Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 863 (2008) 195–205
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

# Simultaneous determination of berberine, palmatine and jatrorrhizine by liquid chromatography–tandem mass spectrometry in rat plasma and its application in a pharmacokinetic study after oral administration of coptis–evodia herb couple

Yating Deng [a,b], Qiongfeng Liao [c], Suhua Li [a], Kaishun Bi [b], Biyan Pan [a], Zhiyong Xie [a,*]

[a] *Laboratory of Pharmaceutical Analysis & Quality Assessment, School of Pharmaceutical Sciences, Sun Yat-sen University, 74 Zhongshan Road II, Guangzhou 510080, China*
[b] *School of Pharmacy, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang 110016, China*
[c] *Department of Pharmaceutical Analysis, College of Chinese Traditional Medicine, Guangzhou University of Chinese Medicine, 232 East of Waihuan Road, University Town, Guangzhou 510006, China*

Received 28 August 2007; accepted 24 December 2007
Available online 21 January 2008

**Abstract**

A rapid and sensitive liquid chromatography–tandem mass spectrometry (LC–MS/MS) method was developed to simultaneously determine berberine, palmatine and jatrorrhizine in rat plasma. After mixing with the internal standard (IS) tetrahydropalmatine, plasma samples were pretreated by protein precipitation with acetonitrile–methanol (1:2, v/v). Chromatographic separation was carried out on a C18 column using a mixture of water (containing 0.1% formic acid) and acetonitrile (30:70, v/v) as mobile phase. The detection was performed by selected reaction monitoring (SRM) mode via electrospray ionization (ESI) source operating in the positive ionization mode. The method was linear over the concentration range of 1.0–250.0 ng/mL for all components. The intra- and inter-day precision values were less than 14.6% and the deviations were within ±4.0%. The fully validated LC–MS/MS method has been successfully applied to the pharmacokinetic study of berberine, palmatine and jatrorrhizine in rat plasma after oral administration of coptis–evodia herb couple. Three peaks were observed in both individual and mean plasma–concentration curves of berberine, palmatine and jatrorrhizine, which may be attributed to distribution re-absorption and enterohepatic circulation.


*Keywords:* Berberine; Palmatine; Jatrorrhizine; LC–MS/MS; Coptis–evodia herb couple; Pharmacokinetics

## 1. Introduction

Herbs used together in couples are the basic composition units of Chinese herbal formulas and have special clinical significance in Traditional Chinese Medicine (TCM). The herb couples are much simpler than other complicated formulas yet retain the basic therapeutic features [1]. In the clinical practice of TCM, various combinations of Coptis (*Coptidis rhizoma*) and Evodia (*Evodiae fructus*) have been used to treat gastro-intestinal disorders [2]. Zoujinwan, which consists of coptis–evodia powder (6:1, g/g), is most widely used in TCM with berberine, palmatine and jatrorrhizine as the most important pharmacologically active constituents. The chemical structures of berberine, palmatine and jatrorrhizine are shown in Fig. 1.

Earlier publications have described methods for berberine determination in biological samples utilizing HPLC-UV [3–10], HPLC-fluorimetry [11], field desorption-mass spectrometry [12] and GC–chemical ionization-mass spectrometry [13]. Recently, Tong and Yan [14] have developed a method to determine berberine and palmatine in rat plasma by LC–MS/MS in the selected ion-monitoring (SIM) mode. However, the method incorporated a complicated and time-consuming sample preparation procedure including liquid–liquid extraction by ethyl ether and gradient elution with 5.5 min running time for each sample. As far as we know, there was no method published for simultaneous determina-

* Corresponding author. Tel.: +86 20 87333095; fax: +86 20 87334718.
*E-mail address:* xiezy2074@yahoo.com (Z. Xie).

1570-0232/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2007.12.028

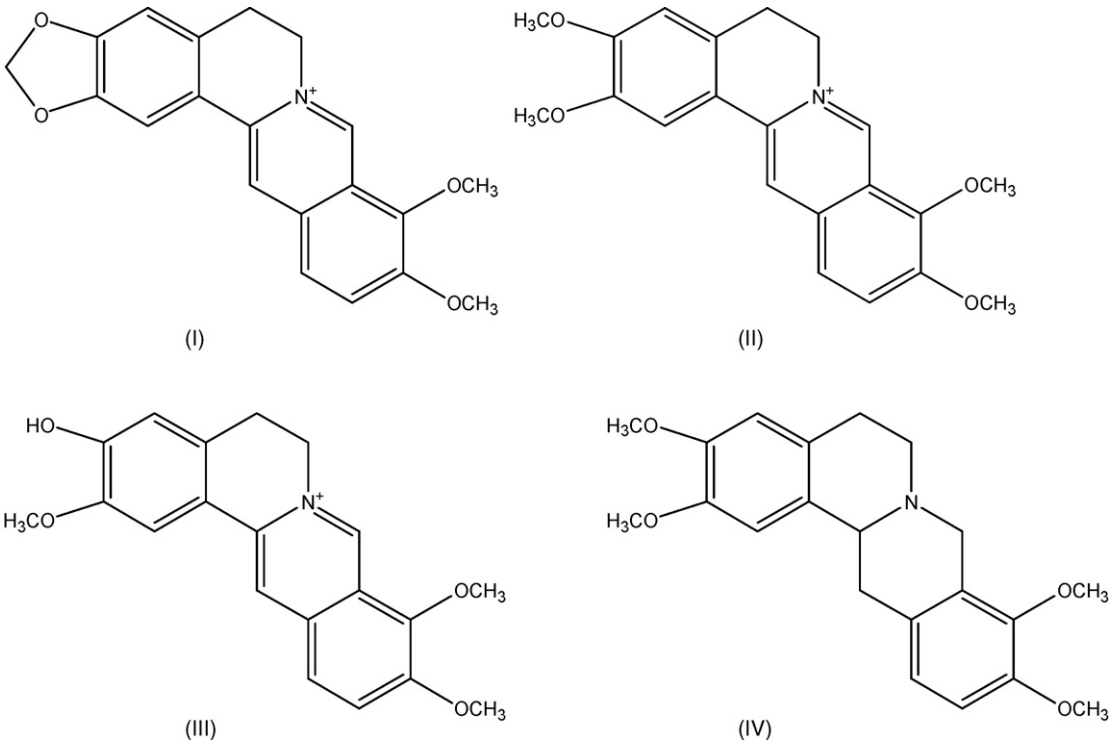


Fig. 1. Chemical structures of berberine (I) palmatine (II) jatrorrhizine (III) and tetrahydropalmatine (IS, IV).

tion of berberine, palmatine and jatrorrhizine in biological fluids.

