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Determination of palmatine in canine plasma by liquid chromatography–tandem mass spectrometry with solid-phase extraction

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Abstract

A sensitive and specific high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS–MS) method has been developed and validated for the determination of palmatine in canine plasma. Palmatine and jatrorrhizine (internal standard, I.S.) were extracted from plasma samples by solid-phase extraction (SPE) using Oasis HLB cartridges. The chromatographic separation was performed on a Waters XTerra MS C_{18} reversed-phase column at 30 °C. The gradient mobile phase, delivered at 0.25 mL/min, was composed of a mixture of acetonitrile −0.1% (v/v) acetic acid aqueous solution adjusted to pH 2.8 with triethylamine. Positive electrospray ionization was utilized as the ionization source. Palmatine and the internal standard (I.S.) were determined using multiple reaction monitoring (MRM) of precursor → product ion transitions at *m/z* 352 → 336 and *m/z* 338 → 322, respectively. The lower limit of quantification (LLOQ) was 0.1 ng/mL using 100 μL plasma samples and the linear calibration range was from 0.1 to 500 ng/mL. The inter-day and intra-day RSDs were lower than 9.9% and the recoveries of palmatine ranged from 87.3 to 100.9%. The mean extraction recoveries of palmatine and the I.S. were 99.2 and 96.8%, respectively. The method has been successfully applied to the pharmacokinetic studies of palmatine in beagle dogs after oral administration and intramuscular injection of palmatine.

Keywords: Palmatine; LC–MS–MS; Pharmacokinetics

1. Introduction

Palmatine is an isoquinoline quaternary ammonium alkaloid extracted from a Chinese herb *Fibaurea recisa* Pierre. It possesses antibiotic activity against bacteria, fungi and viruses, and exerts vasodilatory, sedative, hepatoprotective and antitumor effects [1–6]. In China, palmatine (Fig. 1) has been widely used for the treatment of various inflammatory diseases, such as gynecological inflammation, bacillary dysentery, enteritis, respiratory tract infection, urinary infection, etc.

Clinically, palmatine is commonly administered by intramuscular or oral routes. However, little information about the pharmacokinetics of palmatine after intramuscular or oral administration is available. Since the plasma concentration of palmatine is extremely low after oral administration, it is necessary to develop a sensitive analytical method for the determination of palmatine in complex biological fluids.

High-performance liquid chromatography (HPLC) with UV has been developed for the determination of palmatine in plasma after intravenous administration, but the limit of detection (LOD) was 2 ng/mL, not sensitive enough for oral pharmacokinetic study [7]. A normal-phase (NP) HPLC method with fluorometric detection could obtain an LOD of 0.1 ng/mL [8]. However, the method used a considerable amount of deleterious organic solvents including dichloromethane as mobile phase and chloroform for extraction. Moreover, the linear range (0.1–10 ng/mL) was not broad enough for all samples

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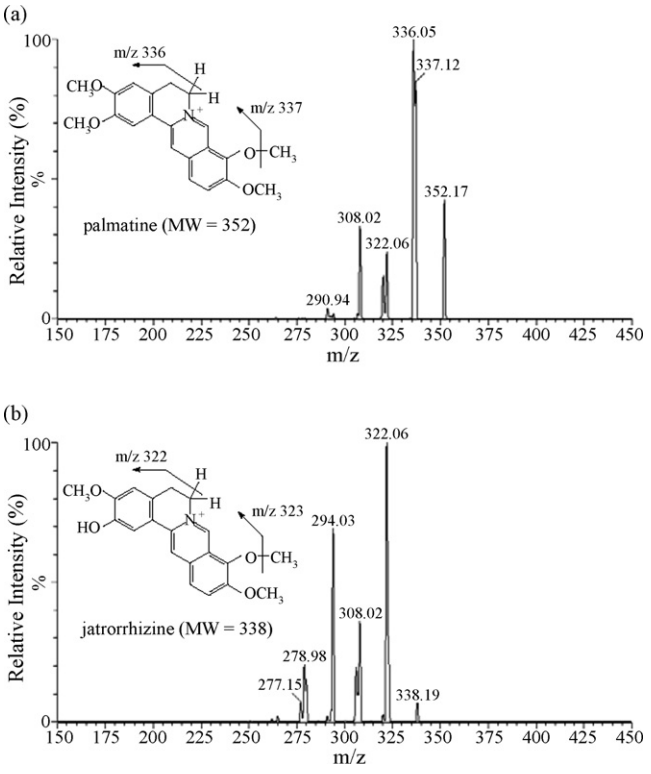

