Journal of Pharmaceutical and Biomedical Analysis 64–65 (2012) 1–7

Contents lists available at SciVerse ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Microdialysis combined with liquid chromatography–tandem mass spectrometry for the determination of levo-tetrahydropalmatine in the rat striatum

Chen Wang[a,b,c,1], Shu Li[d,1], Yuanjun Tang[a,b], Shuowen Wang[a,b], Yalin Zhang[d], Guorong Fan[a,b,*], Liqin Li[c,**], Yi Zhang[d,**]

[a] Department of Pharmaceutical Analysis, School of Pharmacy, Second Military Medical University, No. 325 Guohe Road, Shanghai 200433, PR China
[b] Shanghai Key Laboratory for Pharmaceutical Metabolite Research, No. 325 Guohe Road, Shanghai 200433, PR China
[c] Beijing Research Institute of Pharmaceutical Chemistry, Beijing 102205, PR China
[d] Department of Pharmacy, Navy General Hospital, Beijing 100048, PR China

ARTICLE INFO

Article history:
Received 3 October 2011
Received in revised form 11 January 2012
Accepted 12 January 2012
Available online 11 February 2012

Keywords:
Microdialysis
LC–MS/MS
Levo-tetrahydropalmatine
Pharmacokinetics

ABSTRACT

Levo-tetrahydropalmatine (*l*-THP), one of the main active alkaloids isolated from *Rhizoma corydalis*, was recently found to elicit profound effects on the dopaminergic system in the striatum, which plays an important role in regulating nociception. A rapid and sensitive method based on microdialysis combined with liquid chromatography–tandem mass spectrometry was developed for the determination of *l*-THP in the rat striatum. Microdialysis probes were stereotactically placed in the striatal hemisphere, and *l*-THP was measured from the microdialysates collected using LC–MS/MS. Reverse-phase LC separation was accomplished on a Diamonsil™ C18 column (50 mm × 2.1 mm ID, 5 μm) with the mobile phase composed of methanol–water (50:50, v/v) at a flow rate of 0.2 ml/min. The method had a chromatographic total run time of 5 min. Detection was performed in electrospray positive mode and quantification was executed in selected reaction monitoring mode. The following transitions were monitored: *m*/*z* 356.0 → 191.9 for *l*-THP and 256.0 → 167.1 for the internal standard diphenhydramine. The method was sensitive with a lower limit of quantitation (LLOQ) of 0.1 ng/ml for *l*-THP, with good linearity in the range of 0.1–1000 ng/ml ($r^2 \geq 0.999$). All the validation data, such as accuracy, precision, and inter-day repeatability were within the required limits. The method was successfully applied to pharmacokinetic study of the *l*-THP in the rat striatum.

1. Introduction

In vivo analysis traditionally involves collection of tissue or bodily fluids such as blood in order to investigate the metabolism and final disposition of a pharmaceutical compound. However, collection of tissues is limited by its requirement of at least one animal per time point. The tissue must be also homogenized and complicated sample clean-up performed before analysis. Microdialysis (MD) is a catheter-based sampling technique that is used to collect only the free fraction of the drug, the therapeutically active portion of the dose can be monitored [1]. The size exclusion properties of the probe also simplify any sample clean-up steps that would normally be needed before analysis of tissue samples [2]. In addition, instead of acquiring data at discrete time points in traditional methods, microdialysis is a continuous process. Recent reports have demonstrated that microdialysis is an excellent method for *in vivo* sampling and determination of unbound drug concentrations in plasma [3–5], tissue samples [5,6], and other biological fluids [7,8] for pharmacokinetic studies.

Rhizoma corydalis, the dried tuber of *Corydalis yanhusuo* W.T. Wang, named as Yuanhu in Chinese, has been widely used as medicinal herb to treat spastic pain, abdominal pain, and pain due to injury [9]. Levo-tetrahydropalmatine (*l*-THP), one of the main active alkaloids isolated from *R. corydalis*, was recently found to elicit profound effects on the dopaminergic system in the striatum [10–12], which plays an important role in regulating nociception [13,14]. Therefore, studies of the pharmacokinetics of the *l*-THP in the striatum are of great sense which can provide valuable information for understanding the mechanisms by which *l*-THP alleviates nociception.

Early publications have described methods for the determination of tetrahydropalmatine in plasma for pharmacokinetic studies [15–21]. However, drug concentrations in plasma may not well

* Corresponding author at: Second Military Medical Univ., School of Pharmacy, 325 Guohe Road, Shanghai 200433, China. Tel.: +86 21 8187 1260, fax: +86 21 8187 1260.
** Cocorresponding authors.
E-mail addresses: Guorfan@yahoo.com.cn (G. Fan), llq969696@163.com (L. Li), yizhang229@163.com (Y. Zhang).
[1] These authors contributed equally to this work.

0731-7085/$ – see front matter. Crown Copyright © 2012 Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2012.01.016

reflect the pharmacologic effects since sites of action may not be immediately accessible to drugs, especially for CNS (central nervous system) drugs because of drug–blood–brain barrier (BBB) interactions [22,23]. The pharmacokinetic profile of THP enantiomers in rat brain were investigated by Hong et al. using a LC method, however, it needed five rats per time point [24]. The disadvantages of the method are obvious: (1) a large number of rats were killed; (2) the data of every time point constructing the concentration–time curve was the average values from five rats instead of from one rat.

In the present study, a method based on microdialysis combined with liquid chromatography–tandem mass spectrometry was developed for the determination of *l*-THP in the rat striatum. To the best of our knowledge, studies on microdialysis combined with liquid chromatography–tandem mass spectrometry in the analysis of *l*-THP in the rat striatum have not yet been reported.

