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# Simultaneous determination of tetrahydropalmatine, protopine, and palmatine in rat plasma by LC-ESI-MS and its application to a pharmacokinetic study

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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 simultaneous quantification of tetrahydropalmatine, protopine and palmatine in rat plasma using phenacetin as the internal standard (IS). Two hundred microliters plasma samples were extracted by dichloromethane under a strong basic condition. The analytes were separated by a C18 column and detected with a single quadrupole mass spectrometer. The used mobile phase was acetonitrile–water (40:60, v/v) containing 5 mM ammonium acetate and 0.2% glacial acetic acid. Detection was carried out by positive electrospray ionization in selected ion reaction (SIR) mode at $m/z$ 356.6 for tetrahydropalmatine, 354.6 for protopine, 352.6 for palmatine and 180.4 for the IS, respectively. The method was validated over the concentration range of 1.00–500 ng mL$^{-1}$ and the lower limit of quantification (LLOQ) was 1.00 ng mL$^{-1}$ for all three analytes. The intra- and inter-day precision values were less than 9% relative standard deviation (R.S.D.), and the relative error ranged from −7.4 to 4.8%. The extraction recoveries were on average 91.42% for tetrahydropalmatine, 84.75% for protopine, 57.26% for palmatine, and 83.18% for IS. The validated method was successfully applied to a pharmacokinetic study of tetrahydropalmatine, protopine and palmatine in rats after oral administration of *Rhizoma Corydalis Decumbentis* extract.



## 1. Introduction

Traditional Chinese medicine (TCM) has played an important role in the prevention and treatment of diseases in China for thousands of years. *Rhizoma Corydalis Decumbentis*, a well-known TCM herbal medicine with the Chinese name “Xiatianwu”, was prepared from the dried tubers of *Corydalis decumbens* (Tunb.) Pers. This medicine has been used for centuries for the treatment of hemiplegia, alleviating pain, rheumatic arthritis and infantile paralysis residual, and has been increasingly studied nowadays for its newly found pharmacologic effects against dementia [1,2] and hepatotoxicity [3,4]. The tertiary and quaternary ammonium alkaloid of *Rhizoma Corydalis Decumbentis* has been investigated to some extent, and over 20 alkaloids had been isolated and identified so far [5,6].

Early publications have described methods for the simultaneous determination of tetrahydropalmatine and protopine in herbal medicine and Chinese patent medicine using HPLC-UV [7–9]. An LC–MS/MS and LC-DAD method has been established for the simultaneous determination of ten alkaloids in the ethyl acetate extract, including tetrahydropalmatine, protopine, and palmatine, using linear gradient elution of *Corydalis yanhusuo* extract [10]. HPLC with UV detection has been used for the determination of protopine in rat plasma after intravenous administration [11]. Unfortunately, the limit of detection (LOD) of 50 ng mL$^{-1}$, is not sensitive enough for oral pharmacokinetic study. Lu [12] and Yu [13] have developed a method using linear gradient elution to determine palmatine in the presence of other quaternary alkaloids in rat plasma by LC-ESI-MS. Thus far, there is no documented record on the simultaneous determination of both tertiary and quaternary isoquinoline alkaloids in biological fluids in a single-run process. Meanwhile, little information is available regarding the pharmacokinetic profiles of tetrahydropalmatine, protopine and palmatine.

In this paper, an LC-ESI-MS method has been developed for the simultaneous quantification of tetrahydropalmatine, protopine, and palmatine in rat plasma. This method has been successfully applied to a pharmacokinetic study of the above three alkaloids after oral administration of *Rhizoma Corydalis Decumbentis* extract to rats. The chemical structures of tetrahydropalmatine, protopine, palmatine, and the internal standard (IS) phenacetin are shown in Fig. 1.

