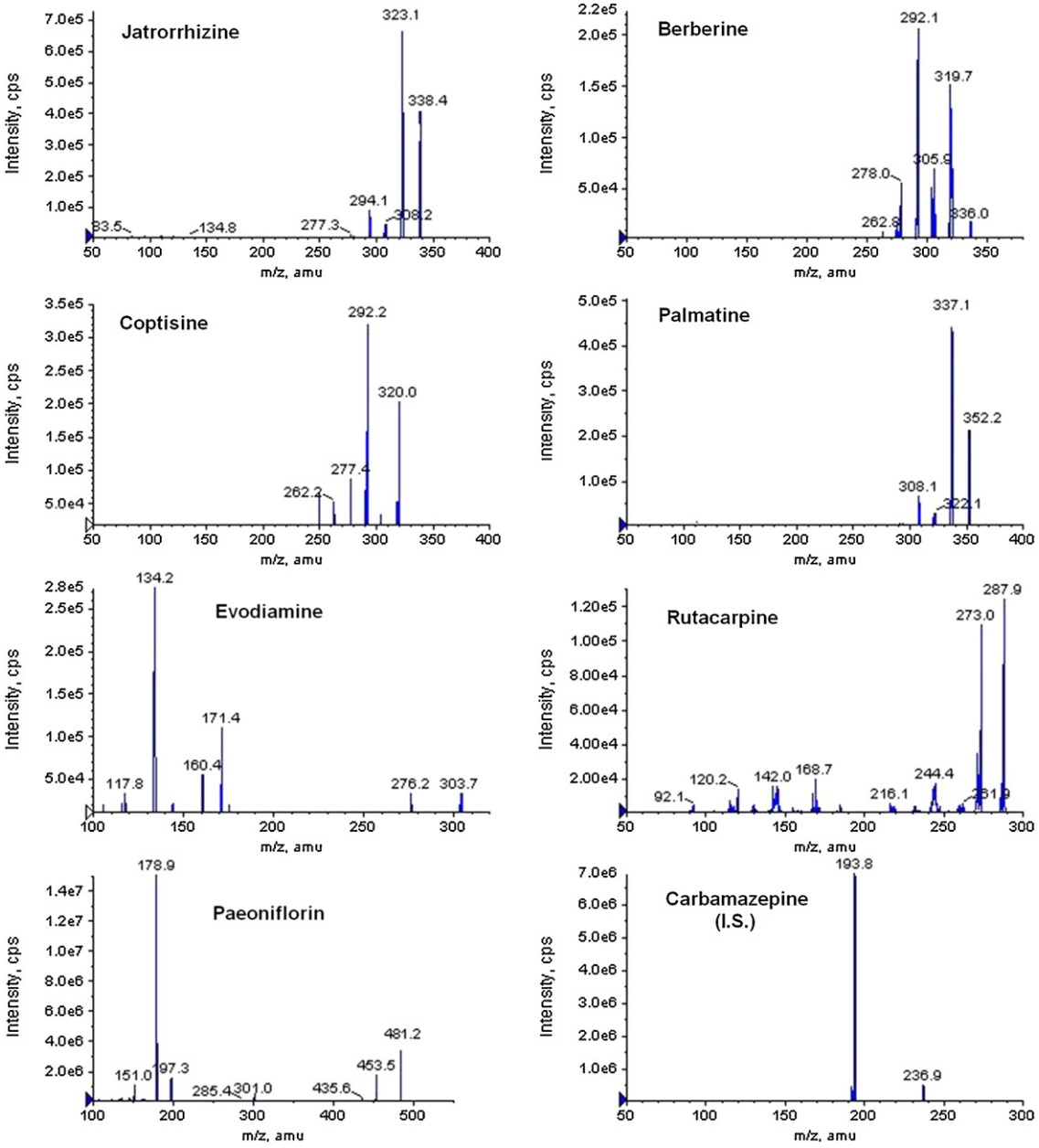

Fig. 2. Electrospray ion MS/MS spectra of jatrorrhizine, berberine, coptisine, palmatine, evodiamine, rutacarpine, paeoniflorin and carbamazepine (IS).