2. Experimental

2.1. Chemicals and reagents

Levo-tetrahydropalmatine sulfate (optical purity ≥ 99.5%) was provided by Nanning Pharmaceuticals (Guangxi, China). Diphenhydramine hydrochloride (IS) was obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). $CaCl_2$, Na_2HPO_4, $MgCl_2$, KCl and NaCl were purchased from WuLian Chemical Factory (Shanghai, China). Methanol (chromatographic grade) was purchased from Merck (Darmstadt, German). Deionized (18 MΩ/cm) water was generated in-house using a Milli-Q System from Millipore (Bedford, MA, USA).

Sprague-Dawley rats were purchased from Shanghai SLAC Laboratory Animal Co. Ltd. (Shanghai, China). All experiments were approved by our local Ethics Committee. Furthermore, all animals received human care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals, which was prepared by the National Academy of Sciences and published by the National Institutes of Health.

2.2. Apparatus and instruments

A Varian LC–MS/MS system (Palo Alto, CA, USA) consisted of a ProStar 410 autosampler, two ProStar 210 pumps, and a 1200l triple quadrupole mass spectrometer equipped with an electrospray ionization source. Varian MS workstation version 6.3 software was used for data acquisition and processing.

Microdialysis sampling was performed by using a BAS microdialyser (BAS Microdialysis,West Lafayette, USA). Microdialysis probes were prepared using single hollow cellulose fiber (200 μm inner diameter, i.d.; 220 μm outer diameter, o.d.; 5 kDa, DM-22 dialyzer, Eicom Ltd., Kyoto, Japan). The fiber was glued with cyanoacrylate glue (Cyberbond Co., Batavia, IL, USA) at both ends to a piece of quartz capillary tubing (98 μm i.d.; 165 μm o.d.; Polymicro Technologies Ltd., USA) [25].

2.3. Liquid chromatographic conditions

The chromatographic separation was performed on a Diamonsil™ C18 column (50 mm × 2.1 mm ID, 5 μm particle size, Dikma Technologies Company, China) thermostated at 30 °C with the mobile phase composed of methanol–water (50:50, v/v) at a flow rate of 0.2 ml/min. Before use, the mobile phase was filtered through a 0.45 μm nylon membrane filter. The injection volume was 10 μl and the analysis time was 5 min per sample.

2.4. Mass spectrometer conditions

The ESI-MS was operated in the positive ion mode. Nitrogen was used as a drying gas for solvent evaporation. The drying gas temperature was kept at 300 °C. Protonated analyte molecules were subjected to collision induced dissociation using argon as the collision gas to yield product ions for each analyte. The collision energy was 25 eV for *l*-THP and 10 eV for the diphenhydramine (IS). The scan time was 1 s and the detector multiplier voltage was set to 1650 V. Selected reaction monitoring of the precursor–product ion transitions m/z 356.0 → 191.9 for analyte and 256.0 → 167.1 for IS was used for quantitation.

In order to prevent contamination of the ion source of MS equipment by the salts in ACSF, the first 2 min of effluent was directed into a waste bottle.

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

A stock solution of 1 mg/ml of *l*-THP in methanol was used to prepare the calibration samples and quality control (QC) samples. This stock solution was prepared weekly and the working solutions were diluted with ACSF solutions (artificial cerebrospinal fluid, composition in mM: 1.2 $CaCl_2$, 2.0 Na_2HPO_4, 1.0 $MgCl_2$, 2.7 KCl, 145 NaCl, pH 7.4) to appropriate concentrations daily. IS was prepared at a concentration of 200 ng/ml in methanol. Calibration standard solutions (*l*-THP concentrations: 0.1, 0.3, 1.0, 3, 10, 30, 100, 300 and 1000 ng/ml) were prepared in ACSF prior to each analytical run. QC samples, which were used in the validation and during the pharmacokinetic study, were prepared from a different stock solution of *l*-THP by independent dilution at three levels for *l*-THP: 0.2, 10 and 800 ng/ml. Two microliters of IS was added to 40 μl of working solution before LC–MS/MS analysis. Calibration curves were established based on the peak area ratios of *l*-THP to IS against nominal *l*-THP concentration using weighted ($w = 1/x$) linear regression analysis.

2.6. In vivo microdialysis procedure

Microdialysis probes (membrane length = 3 mm) were stereotactically placed in the striatal hemisphere using the stereotaxic flat skull coordinates [26] [anterioposterior (AP), 1.7 mm; mediolateral (ML), 3.0 mm; and dorsoventral (DV), 6.4 mm)] after anesthesia induction (25% urethane, 0.4 ml/100 g i.p.). Microdialysis probes were perfused with ACSF at a constant flow rate of 1.5 μl/min. Probes were allowed to equilibrate for 60 min before beginning sample collection. The dialysis samples were collected at every 30 min. After the experiments, the rats were decapitated. The correct localizations of the probe were verified and only the rats with appropriate probe placement were included in the experiment. Prior to analysis, 2 μl of the IS working solution was added to 40 μl of dialysates, and *l*-THP was measured using LC–MS/MS system.

2.7. Recovery of microdialysate

For *in vivo* recovery determinations, a retrograde calibration technique was used. Microdialysis probes were inserted into the rats striatum under anesthesia with urethane. After an hour post-surgical stabilization period, the ACSF perfusion solution was switched to solutions containing *l*-THP. For the evaluation of the effects of *l*-THP concentrations on *in vivo* recovery, ACSF solution containing *l*-THP (0.1, 1 and 10 μg/ml, respectively) was perfused through the probe at a constant flow rate of 1.5 μl/min. For the evaluation of with-in day stability of *in vivo* recovery, ACSF solution containing *l*-THP (1 μg/ml) perfused through the probe at a constant flow rate of 1.5 μl/min. The dialysis samples were collected at every 30 min. Dialysates entering (C_{in}) and leaving the probe

were analyzed by LC–MS/MS. The relative recoveries of *l*-THP were estimated by the equation, $\text{recovery}_{in\ vivo} = (C_{in} - C_{out})/C_{in}$ [27].