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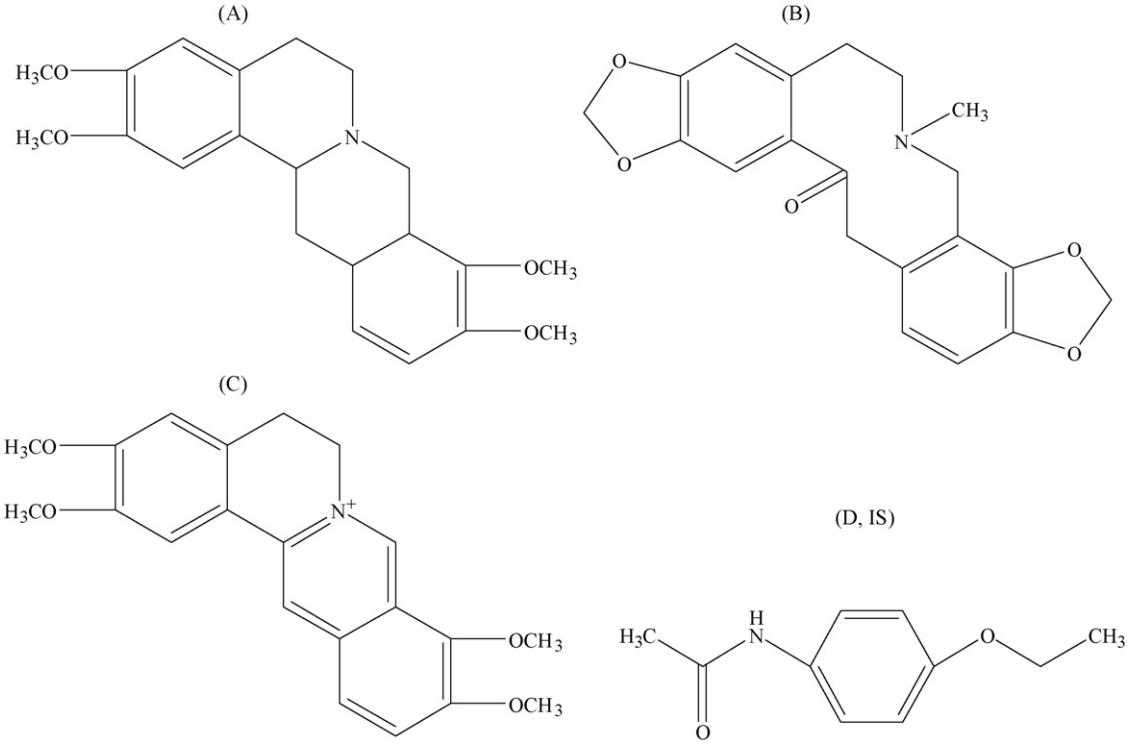


**Fig. 1.** Chemical structure of tetrahydropalmatine (A), protopine (B), palmatine (C), and phenacetin (D, IS).

## 2. Experimental

### 2.1. Chemicals and materials

The reference standards of tetrahydropalmatine, protopine and palmatine and phenacetin were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, PR China). Acetonitrile was of HPLC grade (Fisher Company, USA). Double-distilled water was used. All other reagents were of analytical grade. *Rhizoma Corydalis Decumbentis* was purchased from Shenyang Northern Hospital.

### 2.2. Preparation of Xiatianwu extract

The Xiatianwu extract was prepared according to the *Chinese Pharmacopeia*, and the dried powder was stored at 4 °C before use. To calculate the administration dosage, the tetrahydropalmatine, protopine, and palmatine content in the extract was quantitatively determined. The HPLC analysis method was a modified version of *Chinese Pharmacopeia*. The content of tetrahydropalmatine, protopine, and palmatine were 0.317, 0.772, and 0.601 g, and 100 $g^{-1}$ extract, respectively.

### 2.3. LC–MS conditions

The LC–MS system included a Waters 1525 pump and a ZQ 2000 Micromass mass spectrometer equipped with electrospray ionization source (Waters Co., USA). All data processing was performed with Masslynx 4.0 software.

The separation was achieved on a Diamonsil C18 column (4.6 μm, 250 mm × 4.6 mm, i.d., Dikma Technologies) equipped with an EasyGuard C18 guard column (5 μm, 5 mm × 4.6 mm i.d., Dikma Technologies) maintained at 25 °C. The mobile phase consisted of acetonitrile–water (40:60, v/v) containing 5 mM ammonium acetate and 0.2% glacial acetic acid, and was set to a flow rate of 0.2 mL $min^{-1}$.

The ESI source was set to positive ion mode with ionization conditions as follows: capillary voltage 3.0 kV, cone voltage 30 V, source temperature 105 °C, and desolvation gas (nitrogen) heated at 300 °C (350 L $h^{-1}$). Quantification was performed using selected ion recording (SIR) of *m*/*z* 356.6 for tetrahydropalmatine, 354.6 for protopine, 352.6 for palmatine and 180.4 for phenacetin (IS), respectively, with a scan time of 0.2 s.

### 2.4. Sample preparation

Plasma samples were extracted employing a liquid–liquid extraction technique. Plasma samples were removed from −20 °C storage and thawed under the ambient condition. 200 μL plasma samples were extracted with 3.0 mL dichloromethane after addition of 50 μL IS solution, 50 μL methanol, and 50 μL 0.2 M sodium hydroxide solution. After vortex for 1 min and centrifugation at 3000 × *g* for 10 min, the organic phase was removed and evaporated to dryness in a 40 °C water bath under a gentle stream of nitrogen. The residue was reconstituted in 100 μL mobile phase. Finally, an aliquot of 20 μL was injected into the LC–MS system.

