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# Quantitative determination of hederagenin in rat plasma and cerebrospinal fluid by ultra fast liquid chromatography–tandem mass spectrometry method

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ABSTRACT

A rapid, sensitive and selective method was developed for the quantitative determination of hederagenin in rat plasma and cerebrospinal fluid (CSF) by ultra fast liquid chromatography–tandem mass spectrometry (UFLC–MS/MS). It has been successfully applied in a pharmacokinetic study of hederagenin in the central nervous system (CNS). Sample pretreatment involved a simple protein precipitation with methanol and a one-step extraction with ethyl acetate. Separation was carried out in a Shim-pack XR-ODS II (75 mm × 2.0 mm, i.d., 2.1 μm) column with gradient elution at a flow rate of 0.35 mL/min. The mobile phase was 5 mM ammonium acetate and acetonitrile. Detection was performed in a triple-quadruple tandem mass spectrometer by multiple-reaction-monitoring mode via electrospray ionization. A linear calibration curve for hederagenin was obtained over a concentration range of 0.406 (lower limit of quantification, LLOQ) to 203 ng/mL ($r^2 > 0.99$) for both plasma and CSF. The intra-day and inter-day precision (relative standard deviation, RSD) values were less than 15%. At all quality control (QC) levels, the accuracy (relative error, RE) was within −9.0% and 11.1% for plasma and CSF, respectively. The pharmacokinetics results indicated that hederagenin could pass through the blood–brain barrier. This UFLC–MS/MS method demonstrates higher sensitivity and sample throughput than previous methods. It was also successfully applied to the pharmacokinetic study of hederagenin following oral administration of *Fructus akebiae* extract in rats.



## 1. Introduction

*Fructus akebiae*, the dry fruit of *Akebiae quinata* (Thunb.) Decne., is a well-known medicinal plant, which is widely distributed in China. It is recorded in the Compendium of Materia Medica that *Fructus akebiae* is the major ingredient in some complex prescriptions for treating mental disorders as well as cognitive and behavioral deficits, including insomnia, concentration deficits, phobias and depression etc. Studies reveal that the genus *Akebiae* contains more than thirty types of triterpenoid saponins, and most of these triterpenoid saponins contain hederagenin [1,2]. The predominant hederagenin exhibits a variety of pharmacological effects such as antibiosis, diuresis, depressurization, and anticancer activities [3–5]. In previous studies, hederagenin from the extracts of *Fructus akebiae* was enriched to approximately 70% purity. Moreover, our studies have demonstrated that *Fructus akebiae* extracts (FAE) have antidepressant activity by improving the motivational behavioral deficits [6]. To study the pharmacokinetics of FAE extracts at clinically relevant doses, an accurate, high-sensitivity and rapid quantitative detection method for hederagenin in biological fluids should be established.

Several HPLC methods have been described for the determination of hederagenin in traditional Chinese herbs, such as ELSD detection [7] and UV detection [8–10]. However, these methods have drawbacks of low sensitivity, long analysis times and low throughput that do not meet the requirements for clinical biosample analysis. We noticed that HPLC–MS method has been described to identify the hederagenin in rat feces [11], but could find no report of pharmacokinetic studies of hederagenin or determination of hederagenin concentrations in biological fluids. High performance liquid chromatography (HPLC) coupled with tandem mass spectrometry is a powerful tool for the characterization and identification of active components in Chinese medicines and their metabolites by dissociation of the parent ions into small daughter ions without further isolation and purification. Thus, a method coupled with ultra fast liquid chromatography–tandem mass spectrometry (UFLC–MS/MS) was first developed for continuous monitoring of hederagenin in rat plasma and cerebrospinal fluid (CSF). The validation results showed high sensitivity (an LLOQ as low as 0.406 ng/mL) and short analytical time (4 min per sample).

