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Direct pharmacokinetic analysis of puqietinone by *in vivo* microdialysis sampling and turbulent-flow chromatography coupled with liquid chromatography–mass spectrometry

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ABSTRACT

Sample pretreatment is a key step in bioanalytical process because of possible interference and matrix effects in mass spectrometry analysis. In this work, a novel strategy towards high speed and sensitivity was developed combining *in vivo* microdialysis (MD) sampling, turbulent-flow chromatography (TFC), and liquid chromatography–mass spectrometry (LC–MS). The procedures of cleanup, preconcentration, and separation were completed on-line in one step within 10 min. During the MD optimization procedure, 1% hydroxypropyl-β-cyclodextrin (HP-β-CD) was used to improve the relative recovery of the analyte. Untreated MD samples were directly injected, and a TFC precolumn was flushed for 1 min with aqueous phase of 4 mL/min flow rate to desalt and concentrate biosamples. The retained analytes were then back-flushed by a switching valve onto a fast LC column (4.6 mm × 50 mm, 1.8 μm) for separation. Another diverter valve was employed to prevent the HP-β-CD that interferes with the ESI process from entering the MS. Puqietinone, a lipophilic alkaloid from *Fritillaria puqiensis*, was used as a case for validation. Results showed that the limit of quantification for puqietinone was 0.10 ng/mL, and good linearity (R^2 = 0.9993) was maintained over the range of 1.02–200.02 ng/mL. Accuracy and precision were satisfactory within the range of the standard curve. This approach was able to effectively eliminate the influences of matrix effect and carry-over as the injection volume increased up to 20 μL. The developed method was successfully applied to pharmacokinetic study of puqietinone after intravenous administration to rat. Results demonstrate the potential of using MD with TFC-LC/MS for *in vivo* monitoring experiments.

1. Introduction

Microdialysis (MD) is an *in vivo* technique used for sampling endogenous and exogenous substances. As a method for monitoring dynamically biological systems, it is useful in pharmacokinetics study of drugs providing *in vivo* chronological and real-time information [1,2]. However, a low flow rate at 1–2 μL/min and diluting effect of the dialysis lead to small-volume and low-concentration samples. Highly sensitive and selective analytical methods that can compensate the small volume/low concentration MD samples are required.

Electrospray ionization mass spectrometry (ESI-MS) is preferable for analysis of biological samples but incompatible with buffers and nonvolatile salts present in MD samples [3,4]. Small amounts of salts may affect electrospray stability, reduce the ion signals, and result in weak signal-to-noise ratios because of ionization suppression. MD samples, though relatively clean, highly filtered, and protein-free samples, still contain a large amount of salts (>150 mM). Michotte Y. et al. previously described a microbore column switching method for desalting and purifying brain MD samples prior to ESI-MS [5]. The transformation of this microbore to a capillary and nano-liquid chromatography (LC)–ESI-MS were also discussed [6]. This approach is limited for eliminating salt effects or carry-over when the injection volume exceeded 1 μL.

Turbulent-flow chromatography (TFC) has been recently considered a time-efficient on-line extraction technique for rapid extraction of compounds directly from complex matrices [7–13]. Turbulent flow conditions are achieved using high flow rates of low viscosity solvents in micro-bore columns packed with particles of a large diameter. A guide to the flow characteristics of a mobile phase in a packed column is given by the Reynolds number, $Re = (\mu D_p)/\eta$, where μ is the linear velocity of the mobile phase, D_p the average diameter of the stationary phase particles and η the mobile phase kinematic viscosity [14]. Turbulent flow conditions are achieved by a Reynolds number greater than 1. Large particles are therefore used to encourage turbulent flow conditions whilst simultaneously lowering backpressure on the column,

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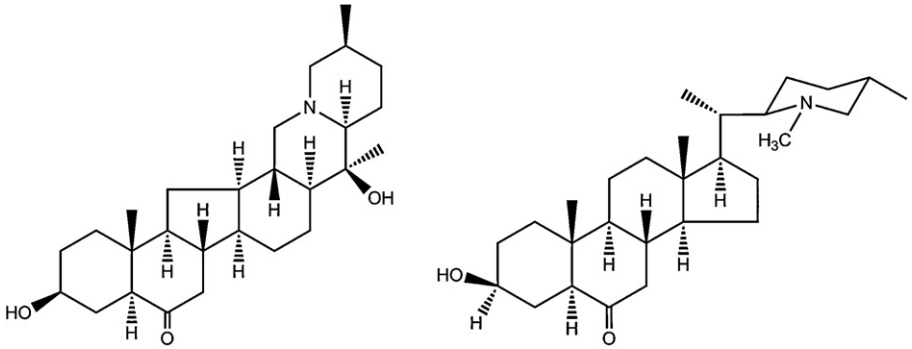

Fig. 1. Chemical structures of puqietinone and verticinone (IS).

which is particularly important due to the high flow rates required during sample loading. This procedure enables the proteins and salts were removed while the low-molecular-weight analytes were retained on the stationary phase. The use of turbulent flow resulted in a faster and more rugged extraction with reduced carry-over. However, complex matrices affected the lifetime of the extraction column (expected 200 injections). Application of relatively clean MD samples might extend the lifetime of extraction columns.