### 2.5. Preparation of calibration standards and quality control (QC) samples

Stock solution of tetrahydropalmatine, protopine, and palmatine was prepared in methanol at concentration of 250 μg $mL^{-1}$.Working solution of analytes between 4.00 and 2000 ng $mL^{-1}$ was prepared by diluting the stock solution with methanol. A 40 μg $mL^{-1}$ stock solution of IS was prepared in water and then diluted to obtain a working solution of 400 ng $mL^{-1}$. All solutions were stored at 4 °C away from light and brought to room temperature before use.

Calibration samples, which covered the concentration range at 1.00, 2.00, 5.00, 10.0, 20.0, 50.0, 100, 250 and 500 ng $mL^{-1}$, were prepared by freshly spiking working solutions into blank plasma, and processed as described in the sample preparation. QC samples

were prepared in a small pool in the same way at concentrations of 2.00, 20.0 and 400 ng mL$^{-1}$, and then divided into small protons and stored at −20 °C until use.

### 2.6. Method validation

#### 2.6.1. Assay specificity

The specificity of the method was assessed by preparing and analyzing six different batches of drug-free rat plasma. Each blank sample was tested for endogenous interference using the proposed extraction procedure and LC–MS conditions. The chromatogram of a blank plasma sample was compared with those obtained with a solution at the concentration of lower limit of quantification (LLOQ). The signal intensity at this concentration was at least 10 times higher response than that of blank plasma samples.

#### 2.6.2. Linearity and LLOQ

The calibration curves of tetrahydropalmatine, protopine, and palmatine were prepared and assayed by the above-mentioned method. The calibration curves were constructed by the plots of the peak-area ratios of three analytes versus the IS ($y$) against the concentrations of the calibration standards ($x$). The concentrations of analytes in unknown samples were determined by interpolation from the calibration curve. The LLOQ of the assay was defined as the lowest concentration on the standard curve that can be quantitated with accuracy within 20% bias of the nominal concentration and precision not exceeding 20% [14].

#### 2.6.3. Accuracy and precision

Accuracy was evaluated as the relative error (R.E.), and precision was determined as the relative standard deviation (R.S.D.). The accuracy and the precision of the assays for intra-day and inter-day determinations were evaluated by the analysis of three QC samples (five samples for each) on the same day and on three consecutive validation days, respectively. The concentration of each sample was calculated using a calibration curve constructed on the same testing day. The criteria employed to assess the suitability of accuracy and precision was as follows: the accuracy should be within 15% of the actual value for QC samples and the R.S.D. should not exceed 15%.

#### 2.6.4. Extraction recovery and matrix effects

The extraction recoveries of analytes were determined by comparing the mean peak areas of the analytes in the extracted QC samples with those obtained from the extracted blank plasma samples post-spiked with corresponding neat solutions ($n$ = 5). Recovery of the IS was also evaluated similarly at 100 ng mL$^{-1}$ ($n$ = 5).

The matrix effect (ME) was examined by comparing the mean peak areas of the analytes and the IS between two different sets of samples. In set 1, reference standards were dissolved in mobile phase and five replicates were analyzed. In set 2, blank plasma samples were extracted and then spiked with the same concentrations of analytes and IS. Samples of both sets were prepared at three QC levels for the analytes and 100 ng mL$^{-1}$ for the IS. The blank plasma samples used in this study were from five different batches of blank rat plasma. The ME was defined as following: ME% = 100 × set 2/set 1. It would indicate the possibility of ionization suppression or enhancement for analytes and IS, and an endogenous matrix effect is implied if the ratio is less than 85% or more than 115% [15].

#### 2.6.5. Stability

The stability of analytes in rat plasma was investigated by using QC samples stored under different temperature conditions for different periods of time, which likely to be encountered during sample storage and the analytical process. The short-term stability was determined by exposing the samples at room temperature (about 25 °C) for 4 h. This duration exceeded the routine preparation time of the samples. Stability of the extracted samples was also assessed at room temperature for 12 h. Freeze–thaw stability was evaluated after three freeze (−20 °C)–thaw (room temperature) cycles. Long-term stability was estimated after storage of spiked QC samples at −20 °C for 2 weeks. In addition, the working solutions were stored for 1 week at 4 °C away from light.

## 3. Application to pharmacokinetic study

The studies were approved by the Animal Ethics Committee of Shenyang Pharmaceutical University. Six male Wistar rats (220–250 g) were purchased from the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). They were kept in an environmentally controlled breeding room at a temperature of 22 ± 2 °C and a relative humidity of 50 ± 10% and provided with standard laboratory food and water ad libitum. The animals were acclimatized to the facilities for 5 days, and then fasted with free access to water overnight prior to the experiment. The *Rhizoma Corydalis Decumbentis* extract was suspended in 0.2% carboxymethyl cellulose sodium (CMC-Na) aqueous solution and was administered to the rats (2.0 g extract kg$^{-1}$ body weight, equivalent to 6.34 mg kg$^{-1}$ of tetrahydropalmatine, 15.44 mg kg$^{-1}$ of protopine and 12.02 mg kg$^{-1}$ of palmatine) by oral gavage. Approximately 500 μL blood samples were obtained from oculi chorioideae vein before dosing and at 0.083, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 h following oral administration. Blood samples were transferred to heparinize eppendorf tubs and centrifuged at 6000 × $g$ for 10 min to separate the plasma. The plasma samples obtained were transferred to clean eppendorf tubes and frozen at −20 °C until analysis.

