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A sensitive and specific liquid chromatography mass spectrometry method for simultaneous determination of berberine, palmatine, coptisine, epiberberine and jatrorrhizine from *Coptidis Rhizoma* in rat plasma

Sen Yu, Xiaoyan Pang, Yuanxiong Deng, Li Liu, Yan Liang, Xiaodong Liu *, Lin Xie, Guangji Wang, Xinting Wang

Key laboratory of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, Nanjing 210009, PR China

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Abstract

A sensitive and specific liquid chromatography–electrospray ionization-mass spectrometry (LC–ESI-MS) method has been developed and validated for the identification and quantification of five protoberberine alkaloids, which are berberine, palmatine, coptisine, epiberberine and jatrorrhizine, in rat plasma using tetrahydroberberine as an internal standard. Following solid-phase extraction, the analytes were separated by linear gradient elution on a Shim-pack ODS (4.6 μm, 150 mm × 2.0 mm i.d.) column and analyzed in selected ion monitoring (SIM) mode with a positive electrospray ionization (ESI) interface using the respective $[M]^+$ and $[M+H]^+$ ions, $[M]^+ = 336$ for berberine; 320 for coptisine; 336 for epiberberine; 338 for jatrorrhizine; 352 for palmatine and $[M+H]^+ = 340$ for the internal standard. The method was validated over the concentration range of 0.31–20 ng mL^{-1} for all the five protoberberine alkaloids. Within-batch and between-batch precisions (R.S.D.%) were all within 15% and accuracy (%Er) ranged from −5 to 5%. The lower limits of quantification were 0.31 ng mL^{-1} for all analytes. The extraction recoveries were on average 80.8% for berberine, 67.0% for coptisine, 66.2% for epiberberine, 71.8% for jatrorrhizine and 73.2% for palmatine. The validated method was used to study the pharmacokinetic profile of the five protoberberine alkaloids in rat plasma after oral administration of *Coptidis Rhizoma* extract.

Keywords: Liquid chromatography–mass spectrometry; Protoberberine alkaloid; Rat plasma; Pharmacokinetics; *Coptidis Rhizoma*

1. Introduction

Coptidis Rhizoma (Huanglian), a widely used Traditional Chinese Medicine, has been used for centuries for the treatment of dysentery, hypertension, inflammation, and liver diseases [1,2]. It is derived from the dried rhizome of ranunculaceous plant such as *Coptis chinensis* Franch., *C. deltoidea* C.Y. Cheng et Hsiao. or *C. teeta* Wall., and is known to contain berberine, palmatine, coptisine, epiberberine and jatrorrhizine (structures shown in Fig. 1) which are all protoberberine alkaloids as its major bioactive components [3–11].

In most previous investigations, as one of the main components, berberine has been reported on the pharmacokinetics in blood, urine, bile and tissues of rats, mice, rabbits, dogs, and humans using non-specific UV spectrophotometric, fluorometric, tritium-labeled berberine, gas chromatography–chemical ionization mass spectrometry and HPLC methods [12–20]. Only a few studies regarding the pharmacokinetics of other protoberberine alkaloids such as, coptisine, dehydroapocavidine and tetradehydroscoulerine, have been carried out [21]. In our previous study, we have developed a liquid chromatography–electrospray ionization-mass spectrometry (LC–ESI-MS) method to determine berberine and palmatine in rat plasma after oral administration of Huang-Lian-Jie-Du decoction [22]. There is merit in characterizing the pharmacokinetics of five active alkaloids from *C. Rhizoma* extract in animals since there was no detailed pharmacokinetic profiles for simultaneous characterization of these alkaloids.

* Corresponding author at: Key Laboratory of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, Nanjing 210009, PR China. Tel.: +86 25 83271006; fax: +86 25 85306750.
E-mail address: xdliu@cpu.edu.cn (X. Liu).