This work is aimed to develop a novel bioanalytical strategy for improved pharmacokinetics study of small-molecular drugs. We combined *in vivo* MD sampling, TFC, and LC–MS for one-step desalting, preconcentration, separation, and detection. Different from common MD sampling of hydrophilic molecules, hydroxypropyl-β-cyclodextrin (HP-β-CD) was added as an enhancer to improve dialysis sampling capability and relative recovery (RR) of lipophilic analytes [15–19]. A diverter valve was installed between the LC column and the ESI-MS to prevent high concentrations of HP-β-CD from entering the ion source. Puqietinone, a steroidal alkaloid from *Fritillaria puqiensis*, was used for validation. Reduced analysis time, improved sensitivity and extended lifetime of extraction column were expected.

2. Experimental Section

2.1. Reagents and materials

LC/MS-grade acetonitrile (ACN) and methanol were purchased from Merck, Darmstadt, Germany. Ammonium formate and 1,2-propanediol of LC grade were purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China). Formic acid (>96% pure) of LC/MS grade was obtained from Tedia, USA. HP-β-CD was purchased from ACROS Organics (Geel, Belgium). Solvents of at least LC-grade purity were used for all experiments described in this study. Solutions were prepared with 18.2 MΩ cm^{-1} Milli-Q (Millipore) deionized water.

The steroidal alkaloid standards puqietinone and verticinone (internal standard, IS) were isolated from *Fritillaria* species in the authors' laboratory, and their identities were confirmed by IR, ^{1}H- and ^{13}C-NMR, and MS analyses [20,21]. The purities of the two steroidal alkaloids were determined to be more than 98% by normalization of the peak areas detected by HPLC with ESI-MS. Their structures are shown in Fig. 1.

2.2. Standard working solutions

The dialysate is a protein-free solution, but endogenous compounds in the biological matrix can still permeate into the dialysate. Therefore, all the samples for validating the assay method were prepared by spiking the blank dialysate of biological matrix with standards to keep consistent with the samples.

Standard stock solution of puqietinone at 1 mg/mL was prepared in methanol, and then diluted with anticoagulant citrate dextrose (ACD) solution to make the working standard solutions of different serials. The IS stock solution of 0.95 μg/mL was also prepared in ACD, and kept at 47.50 ng/mL in each working solution and samples. Calibration samples were prepared by spiking aliquots of the stock standard solutions into blank dialysates to obtain final concentrations in the ranges of 1.02–200.02 ng/mL. Calibration standards were analyzed daily, prior to and throughout the analysis of test portions. The quality control (QC) samples with low, middle and high concentrations (5.04, 80.01 and 200.02 ng/mL) were also prepared daily in the same manner. All solutions were stored at −20 °C before use.

2.3. Apparatus

A schematic diagram of the system based on combining *in vivo* MD sampling, on-line TFC, and LC–MS is shown in Fig. 2. MD systems consisted of a 3-syringe bracket MD pump with a Bee Syringe Pump Controller (Bioanalytical System Inc., West Lafayette, IN, USA). MAB/7 MD probes with 15,000 Da cut-off polyethersulfone membranes were purchased from MAB Mirobiotech (Stockholm, Sweden). The blood MD probes were separately positioned in the rat jugular vein, and then perfused with ACD solution (3.5 mM citric acid, 7.5 mM sodium citrate, and 13.6 mM dextrose) at a flow rate of 2.0 μL/min. After equilibrium of 2 h, dialysates were consecutively collected every 20 min into vials placed on ice cubes.

The on-line TFC–LC/MS instrument set-up was assembled with a column-switching comprising two-channel, two-dimensional LC system configured for TFC and LC, and an Agilent SL G1946D MS with an ESI source. Each channel, for loading or eluting, utilized a quaternary LC pump (Agilent 1100 HPLC system) to combine and pump the appropriate solvents. In this analysis, 1 solvent (water, solvents A) was used in the loading channel and 2 (water with 10 mM ammonium formate and 0.1% formic acid, solvents B and acetonitrile, solvents C) were used in the eluting channel. Only loading pump flow passed through the 50 μL sample injection loop. In this system, a hydrophilic-lipophilic balanced (HLB) reversed-phase column (Oasis HLB, 25 μm, 2.1 mm × 20 mm; Waters, Milford, USA), a fast LC column (Agilent Zorbax StableBond-C18, 4.6 mm × 50 mm, 1.8 μm), and three Rheodyne six-port switching valves were used for different purposes. One of the three switching valves was installed between LC column and