## 4. Data analysis

To determine the pharmacokinetics parameters of three alkaloids, all data were processed by noncompartmental analysis using the DAS 2.0 software package (Chinese Pharmacological Society). The plasma concentration at different times was expressed as mean ± standard deviation (S.D.), and the mean concentration–time curves were plotted. The maximum plasma concentration ($C_{max}$) and the time to reach it were observed directly from the detected value of the concentration–time curves. The area under the plasma concentration–time curve from zero to the time of the final measurable sample ($AUC_{0-t}$) was calculated using the linear-trapezoidal rule up to the last sampling point with the detectable level ($C$). The area under the plasma concentration–time curve from zero to infinity ($AUC_{0-\infty}$) was calculated using the linear-trapezoidal rule with extrapolation to infinity.

## 5. Results and discussion

### 5.1. Optimization of LC–MS for quantitative analysis

Tetrahydropalmatine, protopine and palmatine had stronger signal responses in positive ion mode when compared to negative ion mode, and higher sensitivity was achieved by ESI than ACPI. Under the selected source condition, tertiary alkaloids formed predominantly protonated molecules at $m/z$ 356.6 for tetrahydropalmatine, and at 354.6 for protopine. Palmatine predominantly formed a quaternary ammonium ion $[M]^+$ at 352.6 in the full scan spectra. The protonated molecular ion at 180.4 for the IS was the most abundant ion. The positive ion electrospray mass scan spectra of the analytes and the IS are shown in Fig. 2.

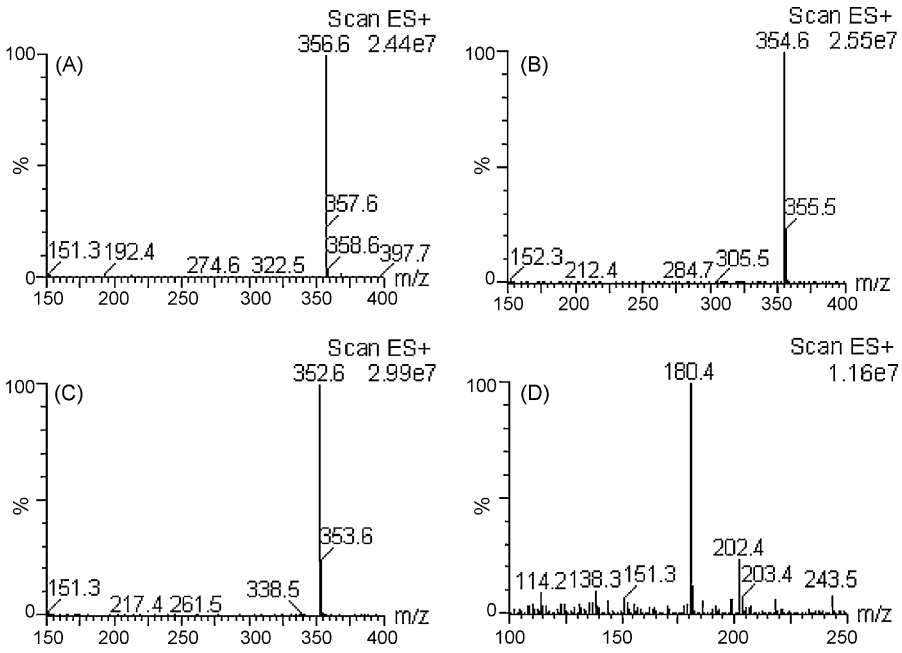


**Fig. 2.** Positive ion electrospray mass scan spectra of tetrahydropalmatine (A), protopine (B), palmatine (C), and phenacetin (D, IS).

In order to obtain maximum sensitivity of the SIR, parameters such as capillary voltage, cone voltage, source temperature, desolvation gas temperature and the nitrogen flow rate were optimized. The other MS parameters were adopted from the recommended values for the instrument.