ion transitions and collision energy with MRM for all target analytes were selected. As shown in Fig. 2, those precursor and product ions were *m*/*z* 338.4/323.1 for jatrorrhizine, *m*/*z* 336.0/292.1 for berberine, *m*/*z* 320.0/292.2 for coptisine, *m*/*z* 352.2/337.1 for palmatine, *m*/*z* 303.7/134.2 for evodiamine, *m*/*z* 287.9/273.0 for rutacarpine, *m*/*z* 481.2/178.9 for paeoniflorin and *m*/*z* 236.9/193.8 for carbamazepine (IS).

3.2. Chromatography conditions

To achieve symmetric peak shape as well as a short run time for the simultaneous analysis of the seven compounds, the chromatographic conditions were optimized through trials and errors. Formic acid with the concentration of 0.1% and ammonium acetate with the concentration of 10 mmol/L were found to be the best as mobile phase to balance the peak shape, sensitivity and retention time of each analyte.

Under the developed chromatographic conditions for simultaneous determination of the seven compounds of Wuji Pill, all analytes were eluted rapidly within 7 min (Fig. 3B and C). The retention time of jatrorrhizine, berberine, coptisine, palmatine, evodiamine, rutacarpine, paeoniflorin and IS were 4.57, 4.88, 4.64, 4.79, 6.16, 6.33, 3.96 and 5.29 min, respectively.

3.3. Optimization of the extraction procedure

One obstacle for multiple components analyses in a biological matrix was the sample preparation owing to their different properties of dissolution, pK_as, stabilities as well as their concentrations in the biological matrix. In order to extract all the analytes and the IS with high recovery and no endogenous interference at the retention time, both protein precipitation and liquid–liquid extraction method were investigated. For protein precipitation, three types of precipitation reagents (methanol, acetonitrile, and methanol, acetonitrile with perchloric acid) were tried, but none of them could