Fig. 1. Chemical structures and product ion mass spectra of molecular ions of palmatine (a) and jatrorrhizine (I.S., b).

when using the same volume of solvent to reconstitute. An additional drawback of the above methods was that at least 1 mL of plasma was needed to achieve the reported LOD. Recently, an HPLC–MS method using selected ion monitoring (SIM) mode was applied to the determination of palmatine in rat plasma after oral administration of Huang-Lian-Jie-Du decoction, in which palmatine was one of the main active components. The method had a lower limit of quantification (LLOQ) of 0.31 ng/mL with 100 μL of rat plasma sample, but the liquid–liquid extraction (LLE) method required a large volume of organic solvents (12.5 times the volume of the sample) to achieve acceptable extraction recovery (62.4–66.7%) [9].

HPLC–MS–MS is well known for its high sensitivity and specificity for the quantitation of drugs and metabolites in biological fluids. In the present work, a sensitive and reliable HPLC–MS–MS method, using multiple reaction monitoring (MRM) mode, was developed and validated for the determination of palmatine in canine plasma. Solid-phase extraction (SPE) using an Oasis HLB cartridge was employed for sample preparation. The method offers the advantages of higher sensitivity (LLOQ 0.1 ng/mL), increased extraction recovery (mean 99.2%), decreased organic solvent consumption, broad linear range and small sample volume (100 μL). The method has been successfully applied to the pharmacokinetic studies of palmatine in beagle dogs after oral administration and intramuscular injection of palmatine, and can be easily extended to the pharmacokinetic study of other species of animal, such as rats.

2. Experimental

2.1. Chemicals and materials

Palmatine chloride (>99% purity) was donated by Yunnan Shanyu Pharmaceutical Co. Ltd. (Yunnan, PR China) and internal standard jatrorrhizine chloride (>98% purity, Fig. 1) was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, PR China). Palmatine chloride tablet was produced by Yunnan Shanyu Pharmaceutical Co. Ltd. (Yunnan, PR China) and palmatine chloride injection was supplied by Kunming Pharmaceutical Co. Ltd. (Yunnan, PR China). Methanol and acetonitrile (HPLC grade) were purchased from Tedia Company Inc. (Fairfield, OH, USA), and other reagents and chemicals (AR grade) were from Shanghai Chemical Reagent Company (Shanghai, PR China). Deionized water was purified by a Milli-Q Reagent Grade Water System from Millipore (Millipore, Bedford, MA, USA). Oasis HLB cartridges (30 mg, 1 mL, Waters, Milford, MA, USA) were used in the procedure of SPE.

2.2. Instrumentation

Chromatographic separation was performed on an Alliance 2695 HPLC system (Waters, Milford, MA, USA) consisting of a quaternary pump, a column heater, an autosampler and a Millennium chromatographic work station. Detection was performed by a Quattro Premier triple-quadrupole mass spectrometer (Micromass, Manchester, UK) using electrospray ionization (ESI) for ion production. Masslynx software (version 4.0) was used for sample control, data acquisition and processing. A 10-ports SPE vacuum manifold system (Agilent, USA) was used for SPE.

2.3. LC–MS–MS conditions

The chromatographic separation was achieved on a Waters XTerra MS C_{18} reversed-phase column (5 μm, 50 mm × 2.1 mm) equipped with a guard column of XTerra C_{18} (5 μm, 10 mm × 2.1 mm). The column temperature was maintained at 30 °C. The mobile phase composed of a premix of solvent A (0.1%, v/v acetic acid aqueous solution adjusted to pH 2.8 with triethylamine) and solvent B (acetonitrile). The mixture was eluted at 0.25 mL/min by a gradient method: 20–65% B from 0 to 1.0 min, 65% B from 1.0 to 2.5 min, 65–90% B from 2.5 to 2.6 min and 90% B from 2.6 to 4.0 min. The temperature of the sample cooler in the autosampler was 4 °C.