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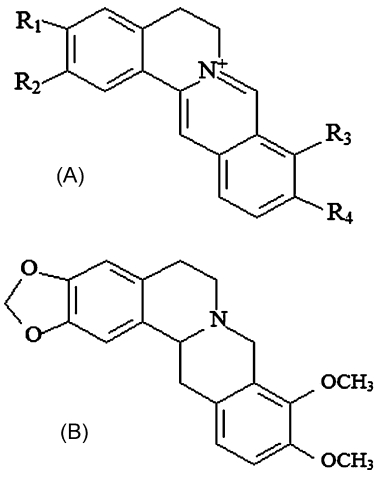

	R_1	R_2	R_3	R_4
berberine	-O-CH_2-O-		-OCH_3	-OCH_3
coptisine	-O-CH_2-O-		-O-CH_2-O-	
epiberberine	-OCH_3	-OCH_3	-O-CH_2-O-	
jatrorrhizine	-OH	-OCH_3	-OCH_3	-OCH_3
palmatine	-OCH_3	-OCH_3	-OCH_3	-OCH_3

Fig. 1. Chemical structures of berberine, coptisine, epiberberine, jatrorrhizine, palmatine (A) and internal standard tetrahydroberberine (B).

In this paper, a sensitive and selective method of liquid chromatography–electrospray ionization-mass spectrometry (LC–ESI-MS) is presented for the simultaneous determination of berberine, palmatine, coptisine, epiberberine and jatrorrhizine in rat plasma. This assay has been successfully applied to a pharmacokinetic study of the five protoberberine alkaloids after oral administration of *C. Rhizoma* extract in rats.

2. Experimental

2.1. Material and methods

2.1.1. Herbal materials

C. Rhizoma (*C. chinensis* Franch.) was purchased from Kaixin Pharmacy (Nanjing, PR China) and identified by Dr. Lina Chen (Department of Pharmacognosy, China Pharmaceutical University, Nanjing, PR China).

2.1.2. Standards and reagents

The reference standards of berberine, palmatine and jatrorrhizine were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, PR China). The reference standards of coptisine and epiberberine, were kind gifts given by Prof. Hao Zhang (West China School of Pharmacy, Sichuan University, Chengdu, PR China). The internal standard (IS), tetrahydroberberine (structure in Fig. 1), was kindly provided by Dr. Can Zhang (Department of Medicinal Chemistry, China Pharmaceutical University, Nanjing, PR China). Solid-phase extraction (SPE) columns (ODS C18, 100 mg) were obtained from Agela (Beijing, PR China). Acetonitrile was of chromatographic grade (Fisher Company Inc., USA). All other reagents were of analytical grade. Milli-Q water (Millipore, Bedford, MA) was used throughout the study.

2.1.3. Preparation of C. Rhizoma extract

C. Rhizoma (1000 g) were extracted twice by refluxing with boiling water (1:10 and then 1:5, w/v) for 1 h, and the solution obtained was concentrated to give an extract (256 g). The dried powder was stored at 4 °C before use.

2.1.4. Contents of five protoberberine alkaloids in C. Rhizoma extract

To calculate the administered dose, the contents of five protoberberine alkaloids in the extract were quantitatively determined. The HPLC analysis of these alkaloids was a modified version of a previously published method [23]. The contents of berberine, coptisine, epiberberine, jatrorrhizine and palmatine were 18.64 ± 0.61, 2.78 ± 0.24, 2.87 ± 0.26, 2.61 ± 0.20 and 3.54 ± 0.14 g $100\,g^{-1}$ extract, respectively.

2.1.5. Liquid chromatographic and mass spectrometric conditions

The LC–MS system consisted of a Shimadzu LC-10AD HPLC series liquid chromatograph and a Shimadzu LC–MS2010A single quadrupole mass spectrometer equipped with an electrospray ionization (ESI) interface and a Q-array-Octapole-Quadrupole mass analyzer. Shimadzu LCMSsolution Version 2.04 was used for data acquisition and processing.

LC separation was achieved using a Shim-pack ODS (4.6 μm, 150 mm × 2.0 mm i.d. Shimadzu) column maintained at 40 °C. The mobile phase consisted of A (0.08% formic acid and 2 mmol L^{-1} ammonium acetate) and B (acetonitrile) with linear gradient elution. The gradient cycle consisted of an initial 3 min isocratic segment (70% A and 30% B). Then, the linear gradient was started, increasing solvent B to 80% within 0.5 min and maintained from 3.5 to 5.5 min. After changing back to 30% solvent B at 6 min, the mobile phase gradient was maintained at this composition from 6 to 8 min for column equilibration. The flow rate was 0.2 mL min^{-1} during the whole gradient cycle.