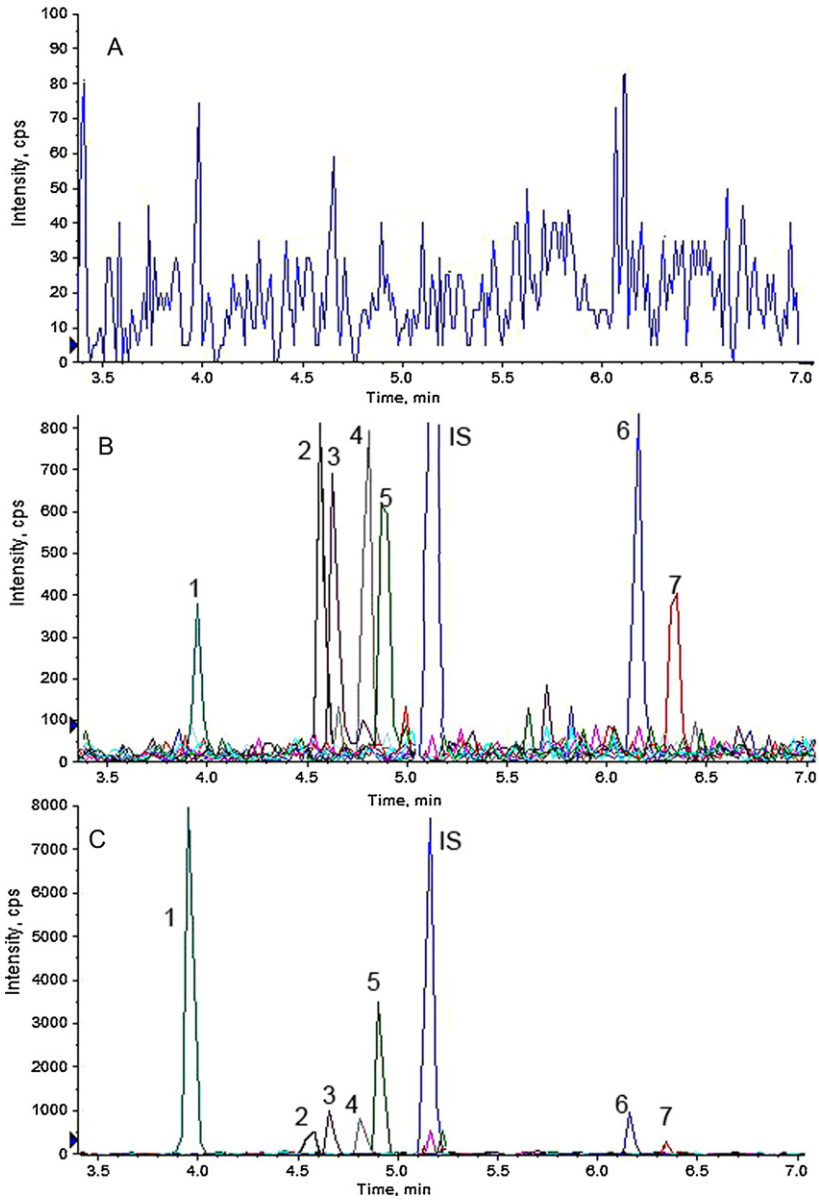

Fig. 3. Chromatograms of the seven compounds in plasma: (A) blank plasma; (B) blank plasma spiked with the seven components in LLOQ: (1) paeoniflorin (4.18 ng/mL), (2) jatrorrhizine (0.44 ng/mL), (3) coptisine (0.52 ng/mL), (4) palmatine (0.42 ng/mL), (5) berberine (0.48 ng/mL), (6) evodiamine (2.38 ng/mL), (7) rutacarpine (2.10 ng/mL), IS: carbamazepine (55.0 ng/mL); and (C) plasma sample obtained 1 h after intragastric administration of Wuji Pill (15 g/kg): (1) paeoniflorin (174.8 ng/mL), (2) jatrorrhizine (12.44 ng/mL), (3) coptisine (11.30 ng/mL), (4) palmatine (25.79 ng/mL), (5) berberine (84.68 ng/mL), (6) evodiamine (21.22 ng/mL), (7) rutacarpine (3.1 ng/mL), IS: carbamazepine (55.0 ng/mL).

result in high sensitivity for the analytes, especially for the paeoniflorin (LLOQ > 50 ng/mL). For liquid–liquid extraction, solvents such as ethyl acetate, diethyl ether and *n*-butanol were attempted, and only ethyl acetate gave the best extraction efficiency for all the analytes and IS.

3.4. Method validation

3.4.1. Selectivity and specificity

Fig. 3A shows the chromatogram of a blank plasma sample showing no endogenous peaks at the retention positions of jatrorrhizine, berberine, coptisine, palmatine, evodiamine, rutacarpine, paeoniflorin and IS. Matrix effect for these analytes was insignificant as demonstrated in Fig. 3B. The seven analytes were clearly detected in the rat plasma after intragastric administration of Wuji Pill using the developed HPLC conditions (Fig. 3C).

3.4.2. Linearity and lower limits of quantification (LLOQ)

Calibration curves which constructed with a linear regression with $1/x$ weighting were shown in Table 1. They all exhibited good linearity. The LLOQs of jatrorrhizine, berberine, coptisine and palmatine were 0.44, 0.48, 0.52, 0.42 ng/mL respectively, and evodiamine and rutacarpine were 2.38, 2.10 ng/mL respectively, and paeoniflorin was 4.18 ng/mL.

3.4.3. Precision and accuracy

Precision and accuracy of the developed method were validated by intra- and inter-day variability assays. Data were obtained by assaying the QC samples at four concentration levels in plasma (Table 2). The intra- and inter-day precision (RSD) ranged 3.2–14.8% and 9.1–14.8%, respectively. The accuracy derived from QC samples was between 80.91 and 115.1% for all the QC levels of all analytes.

Table 1
Regression data and LLOQ of jatrorrhizine, berberine, coptisine, palmatine, evodiamine, rutacarpine, paeoniflorin.