ESI was operated in the positive ion mode with nitrogen as the nebulizer and drying gas. Collision-induced dissociation (CID) was achieved using argon as the collision gas. Following optimization of the settings, the instrument parameters were set at source block temperature of 100 °C, desolvation temperature of 450 °C, desolvation gas flow of 800 L/h, cone gas flow of 60 L/h, collision gas flow of 0.22 L/h, capillary voltage of 3.20 kV, cone voltage of 50 V, extractor of 1.0 V, RF lens voltage of 0.8 V, multiplier of 650 V. The collision energies for palmatine and I.S. were 25 and 30 eV, respectively.

Quantification was performed using multiple reaction monitoring (MRM) of precursor → product ion transitions at *m/z* 352 → 336 for palmatine and *m/z* 338 → 322 for the I.S., respectively. The dwell time was 200 ms.

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

Stock solutions of palmatine and jatrorrhizine (all about 1 mg/mL) were prepared in methanol–water (50:50, v/v). The standard working solutions of palmatine and jatrorrhizine were obtained by further dilution of the stock solutions with water. The I.S. working solution was a 100 ng/mL jatrorrhizine aqueous solution. All solutions were stored at 4 °C and brought to the room temperature before use.

Calibration standards and quality control (QC) samples during validation and pharmacokinetic study were prepared by spiking 100 μL of drug free plasma with 10 μL of standard working solutions of palmatine.

2.5. Sample preparation

A 100 μL aliquot of plasma sample was mixed with 0.5 mL of phosphate buffer (0.05 M, pH 7.2) and 50 μL of I.S. working solution and vortexed for 30 s. The mixture was transferred onto an Oasis HLB cartridge, preconditioned successively with 1 mL of acetonitrile, 1 mL of water and 1 mL of phosphate buffer (0.05 M, pH 7.2), and allowed to run through. The cartridge was washed successively with 1 mL of water and 1 mL of 30% methanol, and purged with air till complete drying of the washing solutions. Palmatine and I.S. were eluted from the cartridge with 0.8 mL of a mixture of acetonitrile–concentrated hydrochloric acid (99.5:0.5, v/v) and the eluate was evaporated to dryness at 40 °C under a stream of nitrogen. The residue was reconstituted with 100 μL of a mixture of acetonitrile −0.1% acetic acid (8:2, v/v) and then 10 μL was injected to the LC–MS–MS system for analysis.

2.6. Method validation

The calibration standards at nine levels over the concentration range of 0.1–500 ng/mL were extracted and assayed by the above-mentioned method. The calibration curves were constructed by the plots of the peak-area ratios of palmatine to I.S. (y) versus the concentrations of the calibration standards (x). A weighted ($1/x^2$) least-squares linear regression analysis was performed for palmatine and the calibration curve was repeated if the correlation coefficient was below 0.990. The concentrations of palmatine in unknown samples were determined by interpolation from the calibration curve.

The accuracy and the precision of the assays for intra-day and inter-day determinations were evaluated by the analysis of QC samples at the concentrations of 0.1, 0.5, 10 and 500 ng/mL ($n = 5$ for each concentration) on the same day and on 5 different days, respectively. These levels were chosen to demonstrate the performance of the method and to determine the LLOQ of the method. The upper limit of quantitation (ULOQ) was given by the highest level of the calibration curve. The accuracy was expressed as the relative recovery (%), calculated as 100 × (the observed concentration)/(the nominal concentration). The precision was expressed as the relative standard deviation (RSD).

The extraction recovery and the matrix effect for the analytes were determined by assaying three batches of samples: neat standard solutions of palmatine and the I.S. (batch 1), plasma extracts spiked with palmatine and the I.S. after extraction (batch 2), and plasma extracts spiked with palmatine and the I.S. before extraction (batch 3). Samples of each batch were prepared at four QC levels of 0.1, 0.5, 10 and 500 ng/mL. By comparing the absolute peak areas of the analytes obtained in batches 1–3, the extraction recoveries of palmatine and the I.S. were calculated as extraction recovery (%) = 100 × batch 3/batch 2, and the matrix effect of palmatine was calculated as matrix effect (%) = 100 × batch 2/batch 1.