2.8. Pharmacokinetic studies

Six SD rats (3 males and 3 females, weighting 220 ± 7 g) were fed with certified standard diet and tap water *ad libitum*. Temperature and humidity were regulated at 21–23 °C and 30–60%, respectively. A light/dark cycle of 12 h on/12 h off was established. After 1 week of acclimatization, microdialysis probes were stereotactically placed in the striatal hemisphere. After probe equilibration, rats received (i.g., intragastric administration) *l*-THP (40 mg/kg). The dialysates were collected every 30 min after *l*-THP administration and stored at −20 °C prior to analysis.

Calibration curves were constructed based on LC–MS/MS analyses of various concentrations of *l*-THP, and the concentrations of *l*-THP in dialysates from rat striatum were calculated from the calibration curves. The concentration–time data were analyzed by non-compartmental method using the Bioavailability Program Package (BAPP, Version 2.0, Center of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University). The maximum concentration (C_{max}) and the time to reach C_{max} (T_{max}) were obtained directly from the concentration–time data. Area under the concentration–time curve from time zero to the last sampling time ($AUC_{0-\tau}$) was calculated by the trapezoidal rule. $AUC_{0-\infty}$ values were estimated by the combination of $AUC_{0-\tau}$ and $AUC_{\tau-\infty}$. Mean residence time (MRT) was estimated from AUMC/AUC.

3. Results and discussion

3.1. Recovery of microdialysate

The recoveries of microdialysate were investigated and the results (Fig. 1) indicated that *l*-THP recovery was independent on the concentration range investigated (0.1–10 μg/ml) and the performance of the microdialysis system was stable over an 8 h study, resulting in a mean *in vivo* recovery of 12.9 ± 1.4%.

3.2. LC–MS/MS optimization

Parameters of MSD (mass spectrometer detector) were tuned according to the MS signal response of the target compound and the results indicated that the positive mode was much more sensitive than the negative mode. It is necessary to use an IS to get high accuracy when a mass spectrometer is equipped with LC as the detector. Diphenhydramine was adopted in the end because of its similarity of retention action, ionization as well as its less endogenous interference at *m*/*z* 256.0. In the positive ESI mode, the analyte and IS formed predominately protonated molecular ions $[M+H]^+$ (*m*/*z* 356.0 and *m*/*z* 256.0) in full scan mass spectra. In the product ion spectra, several fragment ions were obtained, but the ion at *m*/*z* 191.9 and 167.1 were chosen because they displayed much greater intensity than the others in the acquisition of *l*-THP and IS, respectively. Product ion mass spectra for analyte and IS are shown in Fig. 2.

The chromatographic conditions, especially the composition of mobile phase, were optimized through several trials to achieve good resolution and symmetric peak shapes for the analyte and the IS, as well as a short run time. Modifiers, such as ammonium acetate (0.1%, 0.5%) and formic acid (0.1%, 0.5%) were added. The results indicated that ammonium acetate in the mobile phase did not affect the ionization efficiency of *l*-THP and IS. When mobile phase containing formic acid (0.5%) was used, the ionization efficiency of *l*-THP and IS can be enhanced, however, the peak was tailed. At last, methanol–water (50:50, v/v) was adopted as the mobile phase. After careful comparison of many columns (Ultimate XB-C18, 50 mm × 2.1 mm ID, 5 μm; Zorbax Eclipse Plus, 50 mm × 2.1 mm ID, 5 μm; Zorbax SB-C18 column, 100 mm × 3.0 mm, 3.5 μm; and Lichrospher C18 column, 50 mm × 4.6 mm ID, 5 μm), a Diamonsil™ C18 column (50 mm × 2.1 mm ID, 5 μm) was finally used with a flow rate of 0.2 ml/min to produce good peak shapes and permit a run time of 5 min.

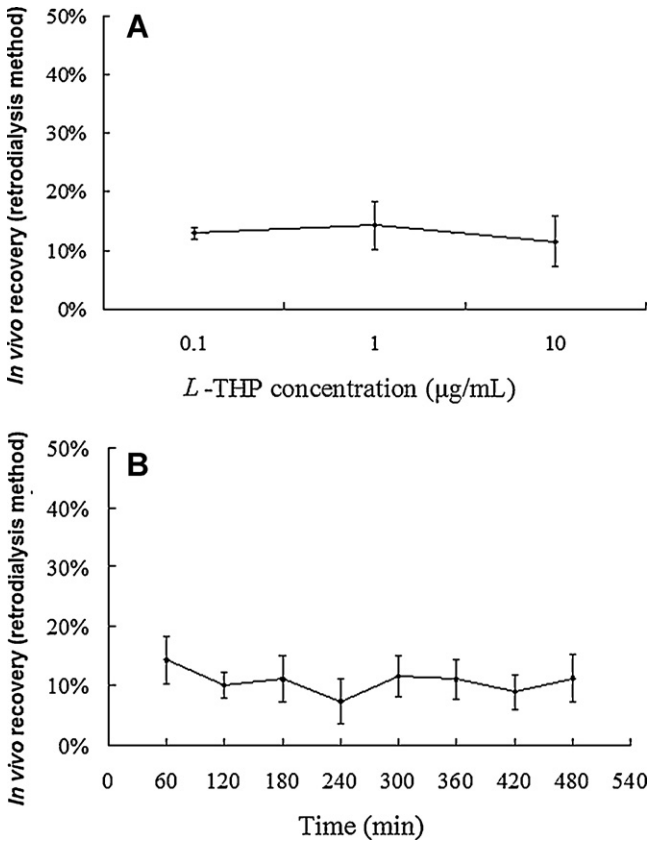

Fig. 1. Effects of *l*-THP concentrations (0.1, 1, and 10 μg/ml) on the *in vivo* recovery from the microdialysis probe ($n = 3$ each point) (A) and within-day stability of *in vivo* recovery (*l*-THP concentration, 1 μg/ml; $n = 3$) (B). Data were presented as means ± SD for three probes.