Compounds	Range (ng/mL)	Linear regression equation	Correlation coefficient	LLOQ (ng/mL)
Jatrorrhizine	0.44–220.0	$y = 0.0095x + 0.00712$	0.9961	0.44
Berberine	0.48–240.0	$y = 0.0098x + 0.00654$	0.9963	0.48
Coptisine	0.52–260.0	$y = 0.0043x + 0.00173$	0.9973	0.52
Palmatine	0.42–208.0	$y = 0.0128x - 0.00156$	0.9984	0.42
Evodiamine	2.38–238.0	$y = 0.0135x + 0.00985$	0.9957	2.38
Rutacarpine	2.10–210.0	$y = 0.0027x + 0.00339$	0.9931	2.10
Paeoniflorin	4.18–418.0	$y = 0.0010x + 0.00038$	0.9985	4.18

These results indicate that the present method has an acceptable accuracy and precision for working in biological media.

3.4.4. Recovery and matrix effect

The extraction recoveries and matrix effects of all seven analytes under the liquid–liquid extraction conditions with ethyl acetate from rat plasma were summarized in Table 3. The recovery of the QC samples of three concentration levels was in the range of 73.59–109.7% and IS was (96.20 ± 8.7%). The matrix effects derived from QC samples was between 79.17% and 119.1% and IS was (95.88 ± 7.5%). The analytes and IS did not exhibit any matrix effects in rat plasma.

3.4.5. Stability experiments

The stability of the analytes during the sample processing procedures and storing was evaluated by analysis three levels of QC samples. The results are shown in Table 4. The results indicated that these analytes in rat plasma were all stable for one-month storage at −70 °C, 24 h in the auto-sampler (4 °C) and three freeze-thaw cycles with accuracy in range of 86.47–108.8%.

3.5. Application

The method described above could be applied to pharmacokinetic study on multiple components and quality control of Wuji Pill. The assay was proved to be sensitive enough for the determination of these analytes except rutacarpine (for its concentrations were lower than the LLOQ in most of the time points) in rat plasma after oral administration of Wuji Pill (Fig. 4). Table 5 shows the estimated pharmacokinetic parameters, the concentration–time courses of jatrorrhizine, berberine, coptisine, palmatine and evodiamine were adequately described by a one-compartment open model, only paeoniflorin was on a two-compartment model. The C_{max} values for jatrorrhizine, berberine, coptisine, palmatine, evodiamine, paeoniflorin were 18.62, 89.57, 33.33, 25.80, 21.22, 210.6 ng/mL, respectively. The mean AUC_{0-t} values for jatrorrhizine, berberine, coptisine, palmatine, evodiamine, paeoniflorin were 61.36, 253.5, 106.5, 81.90, 52.81, 1223.3 μg h/L, respectively. The C_{max} values for jatrorrhizine, berberine, coptisine and palmatine in plasma after oral administration of *Coptidis Rhizoma* were 0.84, 11.39, 1.52 and 1.74 ng/mL, respectively [29]. Interestingly, the C_{max} values for

Table 2
Precision and accuracy of jatrorrhizine, berberine, coptisine, palmatine, evodiamine, rutacarpine, paeoniflorin in rat plasma QC samples.