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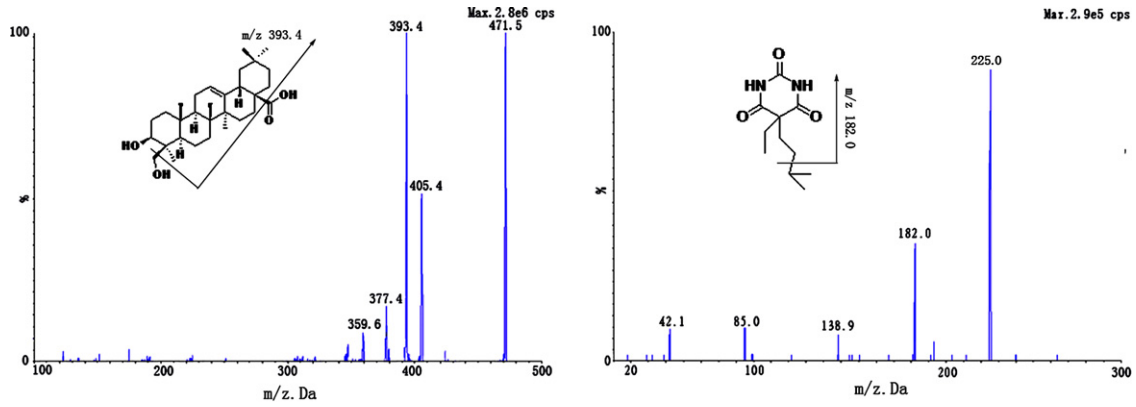


**Fig. 1.** Product ion spectra of hederagenin and amobarbital (IS).

The method was fully validated and successfully applied to the pharmacokinetic study of hederagenin in rat plasma and CSF following oral administration of FAE.

## 2. Experimental

### 2.1. Chemicals and reagents

The hederagenin reference standard (98.8% purity) was provided by TAUTO BIOTECH (Shanghai, China) and Amobarbital (98.5% purity, as internal standard, IS) was obtained from the National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). *Fructus akebiae* extracts (FAE) were provided by the School of Pharmaceutical Sciences of Southern Medical University. Artificial cerebrospinal fluid (ACSF) was purchased from YING CHUANG (Huzhou, China). Acetonitrile, methanol, and ammonium acetate (HPLC grade) were purchased from Dikma (Richmond Hill, NY, USA). All other chemicals were of analytical grade. Ethyl acetate was purchased from Yuwang (Chemical Reagent Plant, Shandong, China). Water was purified by redistillation and filtered through a 0.22 μm membrane filter before use.

### 2.2. Apparatus and operation conditions

#### 2.2.1. Liquid chromatography

Chromatography was performed using the SHIMADZU LC-20A $D_{XR}$ UFLC system with an autosampler and column oven enabling temperature control of the analytical column. A Shim-pack XR-ODS II column (75 mm × 2.0 mm, i.d., 2.1 μm; Shimadzu Corp.) was employed. The column temperature was maintained at 40 °C and chromatographic separations were achieved with a gradient elution. The mobile phase consisted of A (5 mM aqueous ammonium acetate) and B (acetonitrile). The gradient started at 40% mobile phase B and changed linearly to 90% mobile phase B over 0.3 min, was maintained at 90% B for 1.9 min, and then returned to the initial condition. The flow rate was set at 0.35 mL/min. The autosampler was conditioned at room temperature and the injection volume was 10 μL using the partial loop mode for sample injection.

#### 2.2.2. Mass spectrometry

Triple-quadruple tandem mass spectrometric detection was carried out in a Sciex API 4000 Qtrap mass spectrometer (Applied Biosystems, CA, USA). The ESI source was set in negative ionization mode. Quantification was performed using multiple reaction monitoring (MRM) of the transitions of $m/z$ 471.5 → 393.4 for hederagenin and $m/z$ 225 → 182 for amobarbital (IS), with a scan time of 0.10 s per transition. The instrument settings were optimized during analyte infusion to maximize response. The product ion spectra of hederagenin and amobarbital are shown in Fig. 1. All data were acquired and processed using Analyst Software (Applied Biosystems, USA).