The effluent from the HPLC column was directed into the ESI probe. Mass spectrometer conditions were optimized to obtain maximal sensitivity. The curve dissolution line (CDL) temperature and the block temperature were maintained at 250 and 200 °C, respectively. The probe voltage (capillary voltage), CDL voltage and detector voltage were fixed at 4.5 kV, −10 V and 1.6 kV, respectively. Vacuum was obtained by a Turbo molecular pump (Edwards 28, UK). Nitrogen (99.995%, Nanjing University, PR China) was used as the source of nebulizer gas (1.5 L min^{-1}) and drying gas (curtain gas) (4.0 L min^{-1}). Ana-

lytes were quantitated in selected ion monitoring (SIM) mode. $[M]^+ = 336$ for berberine; 320 for coptisine; 336 for epiberberine; 338 for jatrorrhizine; 352 for palmatine and $[M + H]^+ = 340$ for IS were selected as detecting ions. Mass spectra were obtained at a dwell time of 0.2 s in SIM and 1 s in scan mode.

2.1.6. Standard and quality control sample preparation

Stock solutions containing 1 mg mL^{-1} of each reference compound were prepared in methanol and stored at 4 °C until use. Working solutions, ranging from 3.12 to 200 ng mL^{-1}, were prepared by serial dilution with methanol. A solution containing 200 ng mL^{-1} IS was also prepared in methanol.

The samples for standard calibration curves were prepared by spiking the blank rat plasma (100 μL) with 10 μL of the appropriate working solutions to yield the following concentrations: 0.31, 0.625, 1.25, 2.5, 5, 10 and 20 ng mL^{-1}. Quality control (QC) samples were prepared from blank plasma at concentrations of 0.625, 2.5 and 10 ng mL^{-1}.

2.1.7. Solid phase extraction

A 0.1 mL sample of plasma was combined with 10 μL of the internal standard working solution and vortexed for 30 s. SPE columns were preconditioned with 2 mL of methanol and 1 mL of deionized water. Each sample was loaded onto an SPE column and washed with 1 mL deionized water. Analytes were eluted twice with 1 mL of methanol.

The eluant was evaporated to dryness under nitrogen in a 45 °C water bath. The residues were then reconstituted in 100 μL mobile phase followed by centrifugation at 20,000 rpm for 10 min. An aliquot of 10 μL was injected into the LC–MS system.

2.2. Method validation

The method validation assays were carried out according to the currently accepted U.S. Food and Drug Administration (FDA) bioanalytical method validation guidance [24].

2.2.1. Assay specificity

The specificity of the method was evaluated by analyzing blank plasma samples from six rats. Each blank sample was tested for interference using the proposed extraction procedure and chromatographic–mass spectroscopic conditions and compared with those obtained with an aqueous solution of the analyte at a concentration near the lower limit of quantification (LLOQ).

The matrix effect on the ionization of the analytes was evaluated by comparing the peak areas of the analytes resolved in the blank sample (the final solution of blank plasma after extraction and reconstitution) with that resolved in the mobile phase. Three different concentration levels of berberine, coptisine, epiberberine, jatrorrhizine and palmatine (0.625, 2.5 and 10 ng mL^{-1}) and 20 ng mL^{-1} of the IS were evaluated by analyzing the five samples at each level. The blank plasmas used in this study were six different batches of blank rat plasmas. If the ratio is <85% or >115%, an exogenous matrix effect is implied.

2.2.2. Linearity

Calibration curves of seven concentrations of berberine, coptisine, epiberberine, jatrorrhizine and palmatine ranging from 0.31 to 20.0 ng mL^{-1} were extracted and assayed. Blank plasma samples were analyzed to confirm the absence of interferences but were not used to construct the calibration function. The lower limit of detection (LLOD) and the LLOQ were determined as the concentrations at signal-to-noise ratios of 3 and 10, respectively.

2.2.3. Precision and accuracy

The precision of the assay was determined from the QC plasma samples by replicate analyses of three concentration levels of berberine, coptisine, epiberberine, jatrorrhizine and palmatine (0.625, 2.5 and 10 ng mL^{-1}). Within-batch precision and accuracy were determined by repeated analyses of the group of standards on one batch ($n = 5$). Between-batch precision and accuracy were determined by repeated analyses on three consecutive days ($n = 5$ series per day). The concentration of each sample was determined using the calibration curve prepared and analyzed on the same batch.

2.2.4. Extraction recovery

The extraction recoveries of berberine, coptisine, epiberberine, jatrorrhizine and palmatine were determined at low, medium and high concentrations. Recoveries were calculated by comparing the analyte/I.S. peak area ratios (R_1) obtained from extracted plasma samples with those (R_2) from the standard solutions at the same concentration.