3.3. Selectivity

The selectivity of the method was tested by comparing the chromatograms of blank microdialysates ($n = 5$) and the blank microdialysates spiked with *l*-THP at LLOQ (0.1 ng/ml, $n = 5$). All blank microdialysates lots were found to be free of interferences with the compounds of interest. Under the above conditions the retention time of *l*-THP and IS was 3.8 and 2.6 min, respectively (Fig. 3).

Matrix effects are generally caused by molecules originating from the sample matrix that coelute with the compounds of interest. These molecules can interfere with the ionization process in the mass spectrometer, causing ionization suppression or enhancement. These unpredictable effects cause a compound's response to differ when analyzed in a biological matrix compared to a standard solution. Careful consideration must be given to evaluate and eliminate matrix effects when developing an assay [28]. There are two common methods to assess matrix effects: the post-column infusion method [29] and the post-extraction spike method [30]. In the present study, first of all, the post-column infusion method was used to evaluate the matrix effects. Blank microdialysates (10 μl) were injected, and ACSF solution containing *l*-THP and IS (10 ng/ml, both) was post-column infused at a flow rate of 0.05 ml/min. From

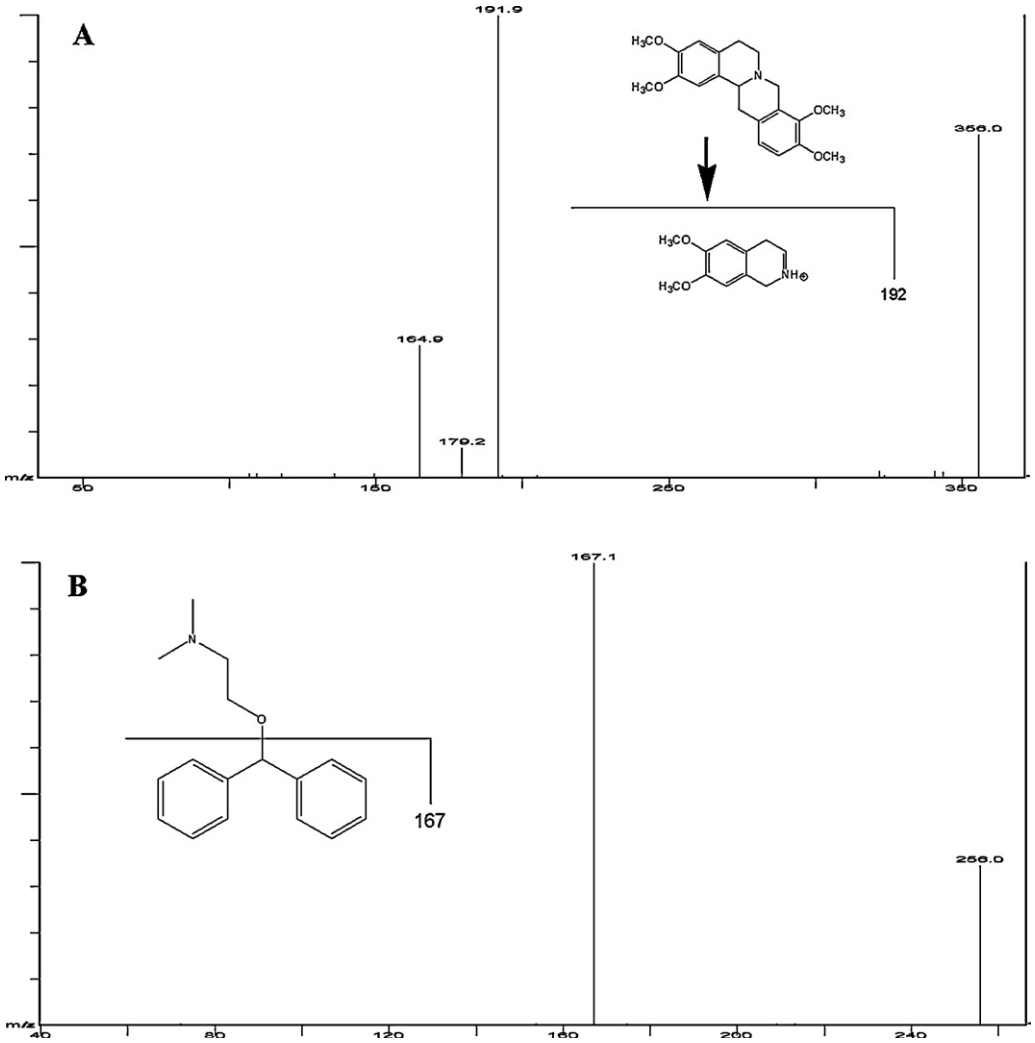

Fig. 2. Chemical structures and product ion spectra of $[M+H]^+$ of *l*-THP (A) and diphenhydramine (B).

Fig. 4, we found that endogenous peaks at the retention time of the analytes of interest were not observed in any of the microdialysates lots ($n = 5$) evaluated, and ion suppression or enhancement from matrix was negligible. Secondly, the post-extraction spike method was applied to determine the matrix effects. The matrix effects were expressed as the ratio of the mean peak area of analytes spiked in blank microdialysates to that of neat standards at different concentrations (0.2, 10 and 800 ng/ml, respectively, $n = 5$). By the analyses of the samples at QC concentrations, matrix effect values were calculated. Average matrix effect values obtained were 97.4% (CV = 3.9%, $n = 5$), 104.6% (CV = 2.1%, $n = 5$) and 98.1% (CV = 2.7%, $n = 5$) for *l*-THP (0.2, 10 and 800 ng/ml, respectively) and 96.9% (CV = 2.1%, $n = 5$) for IS (10 ng/ml). Therefore, ion suppression or enhancement from matrix was negligible for the present method.