The stability of reconstituted samples in autosampler vials was evaluated at 0 and 24 h under the autosampler conditions (4 °C). The short-term stability of palmatine in plasma samples was evaluated by determining the QC samples after being placed at room temperature (about 25 °C) for 2 h. To assess the stability of palmatine in plasma undergoing three freeze (−20 °C)-thaw (ambient) cycles during frozen storage at −20 °C, the QC samples at the concentrations of 0.1, 0.5, 10 and 500 ng/mL were analyzed on day 1 and day 15.

2.7. Application to pharmacokinetic study

Six male healthy beagle dogs weighing between 10.2 ± 0.4 kg were fasted for 12 h before dosing and continued for another 4 h after dosing. Water was freely available. Animal study was approved by the ethics committee for Animal Experimentation of Fudan University. For oral administration, a 300 mg palmatine tablet was given to each dog. Approximately 0.5 mL blood samples were withdrawn before dosing and at 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 24, 36, 48 and 72 h post-dosing. For intramuscular injection, the palmatine injections were given to dogs at a dose of 2 mg/kg. Blood samples were withdrawn before injection and at 0.083, 0.17, 0.33, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 36, 48 and 72 h after injection. All blood samples were taken into heparinized tubes and centrifuged at 5000 × g at 10 °C for 5 min to separate the plasma. The plasma obtained was stored at −20 °C until analysis.

2.8. Pharmacokinetic analysis

Pharmacokinetic analysis was performed with the software package WinNonlin Program (Version 5.1, Pharsight, Mountain View, CA, USA). Pharmacokinetic parameters were calculated with a noncompartmental method using the standard methods. The time to peak (T_{max}) and the peak concentration (C_{max}) were measured directly from the concentration–time curve. The area under the plasma concentration–time curve ($AUC_{0\text{-}t}$) was calculated by the linear trapezoidal rule. The extrapolated area under the curve ($AUC_{0\text{-}\infty}$) was calculated as $AUC_{0\text{-}\infty} = AUC_{0\text{-}t} + C_t/K_{el}$, where C_t is the last quantifiable concentration, t is the time of C_t and K_{el} is the

elimination rate constant determined by log-linear regression analysis on data points on the terminal log-linear phase. The terminal elimination half-life ($T_{1/2}$) was calculated as $0.693/K_{el}$. Relative bioavailability of oral administration to intramuscular injection was calculated by the formula $F\% = AUC_{0\text{-}\infty(po)} \times dose_{(im)}/AUC_{0\text{-}\infty(im)}/dose_{(po)}$.

3. Results and discussion

3.1. Optimization of LC–MS–MS

Chromatographic conditions, especially the composition of mobile phase, were optimized. Since the tandem mass spectrometer allows the specific detection of substances with varying masses or fragments, no or little chromatographic separation is required. Therefore, the selection of mobile phase was focused on good peak shape and appropriate ionization, as well as a short run time. Acetonitrile resulted in better peak shape than methanol, therefore, it was the preferable organic modifier. Modifiers, such as formic acid, acetic acid and acetic acid–triethylamine buffers at different pH values, were investigated. Acetic acid–triethylamine buffers could get the better peak shape than formic acid or acetic acid. Buffer at pH 2.8 gave the best peak shape and appropriate ionization. Gradient elution could improve the peak shape further and elute the residue of endogenous substances from the column.

In positive ion scan mode, palmatine and the I.S. gave molecular ions $[M]^+$ as the most intensive precursor ions, and *m/z* of their precursor ions equaled to their MW. Therefore, $[M]^+$ at *m/z* 352 and 338 were subjected to CID to determine the resulting product ions. The product ion mass spectra of palmatine and the I.S. are shown in Fig. 1. The major product ions at *m/z* 337 and 336 for palmatine were candidates for quantification. When the collision energy was below 20 eV, the product ion at *m/z* 337 was more abundant than the ion at *m/z* 336. However, the product ion at *m/z* 337 was not very stable and the adoption of this ion might cause reproducibility problems during quantitative analysis. With the elevation of collision energy, the abundance of the ion at *m/z* 336 exceeded that of the ion at *m/z* 337 gradually. The product ion at *m/z* 336 was relatively stable and the transition of *m/z* 352 → 336 had adequate intensity for our requirement. Therefore, the transition of *m/z* 352 → 336 was selected for quantification of palmatine. A similar phenomenon was observed in the major product ions at *m/z* 323 and 322 for the I.S., and the precursor → product ion transition of *m/z* 338 → 322 was selected for the I.S. A proposed fragmentation pattern for the formation of ions at *m/z* 336 and 322 is also shown in Fig. 1.