### 2.3. Preparation of standards and quality control samples

The primary stock standard solutions of hederagenin and amobarbital were both prepared in methanol at concentrations of 8.13 μg/mL and 10.0 μg/mL, respectively. The internal standard solution was diluted with methanol to 10.0 ng/mL. Then, the hederagenin solution was serially diluted with methanol to provide working standard solutions of the desired concentrations. All the solutions were stored at −20 °C.

Calibration standards were prepared by spiking 0.2 mL of blank rat plasma and 0.1 mL of ACSF with working standard solutions of hederagenin. The effective concentrations in standard plasma samples and CSF samples were 0.406, 1.02, 5.08, 10.2, 50.8, 102, and 203 ng/mL. The calibration curve was constructed on each analysis day using freshly prepared calibration standards. The quality control samples (QCs) were prepared to evaluate accuracy and precision of this method with blank plasma and ACSF at LLOQ (0.406 ng/mL), low (1.02 ng/mL), mid-range (10.2 ng/mL), and high (102 ng/mL) concentrations. The standards and quality controls were extracted on each analysis day with the same procedure for plasma and CSF samples as described below.

### 2.4. Preparation of plasma and CSF samples

Male SD rats (200–250 g) were obtained from the Laboratory Animal Centre of Southern Medical University (Guangzhou, China) and were acclimated to the facility for 1 week before use in the experiments. Rats were housed 8–12 per cage, and singly housed in standard rat cages. The animals were housed at 23 ± 1 °C with a 12 h/12 h light/dark cycle (lights on at 7:00 a.m.), and were given ad libitum access to water and food. All procedures in this study were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Blood samples (about 500 μL) were collected in heparinized 1.5 mL-polythene tubes immediately pre-dose and post-dose, then centrifuged at 3500 rpm for 10 min at 4 °C. The plasma obtained was stored at −20 °C until analysis.

Internal standard (50 μL) and 0.65 mL methanol were added to a 0.2 mL aliquot of rat plasma in a 1.5 mL centrifuge tube. The mixture was vortex-mixed thoroughly for 1 min, and then centrifuged at 13,000 rpm for 10 min. After centrifugation, the supernatant fluid was transferred to another set of clean glass tubes and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was reconstituted in 100 μL of methanol and transferred to 200 μL

glass vials. Then, a 10 μL aliquot was injected into the UFLC–MS/MS system for analysis.

The rats were anaesthetised using chloral hydrate (Sinopharm Chemical Reagent Co. Ltd.) and CSF removed by puncturing the cisterna magna with a 21G cannula and then euthanased by decapitation. All samples were collected into sterile centrifuge tubes and routinely centrifuged twice at 13,000 rpm to remove any cells or debris from the fluid, which was decanted into another sterile tube. Samples were stored at −20 °C until use.

The internal standard (50 μL of 10 ng/mL stock) and 1.5 mL ethyl acetate were added to a 0.1 mL CSF sample in a 10 mL clean glass tube. The mixture was vortex-mixed for another 60 s, and then agitated in a mechanical shaker for 2 min. After centrifugation at 3500 × *g* for 10 min, the upper organic layer was transferred to another clean glass tube and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was reconstituted in 100 μL of methanol and transferred to a 200 μL glass vial. Then, a 10 μL aliquot was injected into the UFLC–MS/MS system for analysis.

### 2.5. Method validation

The method was validated for selectivity, linearity, LLOQ, accuracy, precision and extraction recovery. Validation runs were conducted on three consecutive days. Each validation run consisted of a minimum of one set of calibration standards and six replicates of LLOQ, QC plasma and ACSF samples at three concentrations. Results from the LLOQ, QC plasma and CSF samples in the three runs were used to evaluate the precision and accuracy of the developed method. The validation parameters and the range of their values for acceptance were in accordance with international guidelines [12] and US Food and Drug Administration guidelines [13].