In the meanwhile, little information is available related to the pharmacokinetic profiles of berberine, palmatine and jatrorrhizine. Zuo et al. [15] have reported the pharmacokinetic behavior of jatrorrhizine as the metabolite of berberine. In addition, most pharmacokinetic studies on berberine were conducted after administration of the active compound berberine instead of herbs containing berberine.

In this study, a rapid and sensitive liquid chromatographic/tandem mass spectrometry (LC–MS/MS) method was developed for simultaneous determination of berberine, palmatine and jatrorrhizine in rat plasma. Using the established method, the pharmacokinetic behaviors of these three active components in rats were studied after oral administration of coptis–evodia (6:1, g/g).

## 2. Experimental

### 2.1. Chemical and reagents

The reference standards of berberine (purity 98.5%), palmatine (purity 98.0%), jatrorrhizine (purity 99.0%) and tetrahydropalmatine (purity 99.0%) (IS) were all obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol, acetonitrile and formic acid are of HPLC grade and 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.

*Coptis rhizome* and *Evodiae fructus* were purchased from the affiliated pharmacies of Guangzhou University of Chinese Medicine and the People's Hospital of Guangdong, respectively.

### 2.2. Content of berberine, palmatine and jatrorrhizine in the coptis decoction

To calculate the administration dosage, the contents of berberine, palmatine and jatrorrhizine in the coptis extract were quantitatively determined. One hundred milligrams of the dry powder was prepared according to the *Chinese Pharmacopeia*. The HPLC analysis of berberine, palmatine and jatrorrhizine was a modified version of a previously published method [16]. The contents of berberine, palmatine and jatrorrhizine were 4.89, 1.08 and 1.33 g/100 g extract, respectively.

### 2.3. LC–MS/MS conditions

The HPLC system consists of a Surveyor MS Pump (Thermo Finnigan, USA) and a Surveyor Autosampler (Thermo Finnigan). Chromatographic separation was carried out on a Hypersil BDS C18 (15 mm × 2.1 mm, 5 μm, Elite, China) with a 4.0 mm × 2.0 mm I.D. Security Guard C18 (5 μm) guard column (Phenomenex, Torrance, CA, USA) kept at 25 °C. The mobile phase consisted of water (containing 0.1% formic acid)/acetonitrile (30:70, v/v), at a flow rate of 500 μL/min. The total running time was 2.5 min for each injection.

Mass spectrometric detection was performed on a Thermo Finnigan TSQ Quantum triple quadrupole mass spectrometer

(San Jose, CA, USA) equipped with an ESI source in the positive ionization mode. The MS operating conditions were optimized as follows: the spray voltage: 3500 V; the source CID voltage: 10 eV; the heated capillary temperature: 350 °C; the sheath gas (nitrogen): 35 psi; the auxiliary gas (nitrogen): 2 psi; the collision gas (argon) pressure: 1.0 mtorr (1 torr = 133.3 Pa); the collision energy: 30 eV for all. Data acquisition was performed by Xcalibur 1.3 software (Thermo Finnigan). Peak integration and calibration were performed using LC Quan software (Thermo Finnigan). Quantification was obtained by using SRM mode of the transitions at *m/z* 336 → 320 for berberine, at *m/z* 352 → 336 for palmatine, at *m/z* 338 → 322 for jatrorrhizine and at *m/z* 356 → 192 for tetrahydropalmatine (IS) respectively, with a scan time of 0.3 s per transition.

### 2.4. Preparation of standard and quality control samples

The stock solution I of berberine, palmatine and jatrorrhizine at the concentration of 40 μg/mL for each was prepared respectively by dissolving the accurately weighed reference compounds in methanol. The stock solution II containing three substances at the concentration of 1.0 μg/mL was obtained by diluting the stock solutions I. Standard working solutions at concentrations over 1.0–250.0 ng/mL for each component were prepared by serially diluting the stock solution II with methanol. A 40 μg/mL stock solution of tetrahydropalmatine (IS) was also prepared in methanol and then diluted to obtain a working solution of 50 ng/mL. All the solutions were stored at 4 °C.

The analytical standard and quality control (QC) samples were prepared as the following procedure: after 20 μL standard working solution was transferred to a 1.0 mL eppendorf tube, solvent was evaporated to dryness under vacuum, and then to the residue 20 μL blank plasma was added. The samples were prepared prior to use during validation and pharmacokinetic study. Calibration samples were made at concentrations of 1, 2, 5, 10, 25, 100 and 250 ng/mL (equal to plasma concentration) for each component. QC samples were prepared at concentrations of 2, 25 and 250 ng/mL.

### 2.5. Sample preparation

To 20 μL rat plasma in a 1.0 mL eppendorf tube, 20 μL of the internal standard solution (50 ng/mL), 20 μL of methanol and 20 μL of acetonitrile were added. After vortex-mixing and centrifugation, the supernatant was separated out and evaporated to dryness under vacuum at 40 °C. Then the residue was reconstituted in 80 μL mobile phase and mixed to make final testing samples. A 20 μL aliquot of the final testing samples was injected onto the LC–MS/MS system for analysis.

### 2.6. Method validation

The method was validated according to the currently accepted USA Food and Drug Administration (FDA) bioanalytical method validation guidance [17].

The selectivity was investigated by preparing and analyzing six individual rat blank plasma samples. The chromatogram of each blank plasma sample was compared with that of samples at the concentration of the lower limit of quantification (LLOQ) which had at least 5 times response more than that of blank plasma samples. Two other blank plasma samples spiked with IS (50 ng/mL) were prepared and analyzed to check the interference of IS.

Calibration curves of berberine, palmatine and jatrorrhizine of seven concentration levels ranging from 1.0 to 250 ng/mL were prepared and assayed. Blank plasma samples were analyzed to confirm the absence of endogenous interference. LLOQ of the assay, defined as the lowest concentration at which both precision and accuracy were less than or equal to 20%, was evaluated by analyzing samples in six replicates.

Accuracy and precision were investigated by determining QC samples at three concentration levels of 2, 25, and 250 ng/mL (six samples for each concentration level) on 3 different validation days. The concentration of each sample was calculated using a calibration curve constructed on the same testing day. Accuracy was described as relative error (RE) and precision was described as relative standard deviation (RSD). The criteria used to assess the suitability of precision and accuracy was as follows: the RSD should not exceed 15% and the accuracy should be within 15% of the actual value for QC samples.