Analytes	Spiked concentration (ng/mL)	Intra-day ($n = 6$)			Inter-day ($n = 18$)		
		Concentration measured (ng/mL)	Precision (RSD, %)	Accuracy (%)	Concentration measured (ng/mL)	Precision (RSD, %)	Accuracy (%)
Jatrorrhizine	0.44	0.39 ± 0.04	11.3	89.39	0.44 ± 0.06	14.2	99.17
	2.20	2.08 ± 0.17	8.4	94.32	2.21 ± 0.24	10.9	100.4
	55.00	60.4 ± 6.9	11.5	109.9	57.54 ± 6.1	10.6	104.6
	220.0	240.3 ± 19.3	8.0	109.2	226.5 ± 28.0	12.4	103.0
Berberine	0.48	0.45 ± 0.06	13.5	93.31	0.48 ± 0.07	13.8	100.5
	2.40	1.94 ± 0.13	6.9	80.91	2.16 ± 0.27	12.6	90.13
	60.00	66.6 ± 5.1	7.7	111.0	61.32 ± 7.8	12.7	102.2
	240.0	270.7 ± 8.8	3.2	112.8	251.9 ± 27.1	10.7	105.0
Coptisine	0.52	0.53 ± 0.07	13.7	102.0	0.50 ± 0.05	10.9	96.18
	2.60	2.19 ± 0.20	9.1	84.09	2.47 ± 0.34	13.9	94.89
	65.00	74.8 ± 8.8	11.8	115.1	68.30 ± 8.4	12.3	105.1
	260.0	289.5 ± 28.8	10.0	111.3	275.7 ± 27.5	10.0	106.0
Palmatine	0.42	0.45 ± 0.02	3.8	108.0	0.42 ± 0.04	10.0	100.7
	2.08	1.98 ± 0.19	9.4	95.17	2.10 ± 0.23	11.0	100.8
	52.00	53.77 ± 2.9	5.4	103.4	53.01 ± 5.5	10.4	101.9
	208.0	211.5 ± 11.4	5.4	101.7	204.9 ± 20.4	10.0	98.53
Evodiamine	2.38	2.31 ± 0.25	10.8	97.11	2.42 ± 0.24	9.8	101.7
	4.76	4.66 ± 0.56	12.0	97.91	4.76 ± 0.59	12.5	99.92
	59.50	64.7 ± 4.5	6.9	108.7	60.50 ± 6.7	11.0	101.7
	238.0	244.8 ± 12.8	5.2	102.9	250.0 ± 22.8	9.1	105.1
Rutacarpine	2.10	1.86 ± 0.22	11.7	88.62	2.04 ± 0.24	11.8	97.08
	4.20	3.47 ± 0.45	13.1	82.71	3.94 ± 0.58	14.8	93.75
	52.50	58.2 ± 3.4	5.8	110.9	55.00 ± 5.9	10.7	104.8
	210.0	209.2 ± 11.9	5.7	99.64	203.5 ± 19.2	9.4	96.89
Paeoniflorin	4.18	4.05 ± 0.55	13.6	96.93	4.28 ± 0.48	11.1	102.4
	8.36	8.58 ± 0.65	7.6	102.7	8.46 ± 1.07	12.6	101.1
	104.5	106.5 ± 15.8	14.8	101.5	99.17 ± 13.1	13.2	94.44
	418.0	462.4 ± 35.9	7.8	110.6	440.9 ± 41.4	9.4	105.5

Table 3
Matrix effects and recovery of jatrorrhizine, berberine, coptisine, palmatine, evodiamine, rutacarpine, paeoniflorin in rat plasma.

Analytes	Spiked concentration (ng/mL)	Matrix effect (%, mean ± SD, $n = 6$)	Recovery (%, mean ± SD, $n = 6$)
Jatrorrhizine	2.20	111.2 ± 9.1	78.4 ± 6.0
	55.00	93.6 ± 8.6	106.8 ± 9.8
	220.0	97.2 ± 5.5	73.6 ± 5.6
Berberine	2.40	79.2 ± 6.4	107.3 ± 3.7
	60.00	95.4 ± 4.1	99.8 ± 3.3
	240.0	94.8 ± 8.5	109.7 ± 5.7
Coptisine	2.60	103.2 ± 6.6	82.2 ± 6.4
	65.00	116.7 ± 6.8	82.5 ± 7.2
	260.0	104.2 ± 11.5	77.0 ± 8.5
Palmatine	2.08	105.2 ± 13.0	80.0 ± 4.9
	52.00	119.1 ± 9.6	74.4 ± 4.6
	208.0	86.3 ± 4.0	85.5 ± 5.0
Evodiamine	4.76	108.4 ± 4.4	78.4 ± 5.1
	59.50	94.9 ± 7.3	102.9 ± 4.0
	238.0	99.8 ± 7.5	78.4 ± 3.8
Rutacarpine	4.20	103.1 ± 5.4	85.0 ± 4.8
	52.50	96.7 ± 6.3	100.5 ± 6.7
	210.0	103.0 ± 9.9	78.1 ± 3.8
Paeoniflorin	8.36	82.7 ± 4.5	109.3 ± 13.4
	104.5	84.6 ± 7.0	109.5 ± 13.0
	418.0	86.1 ± 6.4	92.2 ± 9.1

Table 4
Stability of jatrorrhizine, berberine, coptisine, palmatine, evodiamine, rutacarpine, paeoniflorin in rat plasma QC samples.