To evaluate the matrix effect on the ionization of analyte (the potential ion suppression or enhancement due to the matrix components), hederagenin at three concentration levels were added to the 0.2 mL blank extraced plasma and 0.1 mL extracted ACSF, then dried and reconstituted in 100 μL methanol. Afterwards, the corresponding peak areas (*A*) were compared with those of the hederagenin standard solutions dried directly and reconstituted with the same methanol (*B*). The ratio ($A/B \times 100$)% was used to evaluate the matrix effect. The same procedure was performed for the internal standard (10.0 ng/mL).

The stabilities of the analytes in rat plasma and CSF sample under various storage conditions were evaluated as follows: QC samples were subjected to processed samples kept at 25 °C in autosampler for 4 h, to short-term stability kept at ambient temperature (25 °C) for 4 h, to long-term (7 days) storage conditions (−20 °C), and to three freeze–thaw cycles stability studies. Freezing was performed at −20 °C for 24 h and thawed at ambient temperature. All the stability studies were conducted with QC samples at three concentration levels with 5 determinations for each. The lower limit of quantification (LLOQ) was defined as the lowest concentration level that provided a peak area with a signal-to-noise ratio of 10, with an acceptable accuracy of ±15% and a precision below 15%.

### 2.6. Application to pharmacokinetic study

In this study, FAE (hederagenin of approximately 70% purity) was used instead of pure hederagenin for the pharmacokinetic investigation because of the insufficient availability and high cost of pure hederagenin. The method was applied to determine the plasma and CSF concentrations of hederagenin after oral FAE (400 mg/kg, contains about hederagenin 280 mg/kg). Rat plasma samples were collected before and 0, 5, 10, 15, 20, 30, 60, 90, 120, 150, 180, 210, 240 and 300 min after oral dosing. Rat CSF samples were only collected before and 20 min post-dosing because concentrations of hederagenin in rat CSF were too low to complete a concentration–time curve. Plasma and CSF samples were centrifuged and then stored at −20 °C until analysis.

The maximum plasma concentration ($C_{max}$) and the time ($T_{max}$) were noted directly. The elimination rate constant ($k_e$) was calculated by linear regression of the terminal points from the semi-log plot of plasma concentration against time. Elimination half-life ($t_{1/2}$) was calculated using the formula $t_{1/2} = 0.693/k_e$. The area under the plasma concentration–time curve ($AUC_{0-t}$) to the last measurable plasma concentration ($C_t$) was calculated by the linear trapezoidal rule. The area under the plasma concentration–time curve to time infinity ($AUC_{0-\infty}$) was calculated as: $AUC_{0-\infty} = AUC_{0-t} + C_t/k_e$. DAS 2.1.1 software was used to deal with PK data.

## 3. Results and discussion

### 3.1. Selection of IS

The best internal standard in an LC–MS assay is a deuterated form of the analyte. Unfortunately, deuterated hederagenin was unavailable commercially. Moreover, it was difficult to obtain in our laboratory since a special synthesis of an isotopically labeled standard can be expensive, time consuming and specific protection should be available in the laboratory. On the other hand, a compound structurally or chemically similar to the analyte should be considered. In the primary study, several compounds with similar structure, such as ursone and oleanolic acid were chosen as IS. However, they were not suitable as IS since all of them were found in FAE. The reason is that hederagenin from the extracts of FAE was only enriched to approximately 70% of purity and compounds of similar structure could be found in FAE. Finally, amobarbital was chosen as the internal standard for the assay because the retention time and ionization response in ESI mass spectrometry of amobarbital are similar to hederagenin. Furthermore, the mean extraction recoveries of amobarbital in CSF and plasma samples were both higher than 85%, which indicate that amobarbital has similar extraction and chromatography behavior as hederagenin.