To determine extraction recovery, extracted samples were prepared as the following procedure: 20 μL QC plasma was spiked with 20 μL IS, 20 μL methanol and 20 μL acetonitrile. After vortex-mixing and centrifugation, the supernatant was separated out and mixed with 40 μL methanol before the solvent evaporated. At the same time, spike-after-extraction samples were prepared as the following procedure: 20 μL blank plasma was spiked with 40 μL methanol and 20 μL acetonitrile. After vortex-mixing and centrifugation, the supernatant was separated out and mixed with 20 μL IS and 20 μL working solution before the solvent evaporated. Then both extracted samples and spike-after-extraction samples were evaporated to dryness. The residue was reconstituted in the mobile phase to create final samples for assay. The extraction recoveries of the analytes were determined by comparing the mean peak areas of six extracted low (2 ng/mL), medium (25 ng/mL) and high (250 ng/mL) samples to mean peak areas of six spike-after-extract samples at the same concentrations. Recovery of IS was also evaluated by comparing the mean peak areas of six extracted medium samples to mean peak areas of six reference solutions spiked in extracted plasma samples of the same concentration.

By exposing QC samples to different temperature conditions for different periods of time, the stability of analytes was investigated at three concentration levels (2, 25 and 250 ng/mL for each analyte). The stability studies included: (a) stability at room temperature for 4 h; (b) stability after three freeze–thaw cycles; (c) stability of the extracted samples at room temperature for 24 h. In addition, stock solution stability at 4°C for 2 weeks was also assessed at three concentration levels (2, 25, 250 ng/mL).

The matrix effect (ME) was examined by comparing the peak areas of the analytes and IS between two different sets of samples. In set 1, analyte standards were dissolved in the mobile phase and analyzed at the concentration of 25 ng/mL for the analytes and 50 ng/mL for the IS. These analyses were repea-

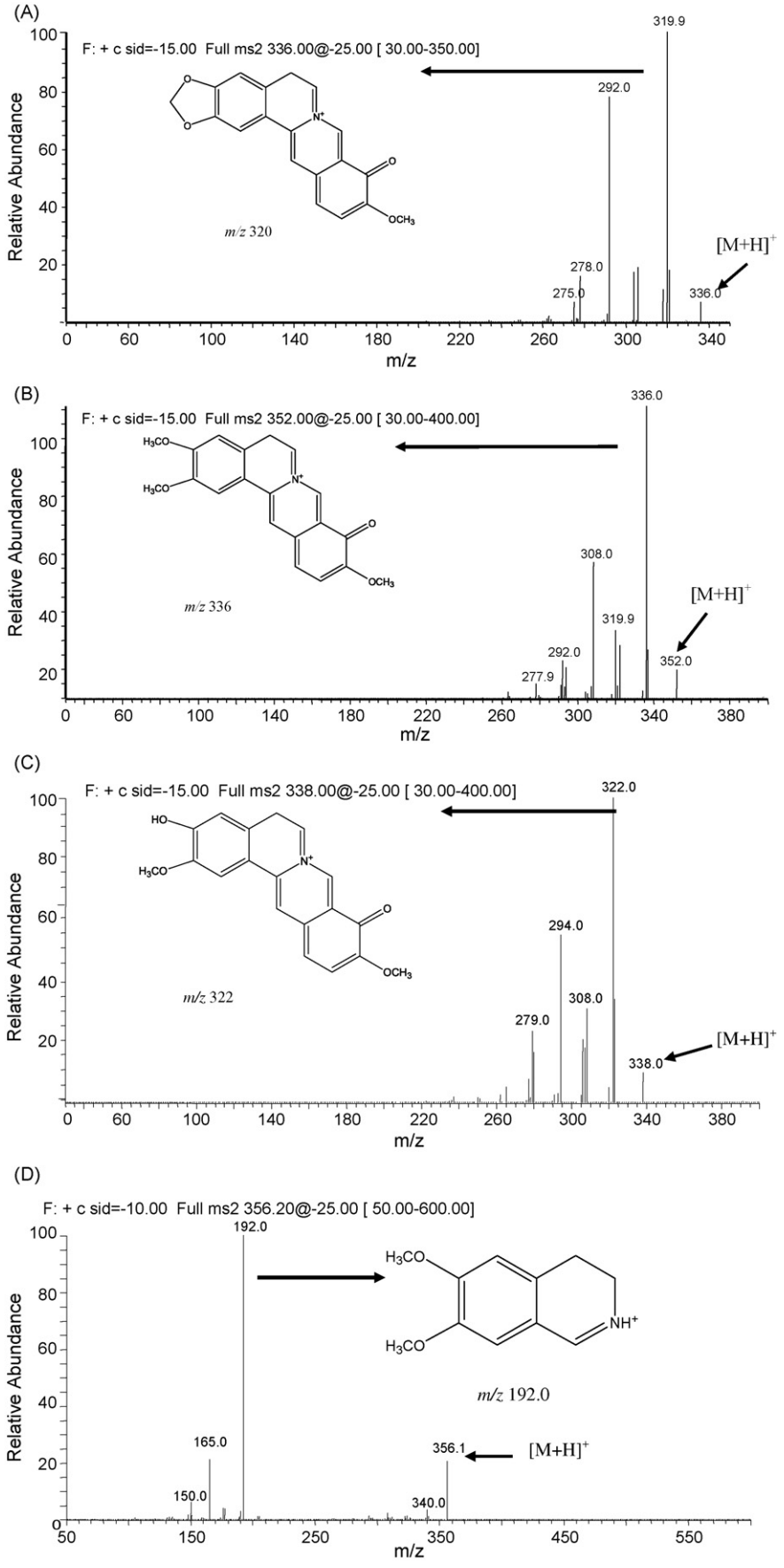


Fig. 2. Product ion mass spectra of $[M+H]^+$ ions of (A) berberine (B) palmatine (C) jatrorrhizine and (D) tetrahydropalmatine.