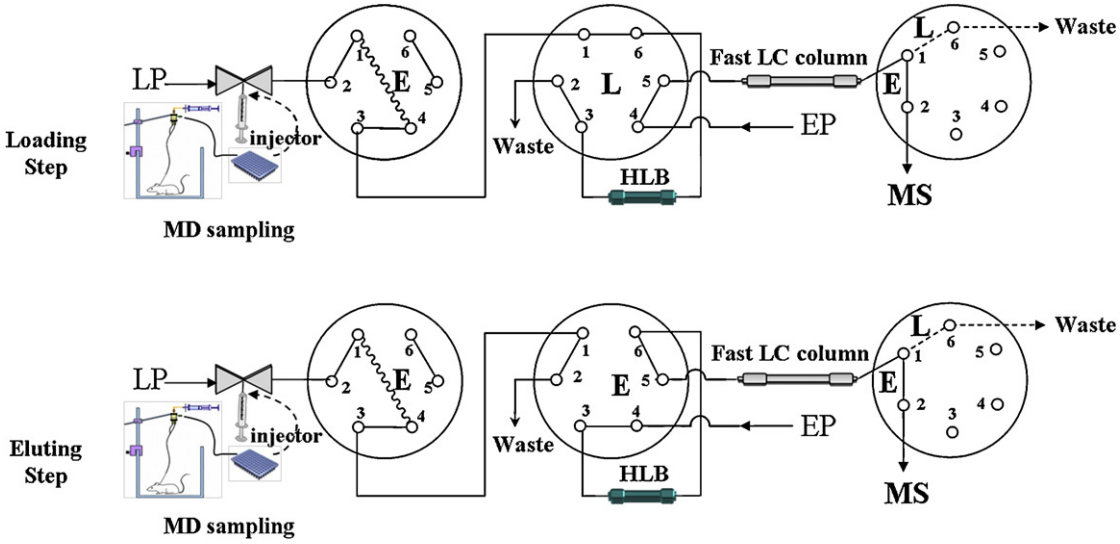

Fig. 2. Configuration of the system based on combining *in vivo* MD sampling, TFC, and LC–MS. *LP*, loading pump for the online extraction, *EP*, eluting pump for the analytical chromatography.

the MS to prevent HP-β-CD from entering the MS within the eluent.

As described above, this study uses TFC in dual column mode, which is performed in three stages. The first is the sample loading stage. In this step, the sample is loaded onto the extraction column with an aqueous mobile phase at a high flow rate. The analytes of interest are retained on the column and polar materials are eluted to waste. Secondly, reverse flow through the extraction column using a gradient elution then takes the analytes onto a fast LC column and through the MS detector. As reported, the typical pressure upper limit of agilent 1100 HPLC pump is 250 bar [22]. Therefore, a low flow rate at 0.5 mL/min was used in the elution process. By using 1.8 μm rapid resolution high throughput columns on common LC system, we achieved rapid analysis in an economic manner. Finally, the extraction column is re-equilibrated with aqueous phase ready for the next load. The detail experimental workflow is described as following.

Experimental workflow: (1) aliquots of 38 μL of MD samples (calibration standards, QC samples and pharmacokinetic MD samples) were mixed with 2.0 μL of ACD containing 0.95 μg/mL of verticinone (IS); (2) a 20 μL injection of the mixed solution was loaded onto the HLB column with solvent A at a flow rate of 4 mL/min for 1 min; the salts and other endogenous compounds were removed while the analytes were retained during this step; (3) after the loading step, the switching valve was switched to the eluting channel, and the Pump 2 started a gradient elution using solvents B and C (Table 1) to elute the analytes from the extraction column to the fast LC column using a flow rate of 0.5 mL/min; (4) the switching valve between the LC column and MS was turned online with the MS at 4.0 min and offline to the waste at 8.0 min, during which the analytes eluted from the column.

2.4. Mass spectrometry

The instrument was operated in the positive ion mode. The source parameters were optimized as follows: capillary voltage was set at 3.0 kV; drying gas (N_2) flow rate was 9.0 L/min; drying gas temperature, 300 °C; nebulizing gas (N_2) pressure, 35 psig; fragmentor, 100 V. Samples were analyzed in selective ion monitoring (SIM) mode by monitoring the molecular ions $[M+H]^+$.

2.5. Method validation

All data presented in this work were obtained by averaging three replicates unless otherwise noted. For the standard curve, the ratio of the peaks area (analyte/IS) was plotted on the *y* axis while the concentration of the analyte on the *x* axis. Linear regression (R^2), calibration curve, and relative standard deviations (RSDs) were calculated. The assay precision was determined from inter- and intra-batch (RSD %) using five determinations per concentration (5.04, 80.01, 200.02 ng/mL). Accuracy was calculated by comparing the experimentally determined concentrations of analyzed standard solutions to their nominal values and expressed as Bias%. The limit of quantification (LOQ) was considered as the concentration that produced a signal-to-noise (S/N) ratio of 10 with acceptable precision and accuracy. In addition, the matrix effect, stability, and carryover of the present method were examined to validate its reliability.

Table 1
Time schedule of the on-line TFC column switching LC procedure.

Step no.	Time (min)	Switch valve 1	Flow LP (mL/min)	Loading A (%)	Switch valve 2	Flow EP (mL/min)	Eluting B (%)	Eluting C (%)	Switch valve 3
1	−1.0[a]	E	4	100	L				L
2	0.0	E	0		E	0.5	75	25	L
3	2	E	0		E	0.5	70	30	L
4	4	E	0		E	0.5	56	44	E
5	5	E	0		E	0.5	50	50	E
6	7	E	0		E	0.5	30	70	E
7	9	E	0		E	0.5	0	100	L
8	10	L	4	100	L	0.5	75	25	L

[a] We defined the LC procedure start point as null point, then the "−1" refers to one minute before the eluting step start.

Table 2
Enhancing effect of different concentrations of HP-β-CD added in perfusion fluid.