### 3.2. Optimization of chromatography and mass spectrometry

The selection of mobile phase components was critical because the separation and ionization of hederagenin and amobarbital were affected by the mobile phase composition. Ammonium acetate was employed to supply the ionic strength and 5 mM of ammonium acetate can achieve this purpose. Gradient elution is usually used in order to extend column life and elute the analyte rapidly. The use of small particles in the stationary phase allowed HPLC to push the limits of both peak capacity (due to higher efficiency) and speed of analysis (due to higher linear velocities) without compromising resolution. However, the column with sub-2 μm particles was more easily blocked. Therefore, gradient elution was used to provide a better peak shape and increase the column life. The retention time for hederagenin was 2.02 min in plasma and 1.99 min in CSF, and the retention time for amobarbital (IS) was 1.42 min. As shown in Figs. 2 and 3, both hederagenin and amobarbital were well separated with excellent peak shapes.

In the full-scan mass spectra of hederagenin, the signal intensity in negative ion mode was much higher than that in the positive ion mode. Thus, all detections were carried out using the predominantly negative ionization mode. The instrument settings were optimized during analyte infusion to maximize response. $MS^2$ spectrum of hederagenin showed a $[M-H]^-$ ion at *m*/*z* 471.5, as well as showed a characterized fragmentation pathway of *m*/*z* 471.5 → 393.4 (loss of $-CH_3OH$ and –HCOOH, respectively). The result was coincident with the literature [11]. $MS^2$ spectrum of IS showed a $[M-H]^-$ ion at *m*/*z* 225 and a characterized fragmenta-