2.2.5. Stability

Freeze and thaw stability: QC plasma samples at three concentration levels were stored at the storage temperature (−20 °C) for 24 h and thawed unassisted at room temperature. When completely thawed, the samples were refrozen for 24 h under the same conditions. The freeze–thaw cycles were repeated twice, and the samples were analyzed after three freeze (−20 °C)-thaw (room temperature) cycles.

Short-term temperature stability: QC plasma samples at three concentration levels were kept at room temperature for a period that exceeded the routine preparation time of the samples (around 6 h).

Long-term stability: QC plasma samples at three concentration levels kept at low temperature (−20 °C) were studied for a period of 2 weeks.

Postpreparative stability: The autosampler stability was conducted by reanalyzing extracted QC samples kept under autosampler conditions (4 °C) for 12 h.

Stock solution stability: The stability of berberine, coptisine, epiberberine, jatrorrhizine and palmatine and the IS working solutions were evaluated at room temperature for 4 days.

2.2.6. Standard curves and quality control samples in each batch

Standard curves in each analytical run were used to calculate the concentrations of berberine, coptisine, epiberberine, jatrorrhizine and palmatine in the unknown samples in the run. Quality

control samples were prepared along with the unknown samples in the same batch and analyzed in the middle of the run.

The QC samples of berberine, coptisine, epiberberine, jatrorrhizine and palmatine in five duplicates at three concentrations (0.625, 2.5 and 10 ng mL^{-1}) were prepared and analyzed with the processed test samples at intervals per batch.

2.3. Safety considerations

No specific safety precautions should be taken for this method but necessary precautions for the handling of chemicals and biofluids.

2.4. Application to pharmacokinetic study

The studies were approved by the Animal Ethics Committee of China Pharmaceutical University. Five male Sprague–Dawley rats (230–250 g) were obtained from Sino-British Sippr/BK Lab Animal Ltd. (Shanghai, PR China). The rats were maintained in an air-conditioned animal quarter at a temperature of 22 ± 2 °C and a relative humidity of 50 ± 10%, having free access to water, and fed with a laboratory rodent chow (Nanjing, China). The animals were acclimatized to the facilities for 5 days, and then fasted, free access to water for 12 h prior to experiment. *C. Rhizoma* extract was dissolved in 0.5% carboxymethyl cellulose sodium salt (CMC-Na) aqueous solution and was administered to the rats (1.3 g extract kg^{-1} body weight) by oral gavage. Blood samples (300 μL) were obtained from the oculi chorioideae vein before dosing and subsequently at 0.25, 0.5, 1.0, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 h following administration, transferred to a heparinized eppendorf tube and centrifuged at 4000 rpm for 10 min. The plasma obtained was frozen at −20 °C until analysis.

To determine the pharmacokinetic parameters of berberine, palmatine, coptisine, epiberberine and jatrorrhizine, the concentration-time data were analyzed by non-compartmental method using the DAS Software (ver. 1.0, Medical College of Wannan, China). C_{max} and t_{max} values were obtained directly from the observed concentration versus time data. All results were expressed as arithmetic mean ± standard deviation (S.D.).

3. Results and discussions

3.1. Selection of internal standard

Tetrahydroberberine was chosen for quantification as the internal standard due to its similarity with the analytes in structure, chromatographic behavior, mass spectrographic behavior and high stability.

3.2. Method development

At the very beginning, HPLC–UV was tried to detect the compounds of interest. However, the analytes could not be separated completely in 30 min due to their similar structures. Moreover, even without considering the recovery, the LLOD in plasma was 15 ng mL^{-1}, while most plasma concentrations of the compounds were below LLOD. So LC–MS was involved.

There is one nitrogen in every structure of the protoberberine alkaloids as well as the IS. During the scan of these, the ESI-MS system revealed the presence of $[M]^+$, and the signal intensity obtained in the positive mode was much stronger than that in the negative mode. Therefore, $[M]^+$ ion was employed for method validation. Addition of 0.08% formic acid and 2 mmol L^{-1} ammonium acetate to the mobile phase A was found to be an essential factor for acquiring the high sensitivity. When the ion adduct $[M]^+$ was selected for determination, the addition of formic acid and ammonium acetate caused a significant sensitivity increase. Comparison were made between the changes caused by modifying the concentrations of formic acid and ammonium acetate singly or both. The best peak shape and ionization were achieved using 2 mmol L^{-1} ammonium acetate buffer, with pH adjusted to 3.5 with formic acid. Linear gradient elution was used to separate the analytes in a time as short as possible and elute endogenous substances residue from the column. During the optimazion of the method, the signal-to-noise ratio detected at the voltage of 1.6 kV was twice as large as that at the voltage of 1.5 kV. Thus, 1.6 kV was utilized as the detector voltage.