Compounds	% HP-β-CD	Recovery (%)	Enhancing effect (%)
Puqietinone	0	25.17 ± 0.98	
	0.01	26.27 ± 0.37	4.35
	0.05	27.45 ± 2.38	9.05
	0.1	31.69 ± 3.13	25.9
	1	51.29 ± 4.03	103.79

2.6. Application to pharmacokinetics study of puqietinone

Animal experiments were carried out in accordance with the Guidelines for Animal Experimentation of China Pharmaceutical University (Nanjing, China) and protocol was approved by the Animal Ethics Committee of this institution. Male Sprague–Dawley rats (270–300 g) purchased from Sino-British Sippr/BK Lab Animal Ltd. (Shanghai, China) were fed on a standard laboratory diet with free access to water under the controlled temperature at 22–25 °C and relative humidity of 50% with 12-h light/dark cycles prior to the study. The rats were fasted overnight before administration of drug with free access to water. For the intravenous route, dosing solutions were prepared by dissolving puqietinone in isotonic saline containing 40% propylene glycol and 4% HCl, and then adjusting the pH value to ~6.0 by NaOH. The blood MD probes were separately positioned within the rat jugular vein, and then perfused with ACD solution at a flow rate of 2.0 μL/min. After 2 h equilibration, rats ($n = 6$) were dosed with puqietinone intravenously (5 mg/kg). Then, MD samples were collected from each probe in 20 min intervals, up to 6 h.

Pharmacokinetic parameters were evaluated from plasma concentration–time curve using non-compartmental (Drug and Statistics 2.0, Mathematical Pharmacology Professional Committee of China, Shanghai, China) analysis. The area under the concentration–time curve (AUC) was calculated according to the log linear trapezoidal method. The half-life ($t_{1/2}$) was calculated as: $t_{1/2} = 0.693/k$ (k is the elimination rate constant). All data were presented as mean ± S.D.

3. Results and discussion

3.1. Method design

MD sampling is a diffusion-based process in which a perfusion fluid is passed through a semipermeable, hollow fiber membrane with a defined molecular weight cut-off. Analytes being sampled can freely diffuse into the probe. The most important parameter in MD is the extraction efficiency, namely the RR.

The RR of puqietinone dissolved in ACD was examined by an *in vitro* study. ACD is a solution of citric acid, sodium citrate and dextrose prepared in water, and it is physiological compatible solution mainly used as an anticoagulant to preserve blood. When ACD was used as the perfusate, the RR of puqietinone across the dialysis membrane was only 25.17 ± 0.98%. The relative lipophilic character of puqietinone resulted in low recovery. To improve the recovery of this hydrophobic analyte, different concentrates of HP-β-CD was added into the perfusate. The results shown in Table 2 indicated that adding 1% HP-β-CD increased the *in vitro* recovery of puqietinone to 51.29 ± 4.03, 2-fold higher than that in blank group. Thus, 1% HP-β-CD containing ACD solution was used to facilitate MD sampling.

In TFC, a high flow rate of aqueous stream was used to produce a turbulent flow. Salts and other endogenous dialysate compounds in the MD sample were removed but the analytes were retained on the extraction column. Then, the extraction column was switched to the eluting channel. The eluting pump started a gradient using solvents B and C to elute the analytes from the extraction column to the fast LC column. Our study demonstrated that the analyte puqietinone and analyte-HP-β-CD complex can be well retained in TFC column in the loading step, and then the analyte-HP-β-CD complex dissociated on the LC column in the eluting step. This finding is consistent with the previous reports [23,24]. Based on the optimal gradient elution, the peaks of HP-β-CD, IS, and puqietinone were well separated within 8 min. The retention times of HP-β-CD, IS, and puqietinone were 2.04, 5.62, and 7.59 min, respectively.

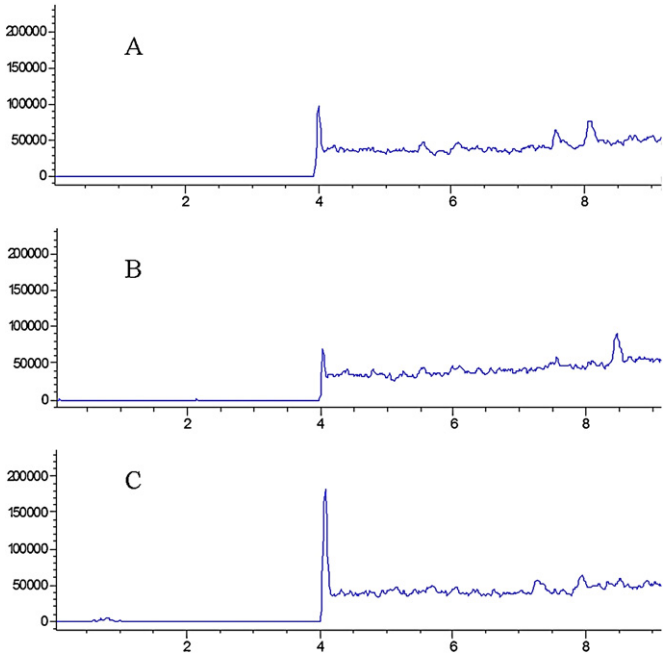

Fig. 3. Positive mode Q1 scan TIC of puqietinone sample containing 0 (A), 0.1% (B), and 1.0% HP-β-CD (C).