Compared with the reported LC–MS method using SIM mode (LLOQ 0.31 ng/mL) [9], the present LC–MS–MS method using MRM mode achieved higher sensitivity (LLOQ 0.1 ng/mL) and better specificity.

3.2. Optimization of sample preparation conditions

LLE and SPE have been used to extract palmatine from plasma. The high hydrophilicity of palmatine makes it difficult to obtain high recovery by LLE. Lu et al. used a large volume of organic solvents (12.5 times the volume of the sample) to achieve acceptable extraction recovery (62.4–66.7%) [9]. Our attempts with LLE also obtained unsatisfactory results and thus an SPE method was established in our earlier study [7]. However, we recently found that the recovery rates varied sometimes. The reason could be that the pH value of eluent (0.5% concentrated hydrochloric acid in acetonitrile) was less than 2, while the conventional C_{18} sorbent in SPE cartridge was unstable. In order to achieve reproducible and high analyte recoveries, we developed a new method based on our earlier approach.

Several stationary phases were initially evaluated in terms of extraction recovery of palmatine and RSD. The cartridges investigated were Phenomenex $C_{18,}$ Oasis HLB and Phenomenex Strata X. Oasis HLB was the best phase and selected for further investigation. The sorbent of Oasis HLB, a relatively new SPE sorbent, incorporates both a lipophilic divinylbenzene and a hydrophilic *N*-vinylpyrrolidone. The advantages of this polymeric cartridge are pH stability range from 0 to 14 for method development flexibility, and the non-requirement of wet cartridges prior to sample loading offering the convenience of continuous vacuuming that saves time in SPE procedure.

In order to establish the optimum conditions for the SPE procedure using Oasis HLB cartridges, the pH value of the sample, the composition of washing solutions, and the composition of eluting solutions were investigated.

To evaluate the effect of sample pH on the extraction recoveries, phosphate buffer solutions, at pH values of 5.0, 7.2, 9.0 and 11.0, were added to the standard plasma samples spiked with palmatine at the level of 500 ng/mL. The mixture was loaded into cartridges, washed with water and eluted with a mixture of acetonitrile–concentrated hydrochloric acid (99.5:0.5, v/v). For Oasis HLB cartridges, the good results were obtained at pH 5.0 and 7.2 with recoveries ranging from 92 to 99%. At higher pH values (pH 9.0 and 11.0), the recoveries decreased remarkably. In our experiments, the buffer pH chosen was 7.2.

Washing solvents with different percentage (5, 10, 20, 30 and 60%) of methanol in water were evaluated. The results indicated that 30% of methanol achieved the best clean-up results without loss of analytes. Composition of the eluting solutions adopted a mixture of acetonitrile–concentrated hydrochloric acid (99.5:0.5, v/v) according to our previous study.

Compared with the SPE method established in our earlier study [7], the SPE method described above achieved higher extraction recovery and better reproducibility of the recovery rate.

3.3. Plasma volume

Different volume of plasma sample (0.1–1 mL) was investigated in our preliminary experiments. Using 1 mL of plasma sample could obtain the highest sensitivity. However, it was difficult to obtain a good linearity over the concentration range of 0.1–500 ng/mL by linear regression with/without weighting fac-