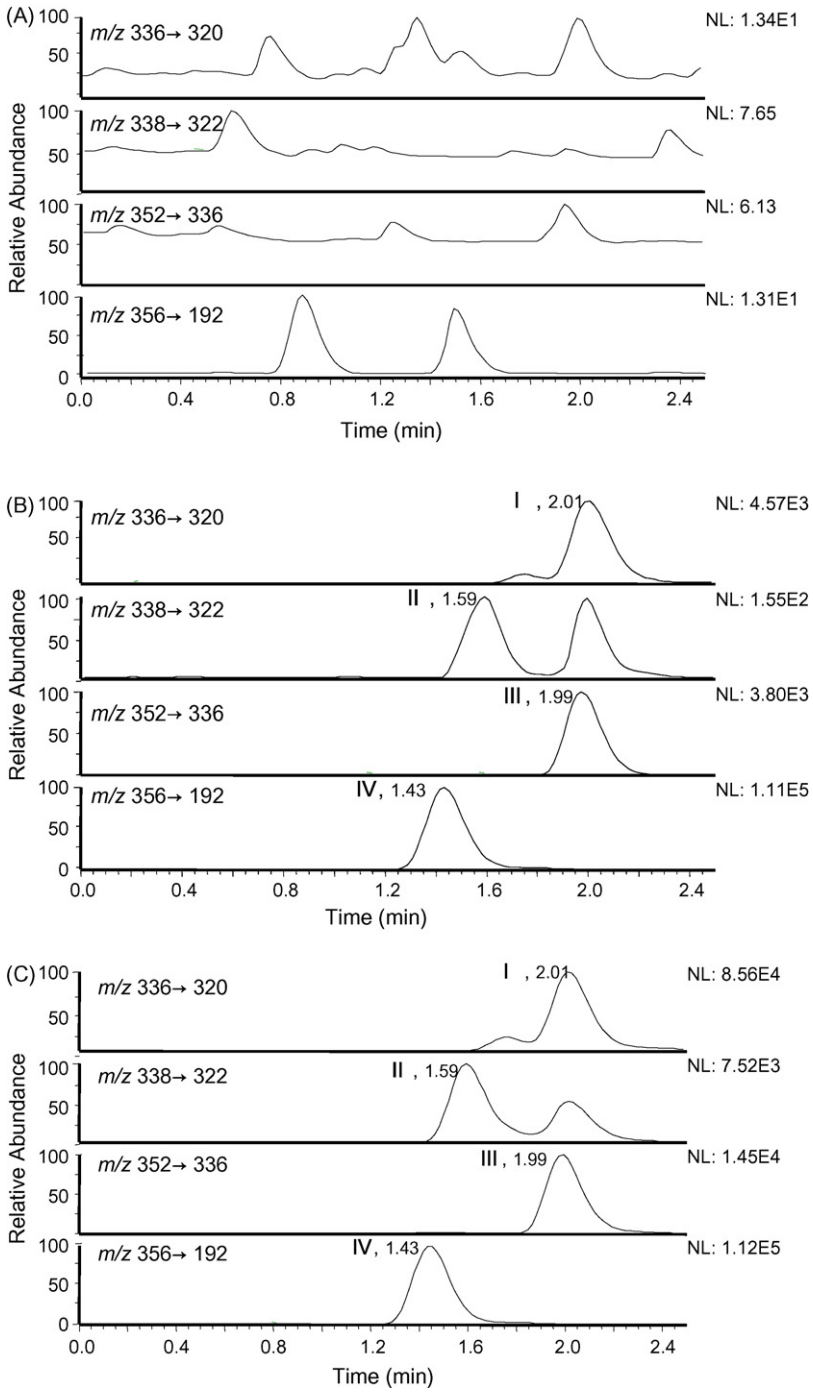


Fig. 3. Representative SRM chromatograms for berberine, palmatine, jatrorrhizine and IS in rat plasma: (A) a blank plasma sample; (B) a blank plasma sample spiked with berberine, palmatine and jatrorrhizine at the LLOQ of 1 ng/mL IS at 50 ng/mL; (C) an incurred plasma sample (5.1 ng/mL for berberine, 14.7 ng/mL for jatrorrhizine, and 2.2 ng/mL for palmatine) I: berberine; II: jatrorrhizine; III: palmatine; IV: tetrahydropalmatine (IS).

ted five times. In set 2, blank plasma samples obtained from five rats were extracted and then spiked with the same concentrations of analytes and IS. Ratio of the mean peak areas of set 2 to that of set 1 would indicate the possibility of ionization suppression or enhancement for analytes and IS. If the ratio is less than 85% or more than 115%, an exogenous matrix effect is implied [18]. The assessment of the relative ME was made by a direct comparison of the analyte peak area values between different sources of plasma.

Cross-talk was also evaluated in our experiment by analyzing standard solutions of individual component separately.

## 3. Applications to pharmacokinetic study

Six male Sprague–Dawley rats, weighing 180–220 g, were supplied by Laboratory Animal Center of the Sun-Yat-Sen University, China. The rats were kept in an air-conditioned animal quarter at a temperature of $22 \pm 2\,^{\circ}C$ and a relative humidity

Table 1
Accuracy and precision results for berberine, palmatine and jatrorrhizine at the plasma concentrations of LLOQ

| Nominal plasma concentration (ng/mL) | Individual measured concentratiXon (ng/mL) | | | Mean measured concentration (ng/mL) | | | Relative error (%) | | | Intra-day RSD (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Berberine | Palmatine | Jatrorrhizine | Berberine | Palmatine | Jatrorrhizine | Berberine | Palmatine | Jatrorrhizine | Berberine | Palmatine | Jatrorrhizine |
| 1.0 | 1.1 | 1.0 | 0.9 | 1.1 | 1.1 | 1.1 | 11.9 | 14.1 | 6.7 | 11.7 | 17.0 | 18.9 |
| | 1.3 | 1.3 | 1.3 | | | | | | | | | |
| | 1.1 | 1.3 | 1.0 | | | | | | | | | |
| | 1.3 | 1.2 | 1.0 | | | | | | | | | |
| | 1.0 | 1.0 | 0.9 | | | | | | | | | |
| | 1.0 | 1.4 | 0.9 | | | | | | | | | |

Table 2
Intra- and inter-day precision and accuracy data for berberine, palmatine and jatrorrhizine in rat plasma (3 days, six replicates per day)

| Nominal plasma concentration (ng/mL) | Mean measured concentration (ng/mL) | | | Relative error (%) | | | Intra-day RSD (%) | | | Inter-day RSD (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Berberine | Palmatine | Jatrorrhizine | Berberine | Palmatine | Jatrorrhizine | Berberine | Palmatine | Jatrorrhizine | Berberine | Palmatine | Jatrorrhizine |
| 2.0 | 1.88 | 1.97 | 1.96 | −2.7 | −1.3 | −2.1 | 8.2 | 10.0 | 10.5 | 14.6 | 11.1 | 12.0 |
| 25.0 | 24.45 | 24.00 | 23.98 | −2.2 | −4.0 | −4.0 | 6.5 | 6.3 | 9.3 | 7.9 | 7.4 | 9.0 |
| 250.0 | 249.10 | 251.94 | 258.44 | −0.3 | 0.8 | 3.6 | 2.9 | 4.6 | 7.0 | 2.3 | 5.4 | 3.6 |

of 50 ± 10%, and had free access to food and water until 18 h prior to experiments. The coptis–evodia powder (6:1, g/g) was dissolved in 0.1% carboxymethyl cellulose sodium (CMC-Na) aqueous solution and was administered to the rats (coptis–evodia (6:1, g/g) powder 1.086 g/kg body weight, containing 46.5 mg berberine, 10.1 mg palmatine and 14.4 mg jatrorrhizine/kg body weight) by oral gavage. Blood samples (60 μL) were obtained from the jugular vein before dosing and subsequently at 5, 10, 15, 45, 90, 150, 180, 210, 300, 420, 480, 720, and 1440 min following administration, transferred to heparinized eppendorf tubes and centrifuged at 12000 rpm for 5 min to separate out plasma. The plasma was then transferred to clean tubes and stored at −20 °C for preservation. All experimental protocols were in accordance with local institutional guidelines for animal care of Sun Yat-sen University, Guangzhou, China.