To prevent the HP-β-CD from entering the MS, a diverter valve was installed between the LC column and MS. Based on the retention time of each analyte, this valve was set to be online with the MS from 4.0 to 8.0 min during the chromatographic separation and was offline for the rest time. To confirm that HP-β-CD was effectively prevented from the ion source, a Q1 scan for the total ion chromatogram (TIC) of puqietinone sample containing 0, 0.1%, and 1.0% HP-β-CD was performed. Fig. 3 shows that no elevation of baseline was observed in the spectrum of puqietinone sample containing HP-β-CD, indicating that the diverter valve effectively prevented HP-β-CD from entering the ion source.

3.2. Method validation

3.2.1. Selectivity

Typical chromatographic profiles of blank dialysate, blank dialysate spiked with puqietinone and IS, and MD sample obtained during 40–60 min after intravenous administration of puqietinone were shown in Fig. 4. No interfering peak from endogenous compounds was observed in blank dialysate, suggesting an acceptable selectivity of the developed method.

3.2.2. Linearity and sensitivity

Calibration curves for puqietinone were analyzed in the concentration range of 1.02–200.2 ng/mL using verticinone as the IS at a concentration of 47.5 ng/mL. The calibration model was selected based on the analysis of the data by linear regression with intercepts and $1/x^2$ weighting factor. Typical equation for the calibration curve was $y = 0.0282x - 0.0018$ with good linear regression

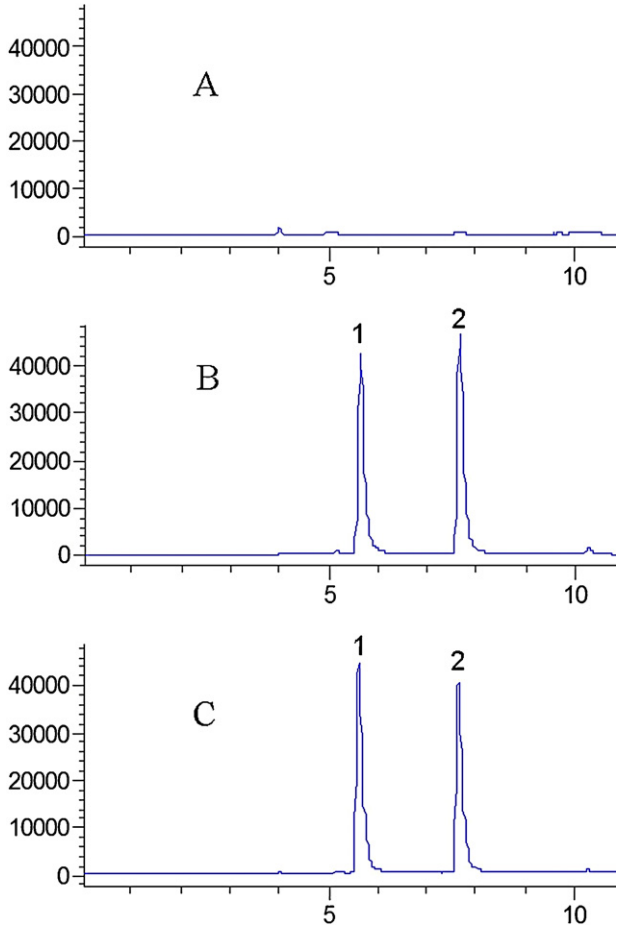

Fig. 4. Selected ion monitoring chromatograms from (A) blank dialysate, (B) blank dialysate spiked with IS (1) and puqietinone (2), and (C) MD sample obtained during 40–60 min after intravenous administration of puqietinone (5 mg/kg).

($R^2 > 0.9993$) in the test range. The LLOQ was determined to be 0.10 ng/mL.

3.2.3. Precision and accuracy

Table 3 shows the intra- and inter-batch precision and accuracy at three concentrations. The intra- and inter-batch accuracy for puqietinone were respectively 96.3–104% and 106.9–109.9% exhibiting minor error bars. We obtained precisions (RSD) all ranging below 10% with a mean of 5.9%. In addition, the RSD values of retention time (t_R) were less than 0.11% and 0.27% in run-to-run and day-to-day analysis, respectively. These results illustrate excellent precision and accuracy of this method, as well as the general ability to serve in the analysis and quantification of small molecules in MD.

3.2.4. Matrix effects

Using direct injection, Michott et al. have found that both ion suppression and ion enhancement can occur in LC/MS assay of the dialysis samples [6]. Their results showed that interfering salts and endogenous dialysis matrix compounds should be removed before introduction into the ESI-MS source. In our study, the online-TFC technique was used to fulfill this purpose.

The effects of salts and other endogenous compounds, present in the dialysate matrix, were investigated by the developed method. The salt matrix effect was evaluated by comparing the peak areas of puqietinone and IS dissolved in blank dialysis with those of standards prepared in pure solvent. Three different concentration levels of analytes were evaluated by analyzing in triplicate at each concentration level. As shown in Table 4, there was no significant interference on the ionization of puqietinone and IS from the dialysate matrix, suggesting that by using high flow rate of aqueous phase in TFC salts and endogenous substances were efficiently washed to waste.