3.4. Sensitivity and linearity

The lower limit of quantitation (LLOQ) of the assay, defined as the lowest concentration on the standard curve that can be quantitated with accuracy within 20% of nominal and precision not exceeding 20% CV, was 0.1 ng/ml. The reproducibility of LLOQ was determined by examining five LLOQ samples independent from the standard curve, and the accuracy and precision was 12.6% and 7.0%, respectively. A typical chromatogram of an LLOQ sample is shown in Fig. 3C.

Calibration curves were constructed by plotting the peak area ratios (*l*-THP/IS) of microdialysates standards *vs.* nominal concentration. The calibration model was selected based on the analysis of the data by linear regression with/without intercepts and weighting factors ($1/x$, $1/x^2$ and none). The best linear fit and least-squares residuals for the calibration curve were achieved with a $1/x$ weighting factor, giving a mean linear regression equation for the calibration curve of: $y = 0.05297x + 0.001$, $r^2 = 0.9997$ ($n = 5$), where y represents the peak area ratios of *l*-THP to that of IS, and x represents the concentration of *l*-THP in ng/ml. Calibration curves were linear in the range 0.1–1000 ng/ml with $r^2 \geq 0.999$. Samples with *l*-THP concentrations exceeding the range were diluted appropriately with ACSF and re-assayed. The difference between the nominal standard concentration and the back-calculated concentration from the weighted linear regression line varied from −9.5% to 14.9% for each point on the standard curve (CV varied from 1.5% to 6.9%).

3.5. Accuracy and precision

The method showed good accuracy and precision. Table 1 shows a summary of intra- and inter-day accuracy and precision for *l*-THP from the QC samples, respectively. In this assay, the intra-day precision was less than 1.8%, the inter-day precision was less than 4%. The accuracy ranged from 95.2 to 111.7%.

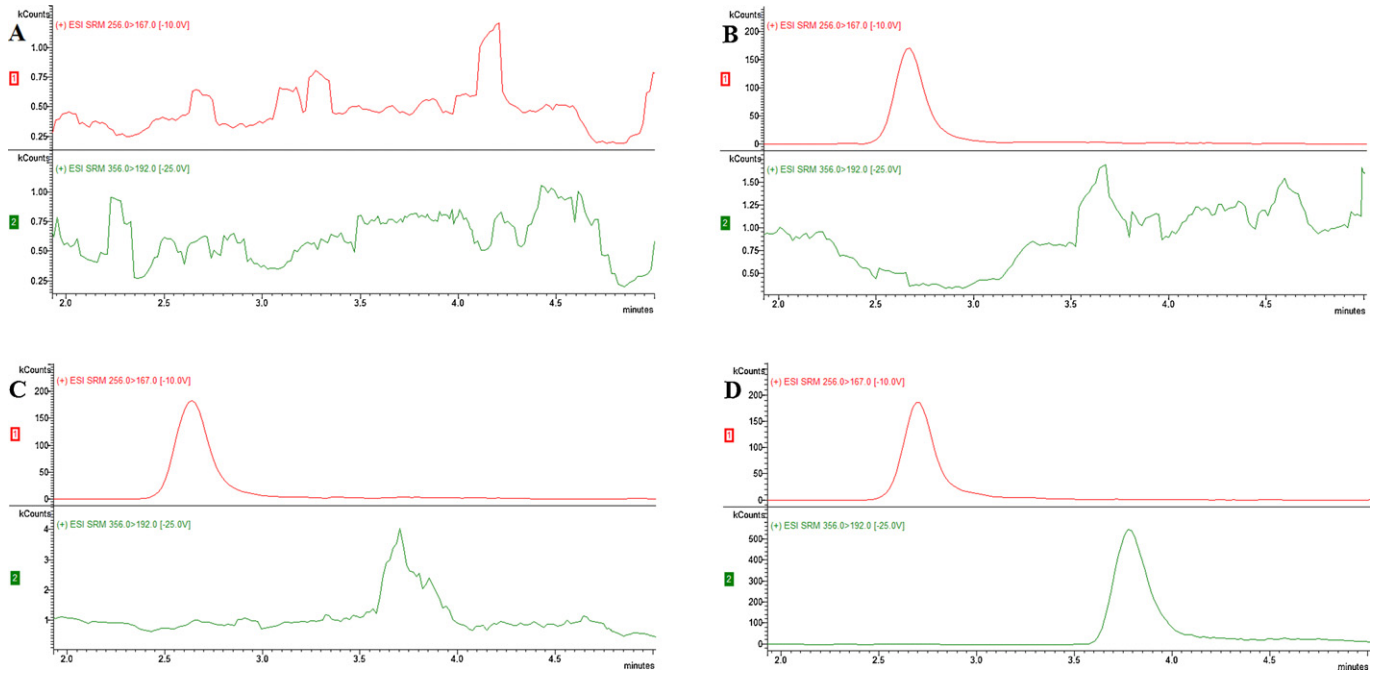

Fig. 3. Representative chromatograms (channel 1 is for IS and 2 for *l*-THP): (A) control microdialysis blank sample; (B) IS standard solution; (C) *l*-THP (LLOQ) and IS (10 ng/ml); and (D) microdialysate sample collected 7.5~8 h after an intragastric administration of *l*-THP (40 mg/kg). The measured concentration of *l*-THP in the microdialysate was 139.4 ng/ml.