Chromatographic conditions, especially the composition of mobile phase, played a critical role in achieving good chromatographic behavior and appropriate ionization. When the chromatographic conditions were optimized, it was found that acetonitrile resulted in lower background noise and better peak shape than methanol. Therefore, the optimized mobile phase composition consisted of acetonitrile–water (40:60, v/v). Modifiers such as formic acid, acetic acid, and glacial acetic acid–ammonium acetate alone or in combination in different concentration were compared. The best peak shape and ionization were achieved using 5 mM ammonium acetate buffer and 0.2% glacial acetic acid, producing a pH of 4.0. Typical chromatograms are shown in Fig. 3.

### 5.2. Sample preparation

Liquid–liquid extraction and a protein precipitation method were compared during sample preparation. The latter was much simpler and less time-consuming, but was still discarded because of a high level of noise and interference by endogenous substances. Liquid–liquid extraction usually offers very clean sample that makes the method more robust and scalable. In the present study, a variety of extraction solvents were evaluated, including ethyl ether, ethyl acetate, and dichloromethane. Dichloromethane was found to be the best due to its low background noise, easy sample preparation, and relative high extraction recovery for analytes and the IS. Fifty microliters of 0.2 M sodium hydroxide solution was added to basify the sample and reduce interference by acidic endogenous compounds.

### 5.3. Method validation

#### 5.3.1. Specificity

Fig. 3 shows representative chromatographs of (1) drug-free plasma, (2) a spiked plasma sample with the analytes (1.0 ng mL$^{-1}$, LLOQ) and IS (100 ng mL$^{-1}$), and (3) a plasma sample after oral administration of *C. decumbens* extracts. The retention times were about 4.1 min for tetrahydropalmatine, 3.0 min for protopine, 3.5 min for palmatine and 5.8 min for the IS, respectively. No endogenous peaks were observed in the drug-free plasma at the retention positions of tetrahydropalmatine, protopine, palmatine and IS.

#### 5.3.2. Linearity and LLOQ

Linear calibration curves were obtained over the concentration range 1.00–500 ng mL$^{-1}$ for all alkaloids in rat plasma. A weighted ($1/x^2$) least-squares linear regression analysis was performed for all alkaloids. Representative regression equations are shown in Table 1. The correlation coefficient ($r^2$) for every calibration curves was over 0.99.

The LLOQ of the compounds was 1.00 ng mL$^{-1}$ with a signal-to-noise (S/N) ratio of 13.4 for tetrahydropalmatine, 36.5 for protopine, and 16.8 for palmatine. This sensitivity has proven useful in the analysis of pharmacokinetic data for TCM orally administrated to rats.

#### 5.3.3. Accuracy and precision

The R.E. of the QC samples was calculated from the nominal concentration ($C_{nom}$) and the mean value of the detected concentration ($C_{det}$) as follows: R.E.% = [($C_{det} - C_{nom}$)/$C_{nom}$] × 100. The results are presented in Table 2. The intra- and inter-day precision values (R.S.D.%) were both less than 9%, while the deviation of assay accuracies ranged from −7.4 to 4.8%. These results, which were within the acceptable criteria for accuracy and precision, indicate that the method is reliable and reproducible for the quantitative analysis of three alkaloids in rat plasma samples.

**Table 1**
Calibration curves of three alkaloids in rat plasma.

| Compounds | Regression equation | Correlation coefficient ($r^2$) |
|---|---|---|
| Tetrahydropalmatine | $y = 2.068 \times 10^{-3} x + 2.934 \times 10^{-3}$ | 0.994 |
| Protopine | $y = 1.608 \times 10^{-3} x + 2.490 \times 10^{-2}$ | 0.995 |
| Palmatine | $y = 2.039 \times 10^{-3} x + 2.688 \times 10^{-3}$ | 0.995 |

$y$: peak-area ratios of analytes to IS; $x$: concentration of alkaloid in plasma (ng mL$^{-1}$).