## 4. Data analysis

To obtain the pharmacokinetic parameters of berberine, palmatine and jatrorrhizine, the concentration–time data were analyzed by non-compartmental methods using the WinNonlin5.0.1 (provided by Mountain View, CA., USA). The maximum plasma drug concentration ($C_{max}$) and the time to reach the maximum plasma drug concentration ($t_{max}$) values were observed directly from the detected concentration versus time data. The area under the curve ($AUC_{0-t}$) was calculated using the linear trapezoidal rule, with extrapolation to infinity ($AUC_{0-\infty}$) from the last detectable concentration using the terminal elimination rate constant ($k_e$) calculated by linear regression of the final log-linear part of the drug concentration–time curve. Apparent elimination half-life ($t_{1/2}$) was calculated as $t_{1/2} = 0.693/k_e$. All results were expressed as arithmetic mean ± standard deviation (S.D.).

## 5. Results and discussion

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

In full-scan spectra, all the three substances formed predominantly quaternary ammonium ion $[M + H]^+$ at *m/z* 336 for berberine, at *m/z* 352 for palmatine, at *m/z* 338 for jatrorrhizine. The IS formed a predominantly protonated molecule $[M + H]^+$ at *m/z* 356. The product ion spectra of the substances can be seen in Fig. 2. The most abundant fragment ion was found at *m/z* 320 for berberine, at *m/z* 336 for palmatine, at *m/z* 322 for jatrorrhizine, and at *m/z* 192 for IS, so the SRM transitions at *m/z* 336 → 320, at *m/z* 352 → 336, at *m/z* 338 → 322 and at *m/z* 356 → 192 were selected to analyze berberine, palmatine, jatrorrhizine and IS, respectively.

To obtain maximum sensitivity of the SRM, some parameters such as spray voltage, capillary temperature, source CID, sheath gas (nitrogen) pressure, auxiliary gas (nitrogen) pressure, collision gas (argon) pressure, and collision energy were optimized. The other MS parameters were adopted from the recommended values for the instrument.

The mobile phase played a critical role in achieving good chromatographic behavior and appropriate ionization. The selected mobile phase was composed of 30% water (containing 0.1% formic acid) and 70% acetonitrile and provided low background noise and proper retention time. Each chromatographic run was completed within 2.5 min.

### 5.2. Sample preparation

In the present study, a simple single-step protein precipitation procedure was used. Different kinds of extraction procedures, including liquid–liquid extraction (LLE) were evaluated during our method development. The results showed that the liquid–liquid extraction with ether–dichloromethane (3:2, v/v) had a similar recovery to that of protein precipitation with acetonitrile–methanol (1:2, v/v). The latter extraction procedure was much simpler and less time-consuming and therefore utilized in the study.

### 5.3. Method validation

Assay specificity was assessed by comparing the chromatograms of six different sources of blank rat plasma with the corresponding spiked plasma. Fig. 3 shows the typical chromatograms of a blank, a spiked plasma sample with berberine, palmatine and jatrorrhizine (1.0 ng/mL, LLOQ) and IS (50.0 ng/mL), and an incurred plasma sample (5.1 ng/mL for berberine, 14.7 ng/mL for jatrorrhizine, and 2.2 ng/mL for palmatine). As shown in Fig. 3, no significant interfering peaks were observed in the drug-free plasma. The retention times for berberine, palmatine, jatrorrhizine and IS were 2.01, 1.99, 1.55 and 1.43 min, respectively.

All calibration curves showed excellent linearity over the range 1.0–250 ng/mL in rat plasma. Typical equations of the calibration curve using weighted ($1/x^2$) least squares linear regression were as following: $y = 2.822 \times 10^{-2} + 2.352 \times 10^{-2}x$, $r = 0.9991$ (berberine), $y = 3.828 \times 10^{-2} + 2.058 \times 10^{-2}x$, $r = 0.9985$ (palmatine),

Table 3
The extraction recovery results for berberine, palmatine and jatrorrhizine in rat plasma ($n = 6$)

| Nominal plasma concentration (ng/mL) | Recovery (%) (mean ± S.D.) | | | RSD (%) | | |
|---|---|---|---|---|---|---|
| | Berberine | Palmatine | Jatrorrhizine | Berberine | Palmatine | Jatrorrhizine |
| 2.0 | 54.3 ± 6.4 | 77.4 ± 8.1 | 74.5 ± 8.9 | 11.8 | 10.5 | 11.9 |
| 25.0 | 54.8 ± 1.1 | 60.7 ± 4.4 | 73.3 ± 3.2 | 2.0 | 7.2 | 4.4 |
| 250.0 | 55.0 ± 2.3 | 70.3 ± 2.4 | 72.8 ± 2.3 | 4.2 | 3.4 | 3.2 |