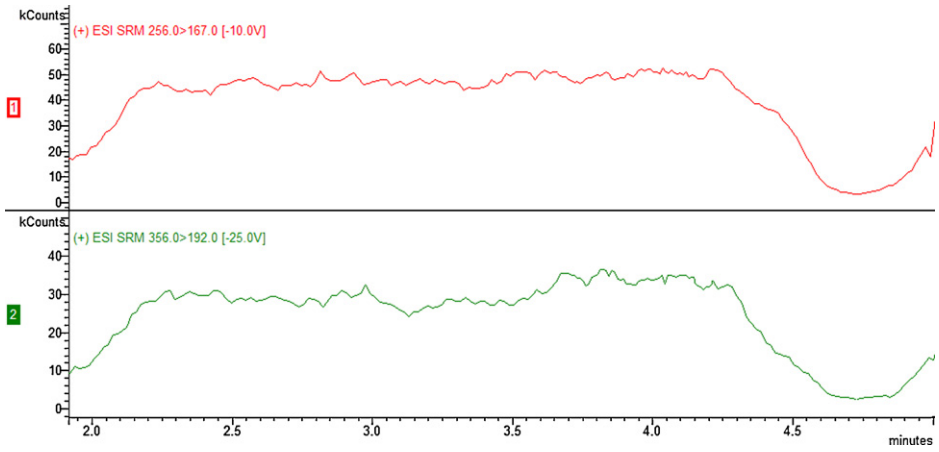

Fig. 4. Evaluation of matrix effects by the post-column infusion method. Blank microdialysates (10 μl) were injected, and ACSF solution containing *l*-THP and IS (10 ng/ml, both) was post-column infused at a flow rate of 0.05 ml/min.

Table 1
Summary of precision and accuracy from QC sample in ASCF.

Day		Low QC 0.2 ng/ml	Medium QC 10 ng/ml	High QC 800 ng/ml
Day 1	Mean ± SD	0.223 ± 0.004	9.529 ± 0.103	798.0 ± 14.8
	Accuracy (%)	111.7 ± 2.1	95.2 ± 1.03	99.8 ± 1.9
	RSD (%)	1.8	1.1	1.8
	n	5	5	5
Day 2	Mean ± SD	0.216 ± 0.006	9.692 ± 0.386	813.1 ± 28.3
	Accuracy (%)	108.1 ± 2.8	96.9 ± 3.9	101.6 ± 3.54
	RSD (%)	2.6	4	3.5
	n	5	5	5
Day3	Mean ± SD	0.221 ± 0.005	9.679 ± 0.243	825.8 ± 9.6
	Accuracy (%)	110.5 ± 2.69	96.8 ± 2.43	103.2 ± 1.2
	RSD (%)	2.4	2.5	1.2
	n	5	5	5
Between-day	Mean ± SD	0.220 ± 0.006	9.633 ± 0.261	812.3 ± 21.4
	Accuracy (%)	110.1 ± 2.8	96.3 ± 2.6	101.5 ± 2.7
	RSD (%)	2.5	2.7	2.6
	n	15	15	15

Table 2
Stability of *l*-THP in ACSF ($n = 5$).

Stability		Low QC 0.2 ng/ml	Medium QC 10 ng/ml	High QC 800 ng/ml
Short-term (2 h at 25 °C)	Accuracy (%)	96.0 ± 3.2	101.3 ± 5.1	101.9 ± 2.3
	RSD (%)	3.4	5.1	2.3
Long-term (1 week at −20 °C)	Accuracy (%)	97.8 ± 4.4	102.3 ± 1.4	100.6 ± 3.0
	RSD (%)	4.5	1.4	3.0
Autosampler (8 h at 25 °C)	Accuracy (%)	97.1 ± 4.0	101.3 ± 5.8	104.8 ± 1.0
	RSD (%)	4.1	5.8	1.0
Freeze–thaw (3 cycles)	Accuracy (%)	96.7 ± 5.0	102.0 ± 4.3	104.3 ± 2.4
	RSD (%)	5.1	4.2	2.2

Table 3
Comparsion of the methods for the determination of tetrahydropalmatine.

No.	Biological matrix	Extraction procedure	Detection method	LLOQ (ng/ml)	Ref.
1	Rat plasma	LLE[a]	LC–MS	1	[15]
2	Rabbit plasma	PP[b]	HPLC-DAD	2.05	[16]
3	Rat plasma	PP	LC–MS/MS	1	[17]
4	Rat plasma	LLE	HPLC-FLD[c]	2.096	[18]
5	Rat plasma	LLE	HPLC-UV	10	[19]
6	Dog plasma	LLE	HPLC-UV	25	[20]
7	Rat plasma	LLE	HPLC-UV	20	[21]
8	Rat brain	LLE	HPLC-UV	20	[24]
9	Rat striatum	Microdialysis	LC–MS/MS	0.1	Present method

[a] Liquid liquid extraction.
[b] Protein precipitation.
[c] Fluorescence detector.

3.6. Stability

Bench-top stability was investigated to ensure that *l*-THP was not degraded in microdialysates samples at room temperature for a time period to cover the sample preparation, and was assessed by exposing the QC samples to ambient laboratory conditions for 10 h. Freeze–thaw stability was assessed over three cycles. QC samples were thawed at room temperature and refrozen at −20 °C over three cycles and assayed. Due to the need for occasional delayed injection or reinjection of extraction samples, the stability of reconstituted samples in autosampler vials was assessed at ambient temperature for over 24 h. The freezer storage stability of *l*-THP in microdialysates at −20 °C was evaluated by assaying QC samples at beginning and 2 weeks later. Five replicates of all stability QC samples were analyzed. The result indicated that *l*-THP had stability within 96.0–104.8% under those conditions, as shown in Table 2.

3.7. Comparison of methods

There are some references on determination of tetrahydropalmatine in biological matrix. The methods on determination of tetrahydropalmatine for pharmacokinetic studies were summarized and compared, as shown in Table 3. Compared with the methods listed, our method is more sensitive for determination of *l*-THP with LLOQ of 0.1 ng/ml. With microdialysis, no sample clean-up steps were needed before analysis, and no interference was found from sample matrix.