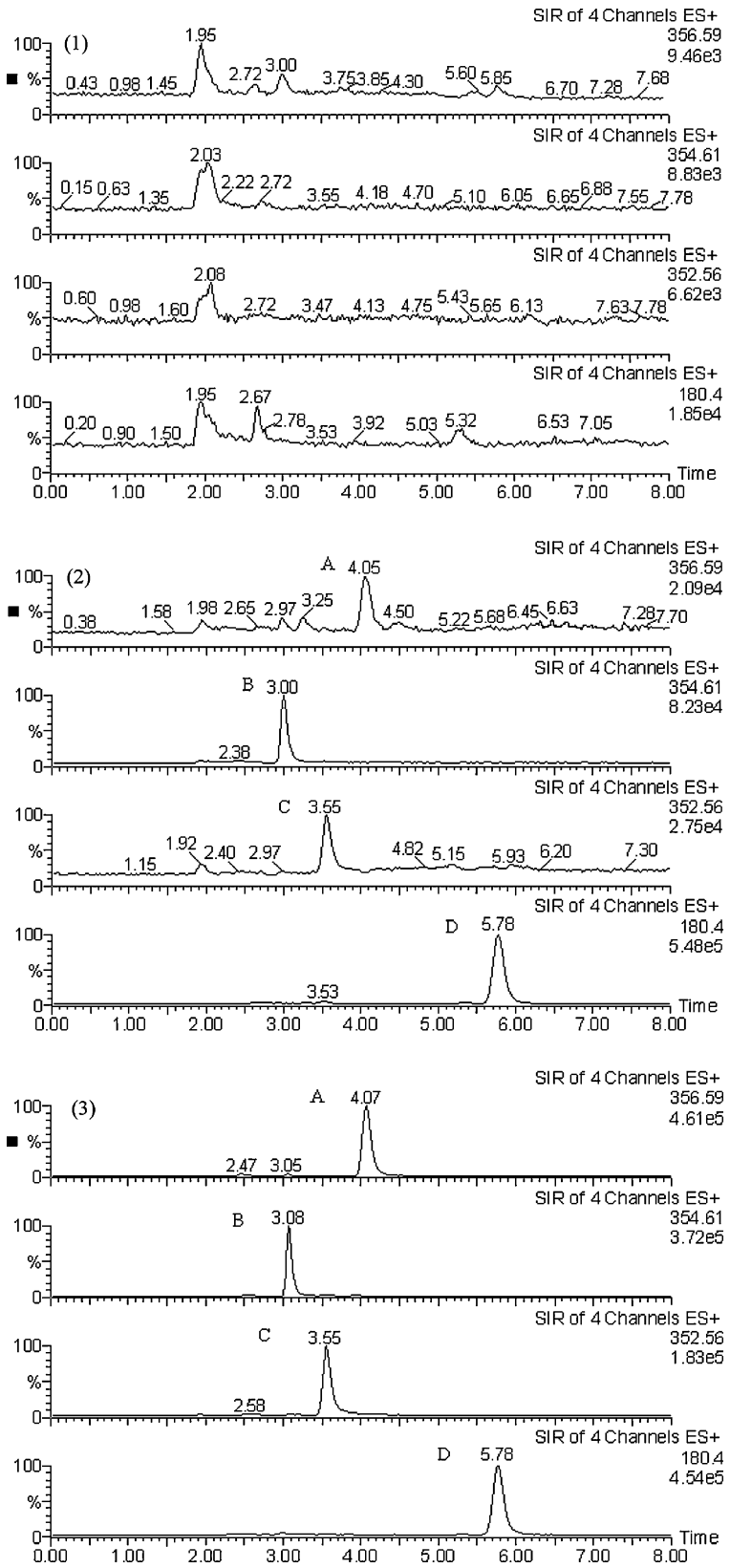


**Fig. 3.** Representative SIR chromatograms of analytes (A–C) and IS (D) in rat plasma: (1) drug-free rat plasma; (2) a rat plasma spiked with analytes at the LLOQ 1.0 ng mL$^{-1}$ and IS of 400 ng mL$^{-1}$; (3) an incurred plasma sample of analytes from a rat after an oral administration (39.07 ng mL$^{-1}$ for tetrahydropalmatine, 24.65 ng mL$^{-1}$ for protopine, and 5.02 ng mL$^{-1}$ for palmatine).

**Table 2**
Accuracy and precision results for analysis of three alkaloids in rat plasma (intra-day: $n = 5$; inter-day: $n = 15$).

| Compound | $C_{nom}$ (ng mL$^{-1}$) | $C_{det}$ (ng mL$^{-1}$) | Accuracy (R.E.%) | Precision (R.S.D.%) |
|---|---|---|---|---|
| Tetrahydropalmatine | | | | |
| Intra-day | 2.00 | 1.89 ± 0.14 | −5.6 | 3.3 |
| | 20.0 | 20.9 ± 1.47 | 4.8 | 7.0 |
| | 400 | 387 ± 17.3 | −3.3 | 4.5 |
| Inter-day | 2.00 | 1.86 ± 0.11 | −7.1 | 6.0 |
| | 20.0 | 20.7 ± 1.26 | 3.3 | 6.1 |
| | 400 | 391 ± 27.9 | −2.2 | 7.1 |
| Protopine | | | | |
| Intra-day | 2.00 | 1.93 ± 0.17 | −3.4 | 8.5 |
| | 20.0 | 19.8 ± 1.69 | −1.2 | 8.6 |
| | 400 | 419 ± 17.8 | 4.6 | 4.3 |
| Inter-day | 2.00 | 1.87 ± 0.13 | −6.1 | 6.8 |
| | 20.0 | 20.6 ± 1.69 | 3.1 | 8.2 |
| | 400 | 410 ± 23.4 | 2.5 | 5.7 |
| Palmatine | | | | |
| Intra-day | 2.00 | 1.88 ± 0.10 | −6.1 | 5.1 |
| | 20.0 | 20.5 ± 1.38 | 2.5 | 6.7 |
| | 400 | 383 ± 19.4 | −4.2 | 5.1 |
| Inter-day | 2.00 | 1.85 ± 0.10 | −7.4 | 5.3 |
| | 20.0 | 20.8 ± 1.30 | 3.8 | 6.2 |
| | 400 | 394 ± 19.7 | −1.5 | 5.0 |