Ethyl acetate and diethyl ether were all tested as extraction solvent before, but poor extraction efficiency was achieved. SPE cartridge was finally adopted because of its best extraction efficiency. Subsequent thorough washings resulted in minimized polar matrix interference and increased specificity. The extraction procedure described here offers a effective way to isolate five protoberberine alkaloids and tetrahydroberberine (IS) from the plasma matrix and provides clean injection extracts.

Acetonitrile, methanol and mobile phase were tested as final solution. The shapes of peaks were found to be best when solutions were prepared in mobile phase, with methanol as the second choice.

3.3. Method validation

All samples were found to be free of interferences with the compound of interest. All the ratios of the peak area resolved in the blank sample, compared with that resolved by the mobile phase, are between 85 and 115%, which means no matrix effect is observed for berberine, coptisine, epiberberine, jatrorrhizine, palmatine and tetrahydroberberine in this method. Fig. 2 shows positive ion electrospray mass scan spectra of the analytes and IS. Representative SIM chromatograms are shown in Fig. 3, indicating no endogenous peaks at the retention positions of berberine, coptisine, epiberberine, jatrorrhizine, palmatine or the IS (tetrahydroberberine).

The calibration curves of the analytes showed good linearity in the range 0.31–20.0 ng mL^{-1} for berberine, coptisine, epiberberine, jatrorrhizine and palmatine. The mean regression equations from five replicate calibration curves on different days were: $R = 0.1127(0.0047)C + 0.0251(0.0039)$, $r = 0.9999$ for berberine; $R = 0.0540(0.0016)C + 0.0062(0.0036)$, $r = 0.9993$ for coptisine; $R = 0.0240(0.0013)C + 0.0004(0.0005)$, $r = 0.9997$ for epiberberine; $R = 0.0639(0.0012)C + 0.0114(0.0083)$, $r = 1.0000$ for jatrorrhizine and $R = 0.0794(0.0011)C + 0.0115(0.0083)$, $r = 0.9999$ for palmatine, where R stands for the peak area ratio of berberine, coptisine, epiberberine, jatror-