3.2.5. Stability

The stability of puqietinone was evaluated by analyzing QC samples under conditions mimicking situations likely to be encountered during sample storage and the analytical process. Three different conditions were mimicked: modeling three freeze/thaw cycles, 12 h storage at room temperature and frozen at −70 °C for 1 month. Stability data are summarized in Table 5 and indicated that puqietinone was stable in dialysate under three different conditions. The short-term stability and freeze-thaw stability data showed that the samples were stable in sampling and analytical process. Moreover, the results showed that the analyte was stable for at least 1 month when kept frozen at −70 °C.

3.2.6. Carry-over

To address the issue of carry-over, multiple blank solutions with undetectable levels of puqietinone were run before and after the injection of a high concentrate QC sample (200.02 ng/mL). The response of puqietinone was comparable for all of the blank injections, and there was no significant increase in response following the injection of QC sample (Fig. 5). These data indicate that through the rigorous wash program carry-over can be eliminated effectively.

3.2.7. Extraction column pressure

For an online extraction column (*e.g.*, HLB), column backpressure will increase slowly as the injection of biological samples. Generally, the maximum sustainable injections were about 200 samples for a new column. Yu et al. found that using an alkyldiol silica column, the retention decreased by 20% and the pressure increased 30 bars after 150 plasma injections without reconditioning procedure [25]. This is because proteins could eventually be unspecifically adsorbed on the hydrophobic ligand of the support.

MD provides the advantage of protein-free samples. Extraction column for pretreatment might benefit from these clean samples. In this study, a new HLB column was used to monitor the

Table 3
Precision and accuracy of puqietinone.

Compound	Spiked concentration (ng/mL)	Measured concentration (ng/mL) (mean ± S.D.)	R.S.D. (%)	Accuracy (%)	Bias (%)
Puqietinone					
Intra-day ($n = 5$)	5.04	5.27 ± 0.27	5.12	106.90	4.55
	80.01	87.98 ± 5.79	6.58	110.00	9.96
	200.02	214.03 ± 8.51	3.97	107.00	7.00
Inter-day ($n = 3 \times 3$)	5.04	5.02 ± 0.39	7.85	99.60	-0.40
	80.01	83.83 ± 8.28	9.88	104.80	4.78
	200.02	203.24 ± 12.55	6.17	101.60	1.61

Table 4
Matrix effect evaluation of puqietinone ($n = 3$).

Compound	Spiked concentration (ng/mL)	Set 1 (mean ± S.D.)	Set 2 (mean ± S.D.)	Absolute matrix (%)
Puqietinone	5.04	$4.91 \times 10^4 \pm 0.45$	$4.61 \times 10^4 \pm 0.63$	93.80
	80.01	$7.85 \times 10^5 \pm 3.37 \times 10^4$	$6.84 \times 10^5 \pm 1.05 \times 10^4$	87.20
	200.02	$1.85 \times 10^6 \pm 9.04 \times 10^4$	$1.98 \times 10^6 \pm 3.65 \times 10^4$	107.00
IS	47.50	$4.31 \times 10^5 \pm 4.43 \times 10^3$	$4.03 \times 10^5 \pm 2.15 \times 10^3$	93.50

Table 5
Stability of puqietinone ($n = 3$).

Compound	Spiked concentration (ng/mL)	Measured concentration (ng/mL) (mean ± S.D.)		
		After three freeze/thaw cycles in dialysate	At room temperature for 12 h in dialysate	At −70 °C for 1 month in dialysate
Puqietinone	5.04	5.09 ± 0.078	5.08 ± 0.04	5.15 ± 0.20
	80.01	82.39 ± 0.79	83.36 ± 0.62	82.21 ± 0.74
	200.02	199.50 ± 2.50	201.46 ± 1.18	201.65 ± 2.14

pressure change. As shown in Fig. 6, after continuous use for 22 days with approximately 400 injections, the column pressure slightly increased from 12 to 19 bars. When the pressure rose to greater than 170 bar at 4 mL/min flow rate, the column was irreversibly damaged. Therefore, the life span of HLB column is extended greatly using MD samples instead of direct plasma samples. The pharmacokinetics study cost is correspondingly reduced.

3.2.8. Recoveries of MD probes

In vivo recovery rates were assessed according to the retrodialysis method [15]. This method relies on the assumption that the diffusion rate is equal in both directions through the semipermeable membrane. Thus, the analyte can be added to the perfusate and the disappearance rate through the membrane can be taken as the *in vivo* recovery. In our studies, *in vivo* recovery was assessed by dialysing the blood with a perfusion medium containing 200.02 ng/mL and 80.01 ng/mL puqietinone. MD samples were collected from each probe in 1 h intervals, up to 6 h, after 2 h equilibration. The perfusate (C_{perf}) and dialysate (C_{dial}) concentrations of puqietinone were determined by the online TFC–LC/MS system. All experiments were conducted in triplicate using different MD probes ($n = 3$). The *in vivo* RR by retrodialysis was calculated as:

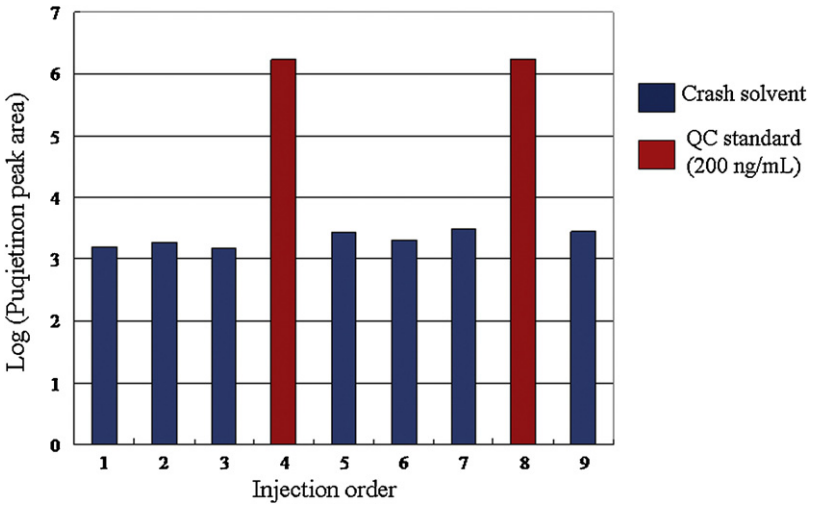

Fig. 5. The logarithm of puqietinone response from consecutive injections of blank crash solvent and 200 ng/mL QC sample.

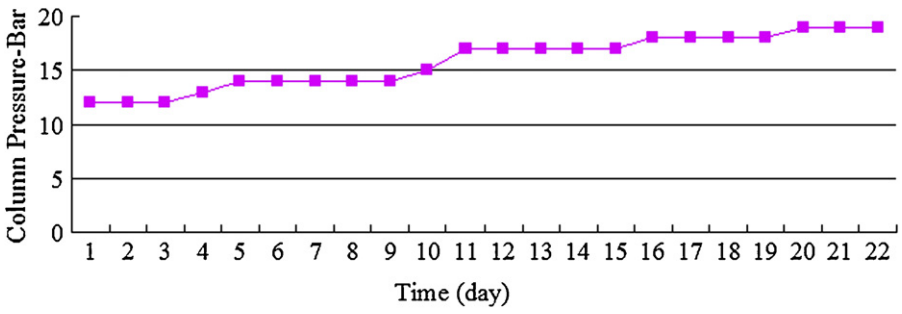

Fig. 6. The trend of changes in pressure of a new HLB column with consecutive injection MD samples for 22 days (20 injections a day on average).

Table 6
In vivo microdialysate recoveries (%) of puqietinone from rat blood dialysate ($n = 3$).

Compound	Microdialysis sampling	Perfused concentr. (ng/mL)	Recovery (%)	Average
Puqietinone	Blood	80.01 200.02	39.04 ± 6.57 36.77 ± 6.67	37.91 ± 1.61

Table 7
Pharmacokinetic parameters of puqitinone after IV administration of 5 mg/kg puqietinone (mean ± S.D., $n = 6$).

Compounds	Parameters					
	$AUC_{(0-t)}$ (μg min/L)	$MRT_{(0-t)}$ (min)	$t_{1/2}$ (min)	CL (L/min/kg)	V_d (L/kg)	C_{max} (μg/L)
Puqietinone	$1.50 \pm 0.51 \times 10^4$	83.55 ± 14.54	141.26 ± 86.47	0.31 ± 0.07	64.97 ± 47.36	155.62 ± 126.47

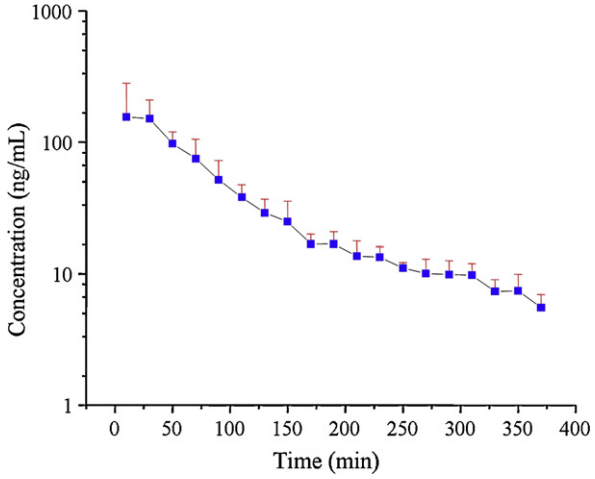

Fig. 7. Mean concentration–time profiles for puqietinone in rat blood dialysates after administration of puqietinone (5 mg/kg).

RR (%) = $[(C_{perf} - C_{dial})/C_{perf}] \times 100$. The mean *in vivo* RR ($n = 3$) of puqietinone in MD probes were 37.91% in blood shown in Table 6. Sample concentrations were corrected by the probe recovery before pharmacokinetic data analysis.

3.3. Results of pharmacokinetics study

Mean unbound puqietinone concentration–time profiles in the rat blood at the dose of 5 mg/kg are presented in Fig. 7, and the main pharmacokinetic parameters in rats are presented in Table 7. After the IV administration, the concentration of puqietinone maintained at 151–155 ng/mL for about 30 min, then the blood level declined biexponentially with an elimination half-life of 141.26 min. Furthermore, a large volume of distribution (V_d) was observed (64.97 L/kg), indicating that puqietinone, a lipophilic alkaloid, was distributed extensively into the extravascular tissues in rats. These results demonstrated that puqietinone was excreted slowly and had a relative long duration of action in rats.