**Table 3**
Extraction recovery results for analysis of three alkaloids and IS in rat plasma ($n = 5$).

| Sample | $C$ (ng mL$^{-1}$) | Recovery (mean ± S.D.) | R.S.D. (%) |
|---|---|---|---|
| Tetrahydropalmatine | 2.00 | 92.55 ± 4.56 | 5.0 |
| | 20.0 | 90.65 ± 5.83 | 6.4 |
| | 400 | 91.06 ± 3.73 | 4.1 |
| Protopine | 2.00 | 83.04 ± 3.77 | 4.5 |
| | 20.0 | 85.19 ± 4.08 | 4.8 |
| | 400 | 86.03 ± 4.42 | 5.2 |
| Palmatine | 2.00 | 56.16 ± 3.70 | 6.6 |
| | 20.0 | 55.39 ± 3.27 | 5.9 |
| | 400 | 60.22 ± 3.72 | 6.2 |
| IS | 100 | 83.18 ± 3.51 | 4.2 |

### 5.3.4. Extraction recovery and matrix effects

As shown in Table 3, the extraction recoveries were on average 91.42% for tetrahydropalmatine, 84.75% for protopine, and 57.26% for palmatine. The data indicates that extraction recovery was consistent and reproducible. All recoveries had R.S.D. values smaller than 7% throughout all QC levels. The mean extraction recovery of the IS was 83.18 ± 3.51%.

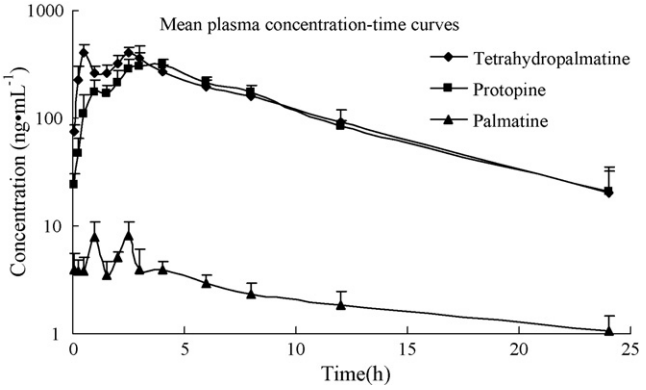


**Fig. 4.** Mean plasma concentration–time curves of three alkaloids after oral administration of *Rhizoma Corydalis Decumbentis* extract at 2.0 g kg$^{-1}$ to rats ($n = 6$).

The average matrix effect values were 98.4%, 101.0%, and 97.5% for tetrahydropalmatine; 98.8%, 95.8%, and 97.2% for protopine; and 97.7%, 99.6%, and 99.5% for palmatine at the three QC concentration levels. The average matrix effect value of the IS was 99.7%. These results indicated that the extracts had little or no detectable co-eluting endogenous substances that could influence the ionization of the analytes and IS.

### 5.3.5. Stability

The working solution was found to be stable for 1 week at 4 °C away form light. The stability test results in different storage conditions are presented in Table 4. All results indicated that samples were stable during our experiment.

## 5.4. Pharmacokinetics study of three alkaloids in rats

The validated LC-ESI-MS method has been successfully applied to the pharmacokinetic study and simultaneous determination of tetrahydropalmatine, protopine, and palmatine in rat plasma for 24 h after oral administration of 2.0 g extract per kg of body weight. The mean plasma concentration–time profiles of the three alkaloids are presented in Fig. 4, and the main pharmacokinetic parameters assessed by noncompartmental analysis are listed in Table 5. Following oral administration of *Rhizoma Corydalis Decumbentis* extract, double peaks were observed in both individual and mean plasma concentration curves of tetrahydropalmatine and palmatine. Deng [16] had reported three peaks in the plasma concentration curve of palmatine, and attributed the phenomenon to distribution, re-absorption and enterohepatic circulation. The time to reach the maximum plasma concentration ($T_{max}$) was 1.50 ± 1.14 h for tetrahydropalmatine, 3.50 ± 0.55 h for protopine,