Analytes	Spiked concentration (ng/mL)	Storage at −70 °C for 1 month		Autosampler 4 °C for 24 h		Three-thaw cycles	
		Measured concentration (ng/mL)	Accuracy (%)	Measured concentration (ng/mL)	Accuracy (%)	Measured concentration (ng/mL)	Accuracy (%)
Jatrorrhizine	2.20	2.31 ± 0.20	105.0	2.29 ± 0.17	104.1	2.34 ± 0.19	106.4
	55.00	56.1 ± 7.0	102.0	56.1 ± 5.9	102.0	51.07 ± 5.2	92.85
	220.0	210.6 ± 23.1	95.71	225.2 ± 29.6	102.4	211.0 ± 25.4	95.91
Berberine	2.40	2.38 ± 0.31	99.13	2.29 ± 0.27	95.48	2.52 ± 0.32	105.0
	60.00	58.9 ± 7.8	98.13	59.3 ± 6.7	98.75	63.39 ± 9.2	105.7
	240.0	254.4 ± 9.7	106.0	224.8 ± 30.0	93.68	220.3 ± 11.2	91.78
Coptisine	2.60	2.46 ± 0.27	94.73	2.29 ± 0.27	88.14	2.46 ± 0.22	102.7
	65.00	68.7 ± 6.9	105.6	59.3 ± 6.7	91.16	64.04 ± 6.5	98.52
	260.0	236.2 ± 28.0	90.85	224.8 ± 30.0	86.47	250.4 ± 21.1	96.32
Palmatine	2.08	2.02 ± 0.17	97.31	2.17 ± 0.21	104.3	1.92 ± 0.20	92.21
	52.00	50.3 ± 6.8	96.59	49.5 ± 6.1	95.17	47.78 ± 4.1	91.88
	208.0	188.3 ± 13.1	90.55	195.7 ± 22.7	94.08	198.0 ± 17.1	95.18
Evodiamine	4.76	4.82 ± 0.54	101.2	4.72 ± 0.51	99.11	4.72 ± 0.51	99.11
	59.50	55.5 ± 4.7	93.24	55.48 ± 4.73	93.24	59.58 ± 6.0	100.1
	238.0	259.0 ± 28.8	108.8	258.9 ± 28.8	108.8	236.9 ± 32.2	99.52
Rutacarpine	4.20	4.41 ± 0.43	105.0	4.38 ± 0.33	104.3	4.02 ± 0.42	95.77
	52.50	54.4 ± 7.3	103.5	51.8 ± 7.1	98.60	56.09 ± 5.6	106.8
	210.0	189.4 ± 15.6	90.20	217.5 ± 23.0	103.6	217.6 ± 10.6	103.6
Paeoniflorin	8.36	7.72 ± 0.87	92.34	8.64 ± 0.87	103.4	8.52 ± 1.18	101.9
	104.5	101.7 ± 12.2	96.86	95.7 ± 7.5	91.18	101.0 ± 13.1	96.15
	418.0	433.4 ± 46.6	103.7	423.0 ± 54.3	101.2	431.5 ± 58.4	103.2

Table 5
Phamacokinetic parameters of jatrorrhizine, berberine, coptisine, palmatine, evodiamine, paeoniflorin after i.g. of Wuji Pill (15 g/kg) to rats ($n = 6$).