3.8. Application of the assay

The method described above was applied to study pharmacokinetics after oral administration of *l*-THP at 40 mg/kg. A representative chromatogram from a post-dose sample is shown in Fig. 3D. The mean concentrations–time profiles of *l*-THP after an oral administration are shown in Fig. 5. The concentration–time data were analyzed by non-compartmental method and the pharmacokinetic parameters are shown in Table 4. The plasma concentrations of *l*-THP reached a C_{max} of 3584 ng/ml at approximately 1.3 h (T_{max}) after dosing and then decreased with a terminal phase $t_{1/2}$ of about 10.2 h. In Hong's studies, the pharmacokinetic profile of THP enantiomers in rat brain and plasma were investigated. The discrepancies of pharmacokinetic parameters (the $t_{1/2}$ and T_{max} values were 0.84 and 0.25 h, respectively) between ours and Hong's may due to the fact that in Hong's study, the data of every time point constructing the concentration–time curve was the average values from five rats instead of from one rat [24]. These experimental data did not seem to be quite reliable. However, our results on t_{max} are similar to those available in

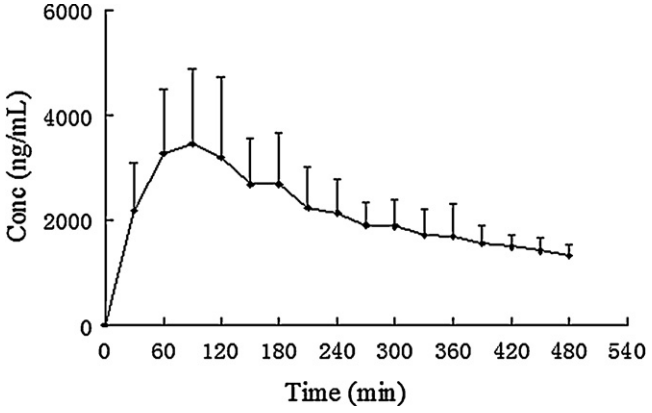

Fig. 5. Recovery-adjusted mean concentration *vs.* time after an intragastric administration of 40 mg/kg of *l*-THP ($n = 6$).

Table 4
Mean pharmacokinetic parameters after administration of 40 mg/kg of *l*-THP ($n = 6$).

Pharmacokinetic parameters	
T_{max} (h)	1.3 ± 0.4
C_{max} (ng/ml)	3584 ± 1430
$t_{1/2}$	10.2 ± 6.4
MRT (h)	14.4 ± 8.7
AUC $_{0-\tau}$ (μg h/ml)	17.1 ± 5.1
AUC $_{0-\infty}$ (μg h/ml)	35.4 ± 7.8

literature on plasma pharmacokinetics of THP [16,17,19]. We might deduce that *l*-THP can quickly pass through the blood–brain barrier (BBB) into striatum, where *l*-THP elicit profound effects on the dopaminergic system. The larger $t_{1/2}$ value in the striatum is also beneficial to *l*-THP induced anti-nociceptive effects.

4. Conclusion

In conclusion, we have successfully evaluated the concentrations of *l*-THP in rat's striatum by microdialysis technique and LC–MS/MS assay. Microdialysis is a powerful sampling method for the *in vivo* monitoring of pharmacokinetics. The method is very sensitive with an LLOQ of 0.1 ng/ml for *l*-THP. Good linearity, precision and accuracy were achieved. All parameters meet the criteria set in international guidelines for bioanalytical methods. The concentration profile of *l*-THP in rat brain was determined. The method was suitable for the pharmacokinetic studies of *l*-THP in rat striatum.

References

[1] G. Cao, H. Cai, X.D. Cong, Y. Zhang, Y.L. Shao, B.C. Cai, Application of microdialysis for pharmacokinetics of traditional Chinese medicine studies, Anal. Lett. 43 (2010) 55–72.

[2] D.J. Weiss, C.E. Lunte, S.M. Lunte, In vivo microdialysis as a tool for monitoring pharmacokinetics, TrAC, Trends Anal. Chem. 19 (2000) 606–616.

[3] T.-H. Tsai, J.-W. Wu, Pharmacokinetics of ciprofloxacin in the rat and its interaction with cyclosporin A: a microdialysis study, Anal. Chim. Acta 448 (2001) 195–199.

[4] V. Kumar, R. Madabushi, M.B.B. Lucchesi, H. Derendorf, Pharmacokinetics of cefpodoxime in plasma and subcutaneous fluid following oral administration of cefpodoxime proxetil in male beagle dogs, J. Vet. Pharmacol. Ther. 34 (2011) 130–135.

[5] Y.J. Zhang, L. Wu, Q.L. Zhang, J. Li, F.X. Yin, Y. Yuan, Pharmacokinetics of phenolic compounds of Danshen extract in rat blood and brain by microdialysis sampling, J. Ethnopharmacol. 136 (2011) 129–136.

[6] Q.L. Zhang, J.H. Hu, Q.G. Zhu, F.Q. Li, J.Y. Liu, D. Wang, Development of a novel HPLC–MS/MS method for the determination of aconitine and its application to in vitro and rat microdialysis samples, Biomed. Chromatogr. 23 (2009) 692–699.

[7] J.P. Qiao, Z. Abliz, F.M. Chu, P.L. Hou, L.Y. Zhao, M. Xia, Y. Chang, Z.R. Guo, Microdialysis combined with liquid chromatography–tandem mass spectrometry for the determination of 6-aminobutylphthalide and its main metabolite in the brains of awake freely moving rats, J. Chromatogr. B 805 (2004) 93–99.