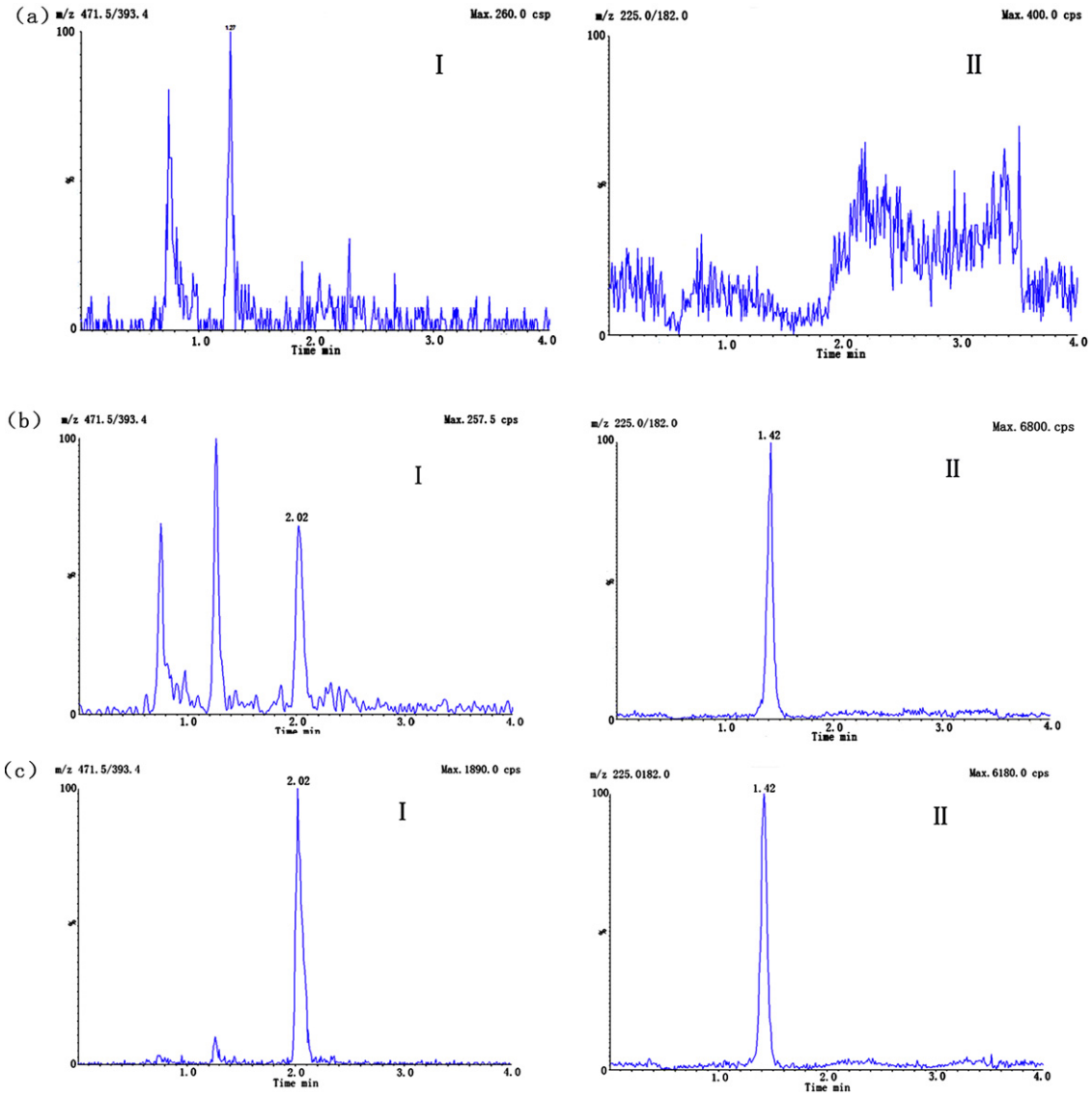


**Fig. 2.** Representative chromatograms of hederagenin and amobarbital (IS) in rat plasma samples are shown in I and II, respectively. (a) A blank plasma sample, (b) a blank plasma sample spiked with hederagenin at the LLOQ of 0.406 ng/mL and amobarbital (IS) (10.0 ng/mL), (c) a plasma sample from a rat 1.0 h after oral administration of FAE.

tion of 182 (loss of $-CH(CH_3)_2$). Following detailed optimization of mass spectrometry conditions, $m/z$ 471.5 → 393.4 was used for quantification of hederagenin and $m/z$ 225 → 182 for IS.

Both hederagenin and amobarbital were rapidly eluted with retention times less than 2.1 min, and the total run time was 4.0 min per sample. The retention time for hederagenin using HPLC–ELSD [7], HPLC–UV [10] and LC–MS [11] were about 7.0 min, 7.3 min and 43.8 min, respectively. The short analysis time may meet the requirement for high sample throughput in bioanalysis. The increasing column efficiency in HPLC also resulted in a higher sensitivity. The LLOQ was 0.406 ng/mL in plasma and CSF, which was lower than the reported LLOQs by HPLC–ELSD and HPLC–UV and meet the norm that LLOQs should be higher than 1.18 μg/mL.

### 3.3. Selection of extraction method

Liquid–liquid extraction (LLE) and solid-phase extraction (SPE) are often used for biological sample preparation to improve the sensitivity and robustness of assays. However, protein precipitation is much simpler than either LLE or SPE, and will cost-effective for high throughput analysis. To obtain a high extraction efficiency, three different protein precipitation agents, acetonitrile, methanol and acetone, were investigated. From the three agents, methanol was the most efficient one in precipitation, thus a simple single-step protein precipitation with methanol was adopted to prepare plasma samples. The mean recovery of hederagenin and the IS in plasma were both higher than 85%. However, in rat CSF, samples after liquid–liquid extraction have higher ionization response than that of the samples after protein precipitation. And the concentrations of hederagenin in rat CSF were much lower than that in plasma samples and the volume of rat CSF was much less. This ionization suppression effects may affect the sensitivity of the determination of hederagenin in rat CSF. Therefore, liquid–liquid extraction was performed to extract the analyte from rat CSF. After investigation of several extract solvent, ethyl acetate was chosen as the extraction solvent to prepare CSF samples and the recovery was satisfactory.