Table 4
Stability results for berberine, palmatine and jatrorrhizine in rat plasma under various storage conditions ($n = 5$)

| Storage condition | Nominal plasma concentration (ng/mL) | Calculated concentration (mean ± S.D.) (ng/mL) | | | RE (%) | | |
|---|---|---|---|---|---|---|---|
| | | Berberine | Palmatine | Jatrorrhizine | Berberine | Palmatine | Jatrorrhizine |
| Room temperature (4 h) | 2.0 | 1.77 ± 0.04 | 1.74 ± 0.02 | 1.94 ± 0.12 | −11.5 | −13.1 | −3.0 |
| | 25.0 | 22.74 ± 0.25 | 22.49 ± 0.05 | 23.45 ± 0.65 | −9.1 | −10.5 | −6.2 |
| | 250.0 | 246.77 ± 4.14 | 245.79 ± 2.07 | 243.30 ± 1.94 | −1.3 | −1.7 | −2.7 |
| Three freeze–thaw cycles | 2.0 | 1.94 ± 0.05 | 1.99 ± 0.05 | 2.05 ± 0.08 | −3.2 | −0.7 | 2.5 |
| | 25.0 | 22.72 ± 0.47 | 22.46 ± 0.32 | 24.34 ± 0.59 | −1.1 | −2.2 | −2.6 |
| | 250.0 | 248.22 ± 3.17 | 252.95 ± 2.07 | 248.83 ± 2.92 | −0.7 | 1.2 | −0.5 |
| At room temperature (24 h extracted samples) | 2.0 | 1.90 ± 0.04 | 1.88 ± 0.00 | 1.92 ± 0.02 | −5.1 | 2.5 | −3.8 |
| | 25.0 | 23.82 ± 0.35 | 23.49 ± 0.11 | 22.09 ± 0.58 | −4.7 | −2.6 | −11.6 |
| | 250.0 | 248.38 ± 3.60 | 247.18 ± 1.91 | 253.35 ± 1.53 | −0.7 | −0.5 | 1.3 |

$y = 6.717 \times 10^{-6} + 1.540 \times 10^{-5}x$, $r = 0.9952$ (jatrorrhizine). The LLOQ was established at 1.0 ng/mL. The precision and accuracy data corresponding to LLOQ are shown in Table 1.

Table 2 contains the intra- and inter-day precision and accuracy data for berberine, palmatine and jatrorrhizine. All values of accuracy and precision were within recommended limits. The intra-day and inter-day precisions were less than 14.6% for each analyte. The bias, determined from QC samples (2, 25, 250 ng/mL), was within ±4.0%.

The extraction recoveries determined for the three analytes were consistent, precise and repeatable. Data are shown in Table 3. The mean extraction recovery of the IS was 77.5 ± 1.1%.

Table 4 summarizes the stability data of QC samples. The bias, determined from standard solutions (2, 25, 250 ng/mL) kept for 2 weeks at 4 °C, was within ±15%. The results showed that all the samples were stable during these tests and there were no stability related problems during the routine analysis of samples for the pharmacokinetic study.

### 5.4. Matrix effects

ME occurs when a biological sample contains a component that does not give a signal in the SRM channel used for the target analyte but co-elutes with the analyte and affects the response of the analyte. The presence of ME can decrease or increase the response of the analyte and thus affects the sensitivity of the method. Therefore studies of ME should be an integral part of the validation of any LC–MS/MS method, especially when ESI is utilized, for ESI has been reported to be much more easily subject to such kind of effects [18,19].

Two approaches have been reported to study ME. In the first approach, a solution of the analyte is constantly infused into the eluent from the column via post-column tee connection using a syringe pump. The continuous post-column infusion produces constant signals in the detector, unless compounds that elute from the column suppress or enhance ionization, which would lead to a decreased or increased detector response, respectively [20–24]. The second approach with a comprehensive strategy has been recently published by Matuszewski et al. [18]. This paper provides excellent guidance on how to perform and evaluate studies on ME in LC–MS/MS analysis. The second approach was introduced to assess ME in this study.

The MEs at the concentration of 25.0 ng/mL for each component and 50 ng/mL for IS in five different sources of rat plasma are presented in Table 5. These observations indicated that endogenous substances slightly suppressed the

Table 5
Matrix effects data for berberine, palmatine and jatrorrhizine at 25.0 ng/mL and IS 50.0 ng/mL in five different sources of rat plasma

| | Matrix effect (%) (mean ± S.D.) | RSD (%) |
|---|---|---|
| Berberine | 87.5 ± 0.95 | 1.1 |
| Palmatine | 77.3 ± 0.75 | 1.0 |
| Jatrorrhizine | 69.3 ± 1.43 | 2.1 |
| IS | 98.3 ± 1.30 | 2.5 |

ionization of berberine, palmatine and jatrarrhizine under the present chromatographic and extraction conditions when the ESI interface was utilized. RSD value of absolute ME in five different sources of rat plasma was below 3.0%, indicating that the relative MEs for the analytes were minimal in this study. In addition, no ionization suppression on IS was observed.

### 5.5. Cross-talk

Cross-talk refers to different forms of instrumental phenomena which compromise the selectivity of a TQMS mass analyzer. Two types of cross-talk exist. The first source is a blending of adjacent SRM transitions attributed to the slower rate of scanning product ions out of the collision cell (Q2) relative to