[8] W.Y. Wang, C. Yao, Y.F. Shao, H.J. Mu, K.X. Sun, Determination of puerarin in rabbit aqueous humor by liquid chromatography tandem mass spectrometry using microdialysis sampling after topical administration of puerarin PAMAM dendrimer complex, J. Pharm. Biomed. Anal. 56 (2011) 825–829.

[9] Pharmacopoeia Commission of the People's Republic of China, China Pharmacopoeia, Part 1, Chemical Industry Press, Beijing, 2005, pp. 94–94.

[10] H.Y. Chu, G.Z. Jin, E. Friedman, X.C. Zhen, Recent development in studies of tetrahydroprotoberberines: mechanism in antinociception and drug addiction, Cell. Mol. Neurobiol. 28 (2008) 491–499.

[11] G. Jin, Discoveries in the Voyage of Corydalis Research, Shanghai Scientific & Technical Publishers, Shanghai, 2001, pp. 50–51.

[12] Z.Z. Ma, W. Xu, N.H. Jensen, B.L. Roth, L.Y. Liu-Chen, D.Y.W. Lee, Isoquinoline alkaloids isolated from *Corydalis yanhusuo* and their binding affinities at the dopamine D-1 receptor, Molecules 13 (2008) 2303–2312.

[13] A.L. Bittencourt, R.N. Takahashi, Mazindol, lidocaine are antinociceptives in the mouse formalin model: involvement of dopamine receptor, Eur. J. Pharmacol. 330 (1997) 109–113.

[14] T. Shimizu, S. Iwata, H. Morioka, T. Masuyama, T. Fukuda, M. Nomoto, Antinociceptive mechanism of L-DOPA, Pain 110 (2004) 246–249.

[15] Z.C. Fan, C.J. Xie, Z.Q. Zhang, Simultaneous quantitation of tetrahydropalmatine and protopine in rabbit plasma by HPLC-PAD, and application to pharmacokinetic studies, Chromatographia 64 (2006) 577–581.

[16] H.D. Ma, Y.J. Wang, T. Guo, Z.G. He, X.Y. Chang, X.H. Pu, Simultaneous determination of tetrahydropalmatine, protopine, and palmatine in rat plasma by LC–ESI-MS and its application to a pharmacokinetic study, J. Pharm. Biomed. Anal. 49 (2009) 440–446.

[17] L. Lin, J.X. Liu, Y. Zhang, C.R. Lin, C.L. Duan, Pharmacokinetic studies of tetrahydropalmatine and dehydrocorydaline in rat after oral administration of yanhusuo extraction by LC–MS/MS method, Yao xue xue bao 43 (2008) 1123–1127.

[18] X.F. Liang, Z.G. Liao, G.F. Wang, G.W. Zhao, C.L. Dai, X.H. Zhang, Influence of combination of extractum Angelicae Dahuricae Siccum and total alkaloids of *Rhizoma Corydalis* on pharmacokinetics of tetrahydropalmatine in rats, Yaoxue Xuebao 44 (2009) 645–650.

[19] Z.Y. Dou, W. Sun, X.L. Mi, L.P. Yao, Comparison of pharmacokinetics of tetrahydropalmatine monomer and extractive of corydalis and corydalis processed with vinegar, Zhong yao cai 30 (2007) 1499–1501.

[20] Z.Y. Hong, G.R. Fan, Y.F. Chai, X.P. Yin, J. Wen, Y.T. Wu, Chiral liquid chromatography resolution and stereoselective pharmacokinetic study of tetrahydropalmatine enantiomers in dogs, J. Chromatogr. B 826 (2005) 108–113.

[21] Z.Y. Hong, J. Le, M. Lin, G.R. Fan, Y.F. Chai, X.P. Yin, Y.T. Wu, Comparative studies on pharmacokinetic fates of tetrahydropalmatine enantiomers in different chemical environments in rats, Chirality 20 (2008) 119–124.

[22] G. Levy, Kinetics of drug action: an overview, J. Allergy Clin. Immunol. 78 (1986) 754–761.

[23] J. Dingemanse, M. Danhof, D.D. Breimer, Pharmacokinetic–pharmacodynamic modeling of CNS drug effects: an overview, Pharmacol. Ther. 38 (1988) 1–52.

[24] Z.Y. Hong, G.R. Fan, J. Le, Y.F. Chai, X.P. Yin, Y.T. Wu, Brain pharmacokinetics and tissue distribution of tetrahydropalmatine enantiomers in rats after oral administration of the racemate, Biopharm. Drug Dispos. 27 (2006) 111–117.

[25] .N. Sun, J. Wen, G.C. Lu, Z.Y. Hong, G.R. Fan, Y.T. Wu, C.Q. Sheng, W.N. Zhang, An ultra-fast LC method for the determination of iodiconazole in microdialysis samples and its application in the calibration of laboratory-made linear probes, J. Pharm. Biomed. Anal. 51 (2010) 248–251.

[26] G. Paxinos, C. Waston, The Rat Brain in Stereotaxic Coordinates, 4th ed., Academic Press, San Diego, 1998, pp. 138–138.

[27] J.K. Hsiao, B.A. Ball, P.F. Morrison, I.N. Mefford, P.M. Bungay, Effects of different semipermeable membranes on in vitro and in vivo performance of microdialysis probes, J. Neurochem. 54 (1990) 1449–1452.

[28] A. Van Eeckhaut, K. Lanckmans, S. Sarre, I. Smolders, Y. Michotte, Validation of bioanalytical LC–MS/MS assays: evaluation of matrix effects, J. Chromatogr. B 877 (2009) 2198–2207.

[29] R. Bonfiglio, R.C. King, T.V. Olah, K. Merkle, The effects of sample preparation methods on the variability of the electrospray ionization response for model drug compounds, Rapid Commun. Mass Spectrom. 13 (1999) 1175–1185.

[30] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC–MS/MS, Anal. Chem. 75 (2003) 3019–3030.