### 3.4. Method validation

#### 3.4.1. Selectivity

Selectivity was assessed by comparing the chromatograms of six different batches of blank plasma and CSF with the corresponding standard plasma samples spiked with hederagenin and

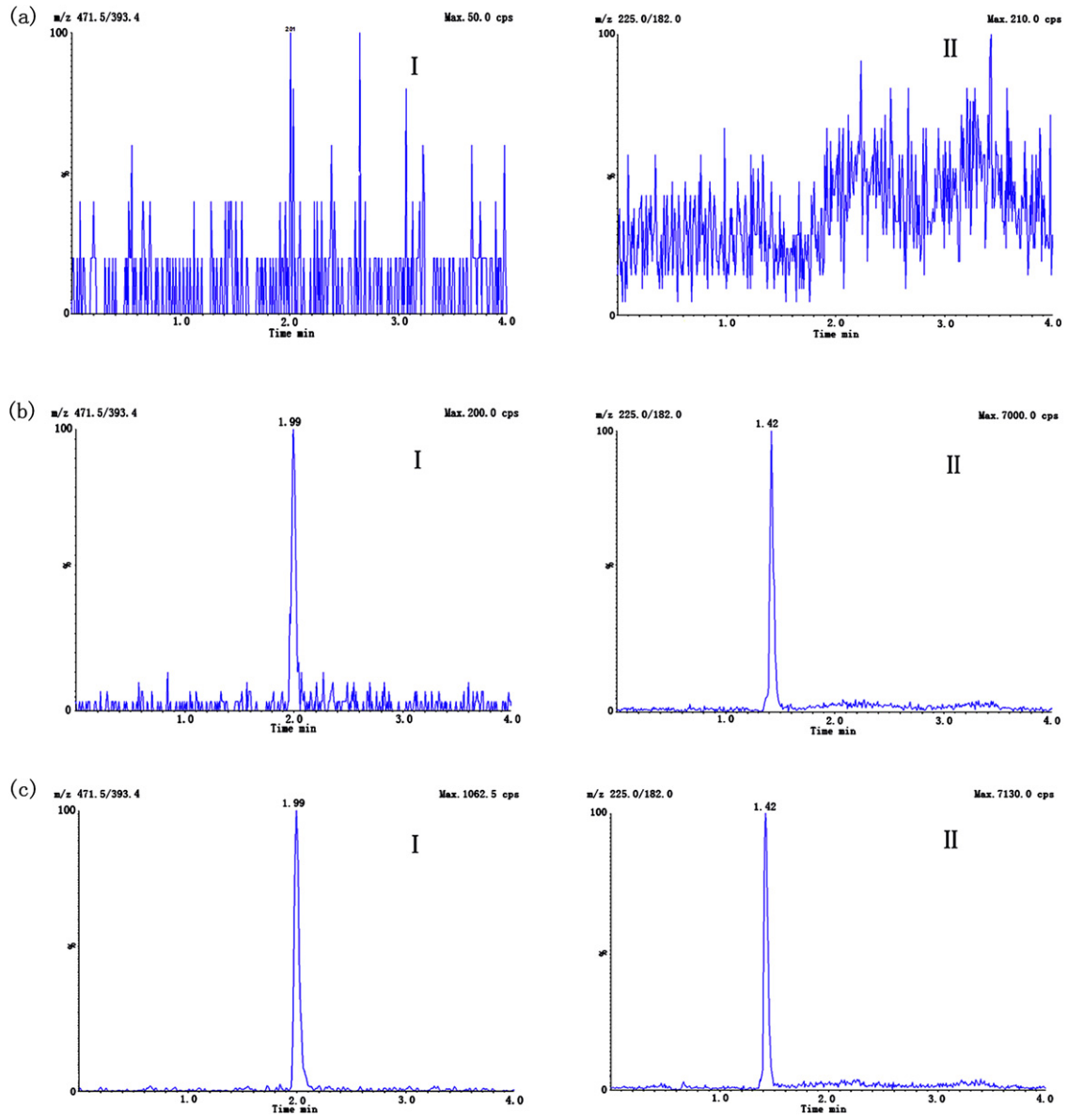


**Fig. 3.** Representative chromatograms of hederagenin and amobarbital (IS) in rat CSF samples are shown in I and II, respectively. (a) A blank CSF sample, (b) a blank CSF sample spiked with hederagenin at the LLOQ of 0.406 ng/mL and amobarbital (IS) (10.0 ng/mL), (c) a CSF sample from a rat 20 min after oral administration of FAE.

amobarbital (IS) as well as plasma and CSF samples after oral doses of hederagenin. As shown in Figs. 2 and 3, no interference from endogenous substances was observed at the retention times of hederagenin and amobarbital.

### 3.4.2. Linearity and LLOQ

The standard calibration curves for hederagenin were linear over the concentration range of 0.406–203 ng/mL ($r^2 > 0.99$) using the weighted least square linear regression analysis with a weight factor of $1/x^2$. A typical equation for the calibration curves in plasma was $y = 0.0245x + 0.00671$, $r = 0.9981$. Typical corresponding values for CSF samples were $y = 0.0520x + 0.0529$ and $r = 0.9960$.

The LLOQ for hederagenin was 0.406 ng/mL from a 10 μL sample injected onto the UFLC column (with precision and accuracy presented in Table 1). The RE was within ±20%, while RSD was lower than 20%. A corresponding chromatogram is given in Figs. 2(b) and 3(b). The high sensitivity can be attributed to the extra resolution and peak sharpness produced by the UFLC chromatographic system and the improved ionization efficiency under the mass spectrometric conditions.