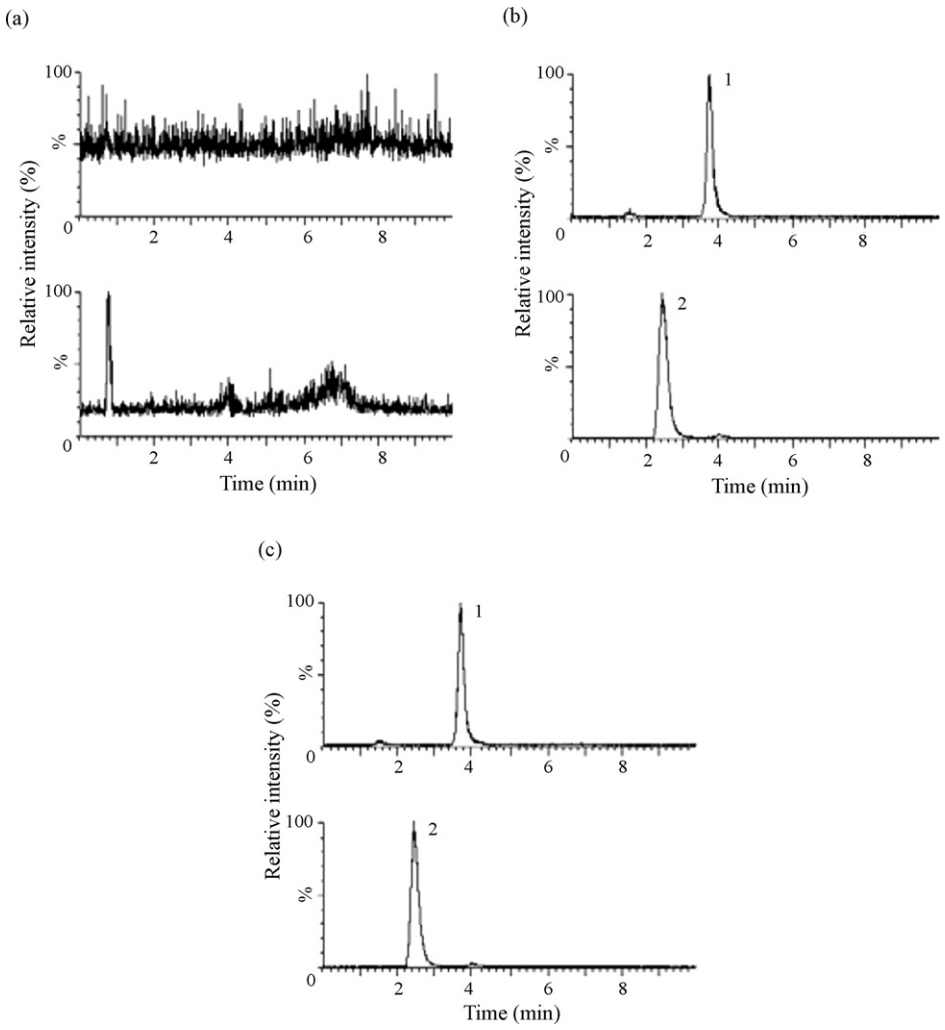

Fig. 2. Representative MRM chromatograms of (a) a blank canine plasma sample; (b) a blank canine plasma sample spiked with palmatine (10 ng/mL) and the I.S. and (c) a plasma sample from a dog after an oral administration of 300 mg palmatine. Peak 1: palmatine; peak 2: I.S.

tors. Using 0.1 mL of plasma sample, the calibration curve was linear over the concentration range 0.1–500 ng/mL, and thus not only the LLOQ but also the ULOQ could meet our requirement. Moreover, a small volume of plasma sample for assay reduced the needed blood volume from dogs. In addition, small volumes of plasma resulted in cleaner samples for analyzing, which was beneficial to instrument maintenance.

3.4. Method validation

3.4.1. Specificity

High specificity was found in MRM mode for the determination of palmatine in canine plasma samples. The representative MRM chromatograms of (a) a blank canine plasma sample, (b) a blank canine plasma sample spiked with palmatine (10 ng/mL) and the I.S., and (c) a plasma sample from a dog after an oral administration of 300 mg palmatine are illustrated in Fig. 2. Palmatine and the I.S. were eluted in 3.7 and 2.4 min, respectively. No interfering peak was eluted at the retention times of interest. In addition, 'cross-talk' was not observed between palmatine and the I.S.

3.4.2. Linearity and sensitivity

The nine-point calibration curve was linear over the concentration range of 0.1–500 ng/mL. The calibration model was selected based on the analysis of the data by linear regression with/without weighting factors, and the best linear fit and least-squares residuals for the calibration curve were achieved with a $1/x^2$ weighting factor. The mean regression equation was $y = 0.0059\ (\pm 0.0011)x + 0.0013\ (\pm 0.0006)$, $r = 0.9904$–0.9984, where y represents the peak-area ratios of palmatine to the I.S. and x represents the concentration of palmatine.