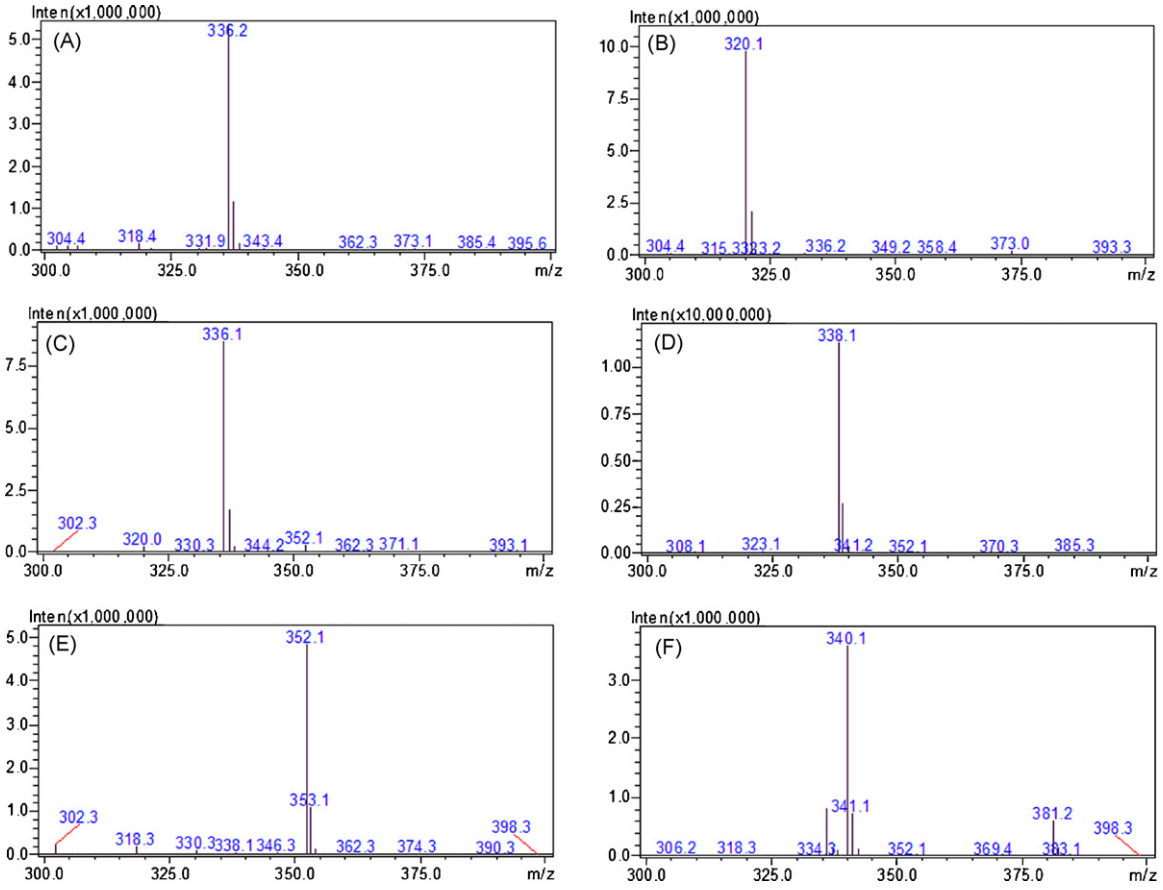

Fig. 2. Positive ion electrospray mass scan spectra of berberine (A), coptisine (B), epiberberine (C), jatrorrhizine (D), palmatine (E) and internal standard tetrahydroberberine (F).

rhizine or palmatine to the IS, and *C* refers to the concentration of berberine, coptisine, epiberberine, jatrorrhizine or palmatine added to plasma.

The lower limits of quantification for berberine, coptisine, epiberberine, jatrorrhizine and palmatine were proved to be 0.31 ng mL^{-1} (LLOQ) and the lower limits of detection (LLOD) were 0.1 ng mL^{-1}.

Data for within-batch and between-batch precision and accuracy of the method for determination of berberine, coptisine, epiberberine, jatrorrhizine and palmatine are presented in Table 1. The accuracy deviation values are within 10% of the actual values. The precision determined at each concentration level does not exceed 10% of the relative standard deviation (R.S.D.). The results have revealed good precision and accuracy.

The extraction recoveries determined for berberine, coptisine, epiberberine, jatrorrhizine and palmatine were shown to be consistent, precise and reproducible. Data are shown in Table 2. The extraction recovery of IS was more than 70%.

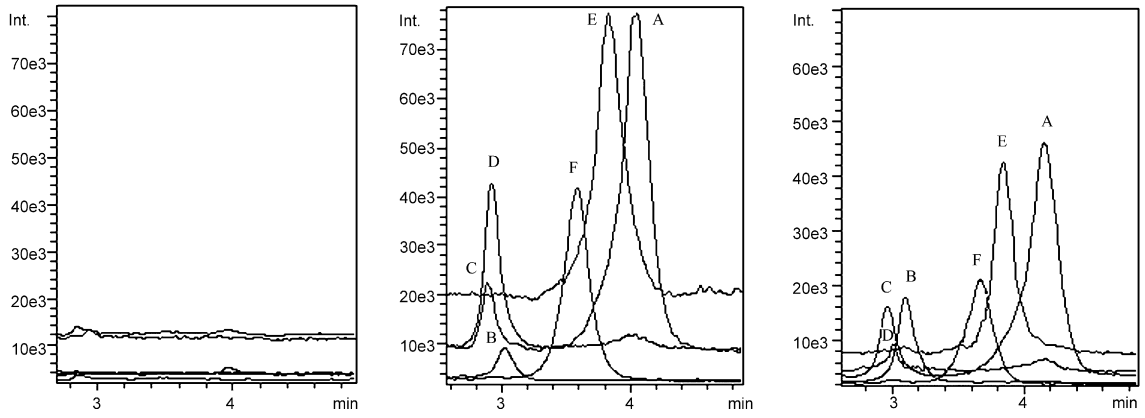

Fig. 3. SIM chromatograms of (A) blank plasma; (B) blank plasma spiked with berberine, coptisine, epiberberine, jatrorrhizine and palmatine (5 ng mL^{-1}) and tetrahydroberberine (20 ng mL^{-1}); (C) samples 3 h after oral administration of *C. Rhizoma* extract. berberine (A), coptisine (B), epiberberine (C), jatrorrhizine (D), palmatine (E) and internal standard tetrahydroberberine (F).