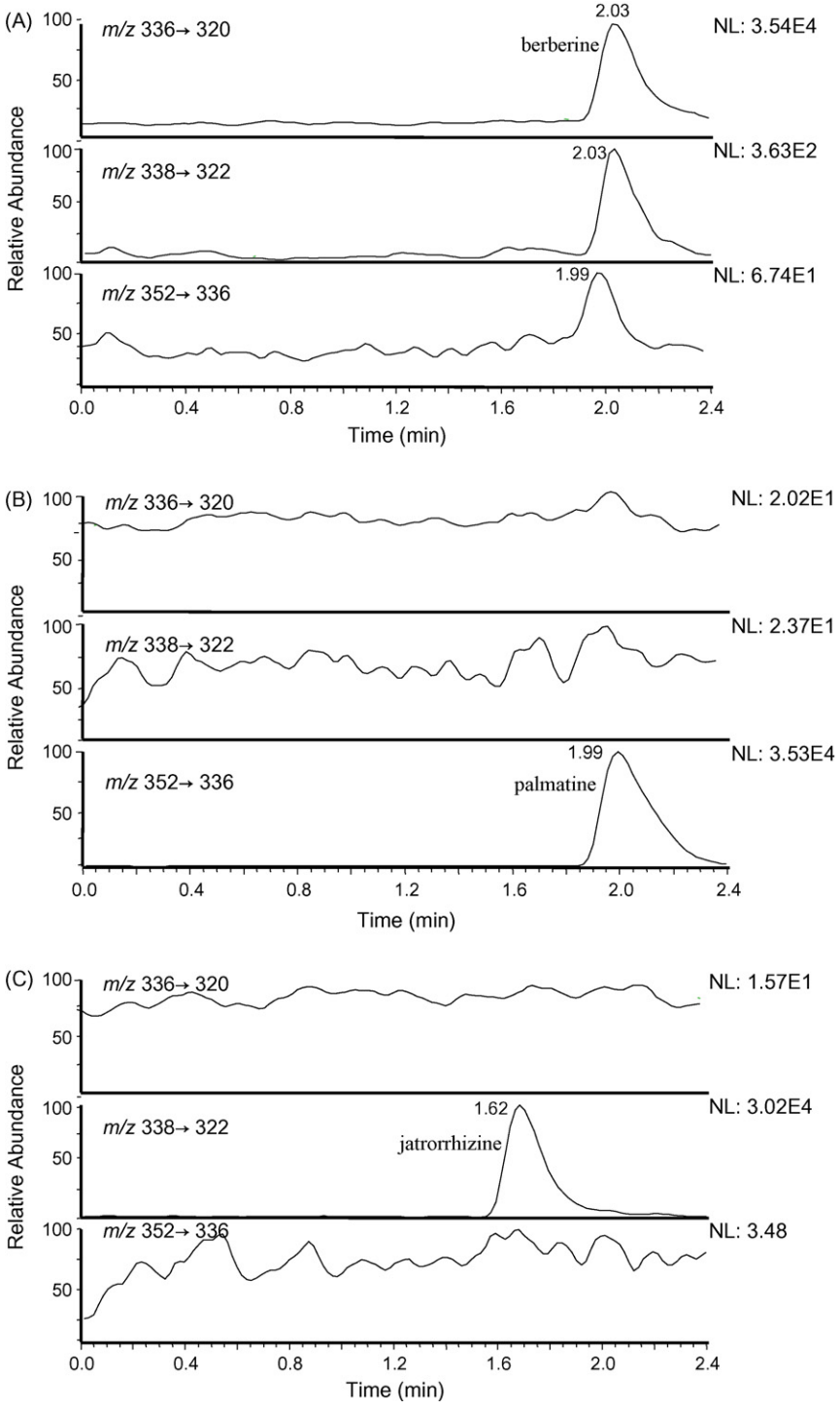


Fig. 4. Representative selected reaction-monitoring (SRM) chromatograms of berberine, palmatine and jatrorrhizine for cross-talk assessment. A blank mobile phase sample spiked with (A) berberine; (B) palmatine; (C) jatrorrhizine.

the response rate of the detector. This effect is most pronounced when consecutive SRM transitions monitor the same product ion *m/z* [25]. The second cross-talk results from fragmentation within the ion source (in-source CID) giving rise to multiple sources for the precursor ion selected by Q1. The three analytes have similar structures and the same fragmentation mechanism, and the SRM transitions selected were at *m/z* 336 → 320 for berberine, at *m/z* 352 → 336 for palmatine, and at *m/z* 338 → 322 for jatrorrhizine, so cross-talk may exist to affect the accuracy of quantification. As is shown in Fig. 4, slight cross-talk was observed between berberine and jatrorrhizine. However, this unremarkable interference did not significantly affect the behaviors of calibration curves or precision and accuracy data due to full chromatographic separation. Therefore, the developed analytical method was reliable and suitable for this study.

### 5.6. Results of pharmacokinetic study

After oral administration of coptis–evodia (6:1, g/g) (1.086 g powder/kg body weight) to six rats, plasma concentrations of berberine, palmatine and jatrorrhizine were simultaneously determined by the described LC–MS/MS method. The mean plasma concentration–time profiles ($n = 6$) are represented in Fig. 5. Three peaks were observed in both individual and mean plasma–concentration curves of berberine, palmatine and jatrorrhizine. It is the first time that multiple blood concentration peaks of berberine, palmatine and jatrorrhizine have been reported. The factors that may contribute to multiple blood concentration peaks are listed as follows:

Distribution re-absorption: if the concentration in tissues is much higher than that of the plasma, it is possible for the drug to transfer from tissues to plasma, which causes another peak in plasma. It was reported that while the berberine was absorbed, it was distributed rapidly with higher concentration in tissues [26].

Enterohepatic circulation: enterohepatic circulation of berberine in rats has been reported in earlier papers. However, no systematic research has been done so far [27]. Much work should be done to confirm whether it is an important cause for multiple blood concentration peaks.

Pharmacokinetic parameters are listed in Table 6. The terminal elimination half-life for berberine, palmatine and jatrorrhizine was 336.9 ± 46.5, 215.7 ± 33.5 and 325.3 ± 8.0 min, respectively. The time to reach the maximum plasma drug concentration was 90.00 ± 0 min for all components. There have been several reports on pharmacokinetic profiles of berberine after oral administration in rats [14,28], Li and Gao [28] reported that berberine was absorbed quickly and eliminated very slowly in rat body after oral administration of xiexin-tang decoction (composed of Radix Scutellariae, Radix et Rhizoma Rhei and Rhizoma Coptidis) at a dose of 20 mL/kg. The mean $t_{max}$ was 0.5 h and the higher concentration lasted for 4.5 h and formed a concentration platform. In addition, the plasma berberine concentration values of different rats fluctuated intensely from rat to rat. Tong and Yan [14] presented pharmacokinetic parameters of berberine and palmatine in rats after a dose of Huang-Lian-Jie-Du decoction (composed of Rhizoma Coptidis, Radix Scutellariae, Cortex Phellodendri and Fructus Gardeniae) as follows: $t_{max}$ was 2.5 ± 1.0, 2.4 ± 0.6 h and $t_{1/2}$ was 8.0 ± 2.9, 10.5 ± 2.8 h for berberine and palmatine, respectively. The results of the reports cited here are vastly different,