The LLOQ (S/N > 10), defined as the lowest concentration at which palmatine can be quantitated with an accuracy of 80–120% and a precision ≤20%, was 0.1 ng/mL. The LOD (S/N > 3) was 0.03 ng/mL.

3.4.3. Accuracy and precision

The data from QC samples were calculated to estimate the intra- and inter-day precision and accuracy of the method. The results are presented in Table 1. The intra- and inter-day % RSDs of palmatine detection ranged from 2.7 to 9.5 and from 6.2 to 9.9, respectively. The relative recoveries of the method for palmatine

Table 1
Summary of the precision and accuracy of the method ($n = 5$)

Spiked concentration (ng/mL)	Concentration found (ng/mL, mean ± SD)	Recovery (%)	RSD (%)
Intra-day			
0.1	0.10 ± 0.01	100.9	9.5
0.5	0.47 ± 0.03	93.0	5.9
10	9.02 ± 0.40	90.2	4.5
500	436.60 ± 11.94	87.3	2.7
Inter-day			
0.1	0.10 ± 0.01	97.0	9.9
0.5	0.45 ± 0.04	90.7	9.4
10	8.91 ± 0.73	89.1	8.2
500	485.69 ± 29.89	97.1	6.2

Table 2
Stability of palmatine in canine sample ($n = 3$)

Sample concentration (ng/mL)	Concentration found (ng/mL)	Recovery (%)	RSD (%)
Stability of the ready-to-inject samples (4 °C, 24 h)			
0.1	0.10 ± 0.01	98.4	8.5
0.5	0.44 ± 0.02	88.8	4.9
10	9.38 ± 0.93	93.8	9.9
500	473.97 ± 38.21	94.8	8.1
Short-term stability (about 25 °C, 2 h)			
0.1	0.11 ± 0.01	106.8	6.2
0.5	0.48 ± 0.01	96.9	1.2
10	8.97 ± 0.49	89.7	5.5
500	440.42 ± 15.18	88.1	3.5
Long-term stability (−20 °C, 15 days) and freeze-thaw stability			
0.1	0.10 ± 0.01	103.7	12.4
0.5	0.47 ± 0.02	94.2	3.7
10	9.77 ± 0.85	97.7	8.7
500	486.11 ± 24.23	97.2	5.0

ranged from 87.3 to 100.9% for intra-day, and from 89.1 to 97.1% for inter-day, respectively. These results were within the acceptable criteria for precision and accuracy.

3.4.4. Extraction recovery

The extraction recoveries for palmatine were 96.2, 95.9, 103.3 and 101.5% for QC samples at the concentration of 0.1, 0.5, 10 and 500 ng/mL, respectively. The mean recovery for palmatine was 99.2%. The extraction recovery of the I.S. was also evaluated and the mean recovery was 96.8%. The RSDs for all recoveries were less than 9.6%.

3.4.5. Matrix effect

The average matrix effect values obtained were 103.1, 94.4, 101.8 and 99.0% for QC samples at concentration of 0.1, 0.5, 10 and 500 ng/mL, respectively. The results indicated that the extracts had little or no detectable co-eluting compounds that could influence ionization of palmatine.

3.4.6. Stability

In the reconstitution solution, palmatine was stable under the autosampler conditions (4 °C) for at least 24 h. In plasma, palmatine was stable after being placed at room temperature (about 25 °C) for 2 h and after undergoing three freeze-thaw cycles during frozen storage at −20 °C for 15 days. Stability data are summarized in Table 2. The stability results in our study and the published papers [7,9] showed that palmatine had good stability in plasma samples.

3.5. Application

The method was successfully applied to the determination of palmatine in canine plasma after oral administration of 300 mg palmatine and intramuscular injection of 2 mg/kg palmatine. Fig. 3 displays the mean plasma concentration–time profiles ($n = 6$). It showed that the concentrations of palmatine in canine plasma were quantifiable for at least 72 h ($C = 0.64 \pm 0.30$ ng/mL) after the oral administration of palmatine, and the peak plasma concentrations of palmatine after intramuscular injection ($C = 334 \pm 35$ ng/mL) could also be determined accurately.