4. Conclusion

MD has received significant attention in bioanalysis primarily for its potential in monitoring concentration dynamics. In this work, we practiced the possibility that TFC can be used to online desalt and pre-concentrate in a sampling and monitoring experiment. The temporal resolution of sampling points was improved by using MD technology, and addition of HP-β-CD helped to enhance the RR. Direct online sample cleanup and enrichment were achieved within 1 min by TFC with high flow rate aqueous phase. Collection with MS after removing salts and the HP-β-CD by TFC and a diverter valve improved the analytical sensitivity and selectivity. The LOQ was 0.10 ng/mL for puqietinone, which is comparable to or even better than those reported methods [26–28]. No significant carry-over and matrix effect were observed. The results presented here suggest that the developed method could also provide a promising technique for monitoring endogenous small molecules, such as intracephalic neurotransmitters. Future research aimed at improving throughput should be directed towards improving sampling and LC separation processes. Extension of the method to other analytical assays and application to other fields will likely yield systems with higher temporal resolution and sensitivity.

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References

[1] M.I. Davies, J.D. Cooper, S.S. Desmond, C.E. Lunte, S.M. Lunte, Adv. Drug Deliv. Rev. 45 (2000) 169.
[2] L.C. Mecker, R.S. Martin, Anal. Chem. 80 (2008) 9257.
[3] C. Liu, S.A. Hofstadler, J.A. Bresson, H.R. Udseth, T. Tsukuda, R.D. Smith, A.P. Synder, Anal. Chem. 70 (1998) 1797.
[4] Y. Chen, M. Mori, A.C. Pastusek, K.A. Schug, P.K. Dasgupta, Anal. Chem. 83 (2011) 1015.
[5] K. Lanckmans, R. Clinckers, A. Van Eeckhaut, S. Sarre, I. Smolders, Y. Michotte, J. Chromatogr. B 831 (2006) 205.
[6] K. Lanckmans, A. Van Eeckhaut, S. Sarre, I. Smolders, Y. Michotte, J. Chromatogr. A 1131 (2006) 166.
[7] D.M. Mueller, B. Duretz, F.A. Espourteille, K.M. Rentsch, Anal. Bioanal. Chem. 400 (2011) 89.
[8] G.Z. Xin, J.L. Zhou, L.W. Qi, C.Y. Li, P. Liu, H.J. Li, X.D. Wen, P. Li, J. Chromatogr. B 878 (2010) 435.
[9] A. Asperger, J. Efer, T. Koal, W. Engewald, J. Chromatogr. A 960 (2002) 109.
[10] P. Mottlier, Y. Hammel, E. Gremaud, P.A. Guy, J. Agric. Food Chem. 56 (2008) 35.
[11] A.A.M. Stolker, R.J.B. Peters, R. Zuiderent, J.M. Dibussolo, C.P.B. Martins, Anal. Bioanal. Chem. 397 (2010) 2841.
[12] J.A. Roach, J.M. DiBussolo, A. Krynitsky, G.O. Noonan, J. Chromatogr. A 1218 (2011) 4284.
[13] J.T. Wu, H. Zeng, M.X. Qian, B.L. Brogdon, S.E. Unger, Anal. Chem. 72 (2000) 61.
[14] R.E. Fairhurst, C. Chassaing, R.F. Venn, A.G. Mayes, Biosens. Bioelectron. 20 (2004) 1098.
[15] M. Klede, H. Schmitz, T. Göen, M. Fartasch, H. Drexler, M. Schmelz, Exp. Dermatol. 14 (2005) 103.
[16] H.J. Fletcher, J.A. Stenken, Anal. Chim. Acta. 620 (2008) 170.
[17] M. Müller, R. Schmid, O. Wagner, B.V. Osten, H. Shayganfar, H.G. Eichler, J. Control. Release 37 (1995) 49.
[18] R.L. Carrier, L.A. Miller, I. Ahmed, J. Control. Release 123 (2007) 78.
[19] S.P. Huang, L.C. Lin, Y.T. Wu, T.H. Tsai, J. Chromatogr. B 877 (2009) 247.
[20] Y. Jiang, H.J. Li, P. Li, Z.H. Cai, W.C. Ye, J. Nat. Prod. 68 (2005) 264.
[21] J.L. Zhou, P. Li, H.J. Li, Y. Jiang, M.T. Ren, Y. Liu, J. Chromatogr. A 1177 (2008) 126.

[22] W. Wang, C.Y. Li, X.D. Wen, P. Li, L.W. Qi, J. Chromatogr. B 877 (2009) 671.
[23] L. Sun, J.A. Stenken, J. Chromatogr. A 1161 (2007) 261.
[24] O. Epemolu, I. Mayer, F. Hope, P. Scullion, P. Desmond, Rapid Commun. Mass Spectrom. 16 (2002) 1946.
[25] Z. Yu, D. Westerlund, K.S. Boos, J. Chromatogr. B 704 (1997) 53.
[26] X. Wu, H. Chen, J. Sun, Y. Peng, Y. Liang, G. Wang, J. Wu, P. Zhang, J. Chromatogr. B 878 (2010) 2067.
[27] Q.L. Zhang, A.Q. Wang, J. Song, J.L. Li, J.R. Cao, J.H. Zhao, Z.X. Tang, Z.Z. Wu, Chin. Pharm. J. 35 (2000) 688.
[28] S.W. Chan, S.L. Li, G. Lin, P. Li, Anal. Biochem. 285 (2000) 172.