**Table 4**
Stability results for analysis of three alkaloids in rat plasma ($n = 5$).

| Storage condition | $C_{nom}$ (ng mL$^{-1}$) | $C_{det}$ (mean ± S.D., ng mL$^{-1}$) | | | Difference from nominal value (%) | | |
|---|---|---|---|---|---|---|---|
| | | Tetrahydropalmatine | Protopine | Palmatine | Tetrahydropalmatine | Protopine | Palmatine |
| Short-term stability (25 °C, 4 h) | 2.00 | 1.87 ± 0.13 | 1.85 ± 0.11 | 1.85 ± 0.12 | −6.6 | −7.4 | −7.1 |
| | 20.0 | 20.5 ± 1.14 | 20.4 ± 0.86 | 21.3 ± 1.24 | 2.3 | 1.8 | 6.5 |
| | 400 | 383 ± 26.7 | 404 ± 18.9 | 393 ± 17.4 | −4.1 | 1.0 | −1.8 |
| Post-preparative stable (25 °C, 12 h) | 2.00 | 1.84 ± 0.09 | 1.89 ± 0.13 | 1.91 ± 0.13 | −8.2 | −5.4 | −4.3 |
| | 20.0 | 19.8 ± 1.10 | 20.8 ± 2.08 | 20.5 ± 1.70 | −0.7 | 3.8 | 2.5 |
| | 400 | 413 ± 23.0 | 387 ± 15.4 | 390 ± 18.8 | 3.2 | −3.2 | −2.6 |
| Three freeze–thaw cycles (−20 °C to 25 °C) | 2.00 | 1.86 ± 0.13 | 1.92 ± 0.13 | 1.88 ± 0.18 | −7.2 | −4.0 | −6.1 |
| | 20.0 | 20.3 ± 1.64 | 20.5 ± 1.97 | 20.9 ± 1.33 | 1.3 | 2.3 | 4.5 |
| | 400 | 403 ± 20.3 | 406 ± 16.7 | 409 ± 20.0 | 0.7 | 1.4 | 2.2 |
| Long-term stability (−20 °C, 14 days) | 2.00 | 1.90 ± 0.16 | 1.89 ± 0.13 | 1.89 ± 0.14 | −5.2 | −5.4 | −5.7 |
| | 20.0 | 20.6 ± 1.37 | 21.0 ± 1.94 | 20.3 ± 1.40 | 3.0 | 4.8 | 1.5 |
| | 400 | 388 ± 18.1 | 414 ± 28.8 | 387 ± 22.6 | −2.9 | 3.5 | −3.4 |

**Table 5**
Mean pharmacokinetic parameters of three alkaloids after oral administration of *Rhizoma Corydalis Decumbentis* extract at 2.0 g kg$^{-1}$ to rats ($n$ = 6).

| Parameter | Tetrahydropalmatine | Protopine | Palmatine |
|---|---|---|---|
| $t_{1/2}$ (h) | 6.68 ± 0.64 | 4.98 ± 1.64 | 12.84 ± 4.58 |
| $T_{max}$ (h) | 1.50 ± 1.14 | 3.50 ± 0.55 | 1.92 ± 0.74 |
| $C_{max}$ (ng mL$^{-1}$) | 435.8 ± 58.5 | 347.9 ± 51.51 | 8.53 ± 2.95 |
| $MRT_{(0-t)}$ (h) | 7.21 ± 0.21 | 7.36 ± 0.64 | 8.10 ± 1.07 |
| $MRT_{(0-\infty)}$ (h) | 9.32 ± 0.58 | 8.84 ± 1.88 | 16.89 ± 6.27 |
| $AUC_{(0-t)}$ (ng h mL$^{-1}$) | 3450.1 ± 480.9 | 2987.0 ± 354.0 | 57.85 ± 13.44 |
| $AUC_{(0-\infty)}$ (ng h mL$^{-1}$) | 3720.1 ± 498.3 | 3150.8 ± 482.8 | 77.58 ± 27.01 |

and 1.92 ± 0.74 h for palmatine. The elimination half-time ($t_{1/2}$) of tetrahydropalmatine, protopine, and palmatine was 6.68 ± 0.64, 4.98 ± 1.64, and 12.84 ± 4.58 h, respectively. The results indicated that the absorption of tetrahydropalmatine and palmatine might be fast indicated by shorter $T_{max}$ (less than 2 h). The elimination of palmatine might be slow because of the longer $t_{1/2}$ and MRT (about 10 h). The information described above might be helpful for further studies on the pharmacokinetics of *Rhizoma Corydalis Decumbentis*, and beneficial for application of this TCM in clinical therapy.

## 6. Conclusions

In this paper a sensitive, specific, reliable, accurate, and precise LC-ESI-MS method has been developed for the simultaneous quantification of tetrahydropalmatine, protopine, and palmatine in plasma. In addition, this method has been successfully applied to pharmacokinetic study of three alkaloids following oral administration of *Rhizoma Corydalis Decumbentis* extract to rats. To our knowledge, this is the first study reporting the pharmacokinetic parameters of protopine after oral administration to rats.

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