The main pharmacokinetic parameters of palmatine in beagle dogs after intramuscular and oral administration were reported for the first time in this paper (Table 3). Following intramuscular injection, palmatine was absorbed rapidly with a T_{max} of 0.2 h. Afterwards, the plasma concentration decreased rapidly. Following oral administration, fluctuation was observed in the concentration–time profile. The absorption appeared slow with

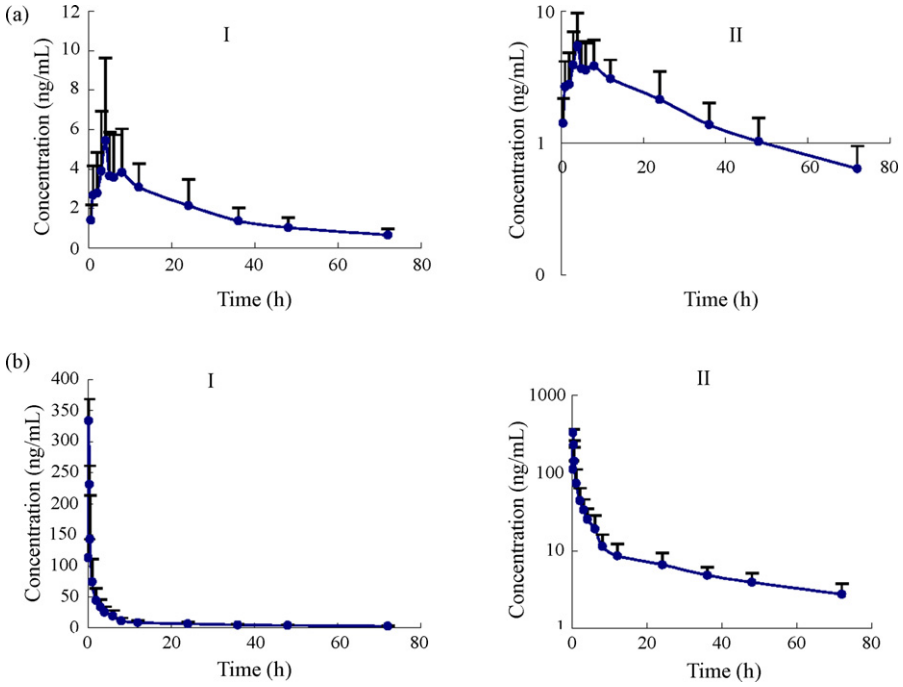

Fig. 3. Mean (±SD) plasma concentration–time profiles of palmatine in dogs ($n = 6$) following (a) an oral administration of 300 mg palmatine and (b) an intramuscular injection of 2 mg/kg palmatine. I: a standard scale for concentration; II: a logarithm scale for concentration.

Table 3
The pharmacokinetic parameters of palmatine in beagle dogs following an intramuscular injection of 2 mg/kg palmatine and an oral administration of 300 mg palmatine ($n = 6$)

Pharmacokinetic parameter	Intramuscular injection	Oral administration
$AUC_{0\text{-}\infty}$ (ng h/mL)	888.7 ± 285.5	161.1 ± 51.4
$AUC_{0\text{-}t}$ (ng h/mL)	690.9 ± 185.7	128.3 ± 43.0
C_{max} (ng/mL)	334.0 ± 34.5	8.0 ± 2.6
T_{max} (h)	0.2 ± 0.0	5.0 ± 1.8
K_{el} (h)	0.019 ± 0.009	0.027 ± 0.013
$T_{1/2}$ (h)	44.6 ± 20.9	31.6 ± 15.8

a T_{max} of 5 h. After T_{max}, the plasma concentration decreased slowly. Elimination half-lives ($T_{1/2}$) after intramuscular and oral dosing were 49 and 56 h, respectively. This value is close to the reported data in dogs after intravenous injection with a $T_{1/2}$ of 60 h [8]. The relative bioavailability of oral administration to intramuscular injection was only 1.31%.

4. Conclusions

In this study, a sensitive and specific HPLC–MS–MS method for the determination of palmatine in canine plasma was developed and validated. The SPE method using Oasis HLB cartridges achieved reproducible and high extraction recoveries of palmatine and the I.S. (jatrorrhizine). Compared with other reported methods, the method has the advantages of higher sensitivity, broad linear range and increased extraction recovery. It has been successfully applied to the pharmacokinetic studies of palmatine in beagle dogs after oral administration and intramuscular injection of palmatine.

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