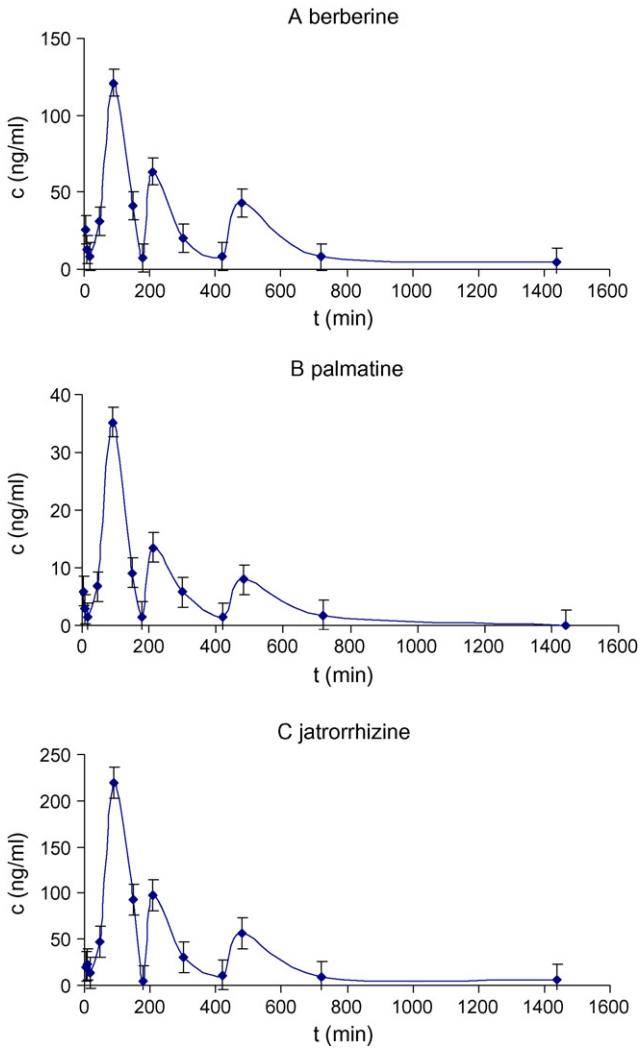


Fig. 5. Mean plasma concentration–time curves of berberine, palmatine and jatrorrhizine after oral administration of coptis–evodia powder (6:1, g/g) 1.086 g/kg body weight.

Table 6
Pharmocokinetic parameters of berberine, palmatine and jatrorrhizine after an oral administration of coptis–evodia powder (6:1, g/g) 1.086 g/kg body weight

| Component | $t_{max}$ (min) | $C_{max}$ (ng/mL) | $AUC_{0\rightarrow\infty}$ (ng min/mL) | $t_{1/2}$ (min) |
|---|---|---|---|---|
| Berberine | 90 ± 0 | 121.1 ± 11.9 | 28341.5 ± 5241.2 | 336.9 ± 46.5 |
| Palmatine | 90 ± 0 | 35.2 ± 9.5 | 6345.5 ± 133.9 | 215.7 ± 33.5 |
| Jatrorrhizine | 90 ± 0 | 219.9 ± 12.8 | 43576.9 ± 4767.8 | 325.3 ± 8.0 |

which may be explained by interaction among multitudinous compounds in formulas.

### 5.7. Means of administration

Zuojinwan is the formula of coptis–evodia powder (6:1, g/g). In our experiment, the coptis–evodia powder (6:1, g/g) was directly dissolved in 0.1% CMC-Na aqueous solution and administered to the rats. The dosage for rat (1.086 g/kg) was calculated by multiplying the clinical dose of people (6.0 g/kg) by the ratio of human body surface area to rat body surface area.

## 6. Conclusions

An LC–MS/MS assay has been developed for the simultaneous determination of berberine, palmatine and jatrorrhizine in rat plasma for the first time. Full validation including matrix effects and cross-talk indicated that the established method was sensitive, reliable and rapid with a total running time of 2.5 min for each sample. The LC–MS/MS assay was applied to the detailed pharmacokinetic studies of berberine, palmatine and jatrorrhizine in plasma. The described LC–MS/MS assay could be slightly modified to make it applicable for determination of other alkaloids of TCM in plasma, if needed.

## Acknowledgement

The authors would like to thank Grants 30600818 and 30600823 of the National Natural Science Foundation of China for providing financial support.

## References

[1] C.L. Lin, H.Y. Hsu. CA 25 (1992) 1.
[2] W. Yang, J. Basic Med. TCM 11 (2003) 49.
[3] S.J. Lin, H.H. Tseng, K.C. Wen, T.T. Suen, J. Chromatogr. A 730 (1996) 17.
[4] F. Zuo, Z.M. Zhou, M.L. Liu, Biol. Pharm. Bull. 24 (2001) 693.
[5] H.T. Liu, K.T. Wang, X.G. Chen, Z.D. Hu, Biomed. Chromatogr. 18 (2004) 288.
[6] Y. Li, J.P. Gao, X. Xu, J. Chin. Pharm. Sci. 14 (2005) 110.
[7] F. Zuo, Z.M. Zhou, Q.Z. Zhang, D. Mao, Y.L. Xiong, et al., Biol. Pharm. Bull. 26 (2003) 911.
[8] X.D. Ran (Ed.), Synopsis of Prescriptions of the Golden Cabinet, Chunqiu Publishing Company, Beijing, 1988, p. 157.
[9] A.P. Jiang, Beijing J. Trad. Chin. Med. 5 (2001) 45.
[10] X. He, Y.H. Shi, C.W. Shi, et al., Chin. Pharm. J. 33 (1998) 232.
[11] X.Z. Li, X. Chen, Y.J. Yang, Y. Ning, Z.Q. Huang, Chin. Pharm. J. 34 (1999) 107.
[12] T. Akao, K. Kawabata, E. Yanagisawa, K. Ishihara, Y. Mizuhara, et al., J. Pharm. Pharmacol. 52 (2000) 1563.
[13] J.H. Lee, J.M. Kim, C. Kim, J. Ethnopharmacol. 84 (2003) 5.
[14] L. Tong, L. Yan, J. Pharm. Biomed. 40 (2006) 1218.
[15] F. Zuo, N. Nakamura, T. Akao, et al., Drug Metab. Dispos. 34 (2006) 2064.
[16] T. Misaki, K. Sagawa, T. Ojima, S. Kakizawa, T. Oshima, H. Yoshizawa, Chem. Pharm. Bull. 30 (1982) 354.
[17] US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Guidance for Industry, Bioanalytical Method Validation, May 2001.
[18] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019.
[19] T.M. Annesley, Clin. Chem. 49 (2003) 1041.
[20] S. Souverain, S. Rudaz, J.-L. Veuthey, J. Chromatogr. A 1058 (2004) 61.
[21] C.R. Mallet, Z. Lu, J.R. Mazzeo, Rapid Commun. Mass Spectrom. 18 (2004) 49.
[22] H.R. Liang, R.L. Foltz, M. Meng, P. Bennett, Rapid Commun. Mass Spectrom. 17 (2003) 2815.
[23] R. Dams, M.A. Huestis, W.E. Lambert, C.M. Murphy, J Am. Soc. Mass Spectrom. 14 (2003) 1290.
[24] C. Muller, P. Schafer, M. Stortzel, S. Vogt, W. Weinmann, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 773 (2002) 47.
[25] T. Covey, B. Stott, in: Development of a High Sensitivity Triple Quadrupole Instrument for Quantitative Analysis. Proceedings of the 46th ASMS Conference on Mass Spectrometry and Allied Topics, Orlando, FL, 1998.
[26] Z.L. Xu, J. Sichuan Med. 1 (1959) 36.
[27] Q.M. Qu, M.F. Zhang, J. Chin. Acad. Med. Mag. Organ. (2002) 31.
[28] Y. Li, J.P. Gao, J. Chromatogr. B 838 (2006) 50.