Table 1
Accuracy and precision for the analysis of berberine, coptisine, epiberberine, jatrorrhizine and palmatine

Analyte	Concentration (ng mL^{-1})	Intra-batch ($n=5$)		Inter-batch ($n=5$)	
		Precision (CV, %)	Accuracy (%Er)[a]	Precision (CV, %)	Accuracy (%Er)[a]
berberine	0.625	7.9	3.8	7.3	4.1
	2.50	3.8	−3.8	4.1	2.6
	10.00	7.2	0.5	6.1	−1.6
coptisine	0.625	7.2	−3.5	6.9	−2.5
	2.50	7.6	2.1	6.9	−3.5
	10.00	3.5	−1.8	3.1	−0.3
epiberberine	0.625	6.5	−3.6	6.8	−3.3
	2.50	4.9	−1.5	4.2	−3.8
	10.00	7.5	−2.8	6.4	0.3
jatrorrhizine	0.625	9.5	3.4	8.9	4.2
	2.50	6.5	−1.1	5.8	−3.3
	10.00	5.9	−0.2	5.2	−1.9
palmatine	0.625	8.2	−2.4	7.5	4.0
	2.50	6.4	3.5	6.1	2.1
	10.00	6.3	−0.6	5.9	−0.7

[a] Relative percentage error = [(overall mean assayed concentration − added concentration)/(added concentration) × 100].

Table 2
Recovery of berberine, coptisine, epiberberine, jatrorrhizine and palmatine from plasma ($n=5$)

Nominal (ng mL^{-1})	0.625		2.50		10.00	
	Recovery (mean ± S.D., %)	R.S.D. (%)	Recovery (mean ± S.D., %)	R.S.D. (%)	Recovery (mean ± S.D., %)	R.S.D. (%)
berberine	78.9 ± 6.8	8.6	83.4 ± 3.6	4.3	80.1 ± 1.8	2.2
coptisine	66.1 ± 6.3	9.5	66.6 ± 4.5	6.8	68.4 ± 2.4	3.5
epiberberine	63.2 ± 5.3	8.4	67.4 ± 3.6	5.3	67.9 ± 3.2	4.7
jatrorrhizine	68.5 ± 5.5	8.0	73.0 ± 5.1	7.0	73.8 ± 2.5	3.4
palmatine	72.7 ± 6.1	8.4	71.9 ± 3.2	4.5	74.9 ± 2.3	3.1

Table 3 summarizes the freeze and thaw stability, short-term stability, long-term stability and postpreparative stability data of berberine, coptisine, epiberberine, jatrorrhizine and palmatine. All the results showed good stability during these tests and there were no stability related problems during the routine analysis of samples for pharmacokinetic study.

The stability of the working solutions was tested at room temperature for 4 days. On the basis of the results obtained, these working solutions were found to be stable within 4 days.

3.4. Pharmacokinetics in rats following oral administration

The extract of *C. Rhizoma* is so hard to dissolve directly in water that 0.5% CMC-Na was used to make it a suspension for oral administration.

Table 3
Data showing the stability of berberine, coptisine, epiberberine, jatrorrhizine and palmatine in rat plasma at different QC levels ($n=5$)

Nominal (ng mL^{-1})		Accuracy (mean ± S.D., %)			
		Freeze and thaw stability	Short-term stability	Long-term stability	Postpreparative stability
berberine	0.625	105.5 ± 9.0	94.2 ± 7.1	95.8 ± 7.8	104.7 ± 7.5
	2.50	96.4 ± 6.2	98.1 ± 6.6	97.3 ± 6.8	101.9 ± 6.0
	10.00	98.7 ± 5.1	101.0 ± 4.8	100.7 ± 5.3	98.7 ± 3.9
coptisine	0.625	93.6 ± 8.6	97.8 ± 8.2	96.7 ± 7.0	104.2 ± 8.1
	2.50	98.0 ± 7.3	104.3 ± 6.9	98.1 ± 6.3	99.8 ± 5.1
	10.00	102.1 ± 5.2	100.8 ± 4.3	99.4 ± 5.8	101.2 ± 4.1
epiberberine	0.625	104.3 ± 7.8	105.8 ± 7.2	95.7 ± 6.8	95.8 ± 6.6
	2.50	97.0 ± 5.9	102.1 ± 5.5	103.8 ± 5.2	103.5 ± 6.4
	10.00	99.0 ± 4.0	99.5 ± 3.0	100.2 ± 4.2	100.9 ± 4.7
jatrorrhizine	0.625	95.9 ± 8.5	97.6 ± 7.4	97.0 ± 7.8	96.2 ± 6.8
	2.50	97.1 ± 6.3	105.9 ± 6.2	99.8 ± 5.9	99.8 ± 5.2
	10.00	99.6 ± 4.7	98.9 ± 3.1	102.8 ± 5.1	102.3 ± 4.6
palmatine	0.625	93.1 ± 8.0	105.3 ± 8.6	96.0 ± 7.6	95.0 ± 6.7
	2.50	103.2 ± 7.0	97.1 ± 6.8	103.5 ± 5.8	103.7 ± 4.7
	10.00	100.6 ± 4.1	98.3 ± 5.1	99.2 ± 4.8	97.4 ± 3.1