Parameter	Jatrorrhizine	Berberine	Coptisine	Palmatine	Evodiamine	Peoniflorin
$t_{1/2}\alpha$ (h)	2.17 ± 0.23	3.08 ± 1.14	5.15 ± 1.63	3.07 ± 0.84	2.44 ± 0.42	1.00 ± 0.45
$t_{1/2}\beta$ (h)	–	–	–	–	–	6.29 ± 2.83
AUC_{0-t} (μg h/L)	61.36 ± 9.74	253.50 ± 70.79	106.46 ± 29.76	81.90 ± 16.70	52.81 ± 8.23	1223.30 ± 153.65
t_{max} (h)	1.42 ± 0.20	0.92 ± 0.20	2.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.50 ± 0.00
C_{max} (μg/L)	18.62 ± 1.43	89.57 ± 16.64	33.33 ± 4.30	25.80 ± 3.27	21.22 ± 4.35	210.59 ± 34.30
CL/F (L/h/kg)	203.66 ± 33.57	61.49 ± 14.37	142.98 ± 51.26	186.48 ± 36.79	287.49 ± 53.21	11.45 ± 1.48
MRT_{0-t} (h)	5.40 ± 0.62	5.10 ± 0.43	5.88 ± 1.27	4.89 ± 0.41	6.91 ± 0.94	6.88 ± 0.70

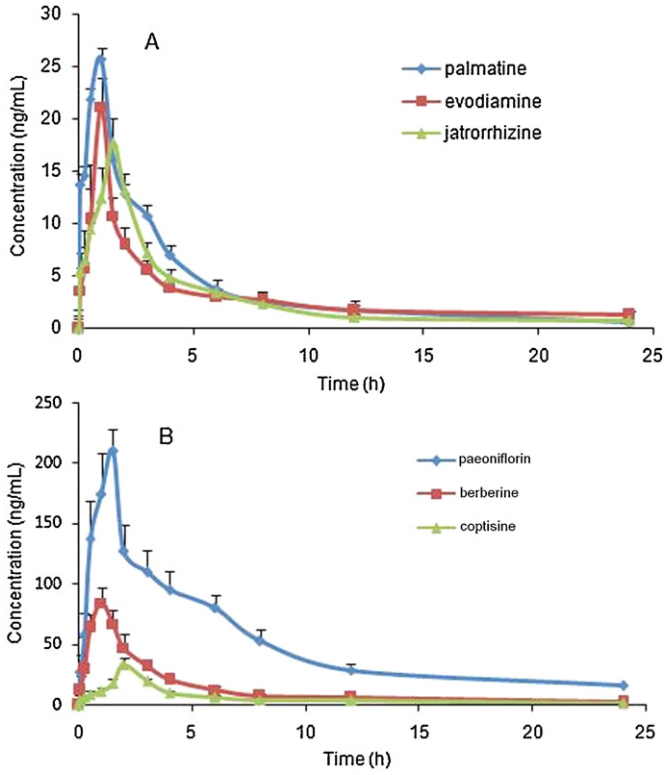

Fig. 4. Mean pharmacokinetic profiles of jatrorrhizine (A, ▲), berberine (B, ■), coptisine (B, ▲), palmatine (A, ♦), evodiamine (A, ■), paeoniflorin (B, ♦) after oral administration of Wuji Pill (15 g/kg).

jatrorrhizine, berberine and palmatine in plasma after oral administration of Coptis-Evodia powder (6:1, g/g) were 219.9, 121.1 and 35.2 ng/mL, respectively [24]. The C_{max} values for paeoniflorin after oral administration of *Radix Paeoniae Alba* was 1460 ng/mL [30]. These huge variations of C_{max} values demonstrate the complexity of bioavailability of these active components in different herbal formulations and the dosage.

4. Conclusions

In this study, we have successfully developed a method using carbamazepine as an internal standard for the analyses of Wuji Pill using LC–MS/MS in rat plasma. The sensitivity and precision of this method allows accurate measurement of jatrorrhizine, berberine, coptisine, palmatine, evodiamine, rutacarpine, paeoniflorin concentrations in plasma. We propose that this method could be useful to determine these compounds in Chinese medicines containing the herbs, *Rhizoma Coptidis*, *Fructus Evodiae Rutaecarpae* and *Radix Paeoniae Alba*.

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