Table 4
Pharmacokinetic parameters of berberine, coptisine, epiberberine, jatrorrhizine and palmatine after oral administration of 1.3 g extract kg^{-1}, each value represents the mean ± S.D. ($n = 5$)

Alkaloids	AUC^{0-24} (ng h mL^{-1})	C_{max} (ng mL^{-1})	t_{max} (h)	MRT (h)
berberine	147.66 ± 14.19	11.39 ± 1.62	3.40 ± 1.47	9.31 ± 0.81
coptisine	11.74 ± 7.24	1.52 ± 0.74	2.25 ± 1.82	6.69 ± 2.07
epiberberine	10.19 ± 6.67	2.40 ± 0.88	1.30 ± 1.56	5.09 ± 2.44
jatrorrhizine	–	0.84 ± 0.47	1.69 ± 1.72	–
palmatine	14.80 ± 2.25	1.74 ± 0.56	0.90 ± 0.95	10.12 ± 1.16

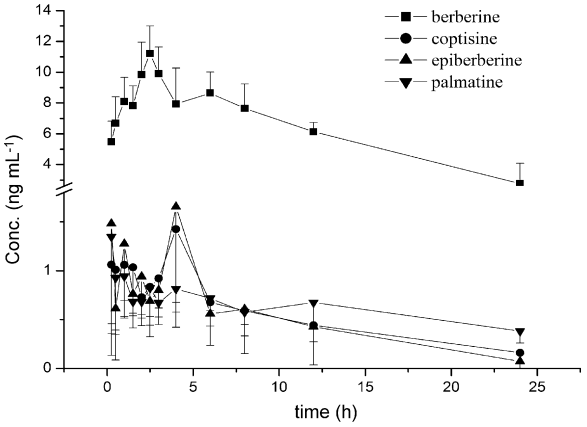

Fig. 4. Mean plasma concentration–time profiles of berberine, coptisine, epiberberine and palmatine in five male rats after oral administration of 1.3 g extract kg^{-1}, each point and bar represents the mean ± S.D. ($n = 5$).

The method described above was successfully applied to the pharmacokinetic study in which plasma concentrations of berberine, coptisine, epiberberine, jatrorrhizine and palmatine were determined for 24 h after oral administration of 1.3 g *C. Rhizoma* extract kg^{-1} (berberine 242.3 mg kg^{-1}, coptisine 36.1 mg kg^{-1}, epiberberine 37.3 mg kg^{-1}, jatrorrhizine 33.9 mg kg^{-1} and palmatine 46.0 mg kg^{-1}). The plasma concentrations of jatrorrhizine in most of the time points were found to be lower than the LLOQ. The pharmacokinetic profiles of berberine, coptisine, epiberberine and palmatine are shown in Fig. 4. Their pharmacokinetic parameters are listed in Table 4.

The poor absorption and extensive metabolism [18,25–27] may take responsibility for the extremely low plasma concentration of berberine (ng mL^{-1} level) after oral administration to rat, Beagle dog and human [15,17,20,28]. Reasonablely, the plasma concentrations of the other four protoberberine alkaloids are lower, to some extent, for their similar structures with berberine and lower contents for adminstration.

4. Conclusions

In this paper, it has been described a sensitive, specific, accurate and precise HPLC–ESI-MS method for the simultaneous determination of berberine, palmatine, coptisine, epiberberine and jatrorrhizine in rat plasma.

To our knowledge, it is the first study reporting the pharmacokinetics of these five protoberberine alkaloids after oral administration of *C. Rhizoma* extract. The pharmacokinetic parameters obtained from this study can give some useful information for further research of *C. Rhizoma*.

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