### 3.4.3. Precision and accuracy

The intra-day and inter-day precision and accuracy for the method are listed in Table 1. The intra-day precision for LLOQ, low, mid-range, and high concentrations in QC samples of hederagenin was between 2.4% and 8.9%, while inter-day precision was between 1.1% and 13.0%, with an accuracy (RE) within −9.0% to 11.1%. The precision and accuracy of the present method conformed to the criteria for the analysis of biological samples set by the USFDA [13], where precision (RSD) determined at each concentration level must not exceed 15%, with RE within ±15% of the actual value.

### 3.4.4. Extraction recovery and matrix effect

The extraction recoveries and matrix effect to determine hederagenin in rat plasma and CSF are shown in Table 2. In terms of matrix effect, all the ratios $(A/B \times 100)\%$ (defined as in Section 2) were between 85% and 115%, which means that there was no

**Table 1**
Precision and accuracy for the hederagenin determination in plasma and CSF (intra-day: $n = 6$; inter-day: $n = 6$ series per day, 3 days).

| Assay | Added C (ng/mL) | Found C (ng/mL) | Intra-run RSD (%) | Inter-run RSD (%) | Accuracy RE (%) |
|---|---|---|---|---|---|
| Plasma | 0.406 (LLOQ) | 0.400 ± 0.01 | 4.3 | 2.5 | −0.7 |
| | 1.02 (low) | 1.02 ± 0.05 | 4.6 | 1.1 | 0.6 |
| | 10.2 (middle) | 10.5 ± 0.50 | 3.8 | 8.1 | 3.8 |
| | 102 (high) | 101 ± 4.80 | 4.2 | 5.6 | 1.6 |
| CSF | 0.406 (LLOQ) | 0.420 ± 0.05 | 8.9 | 13.0 | −1.4 |
| | 1.02 (low) | 1.04 ± 0.08 | 6.2 | 5.3 | 3.0 |
| | 10.2 (middle) | 9.92 ± 0.34 | 2.6 | 3.7 | 11.1 |
| | 102 (high) | 102 ± 4.23 | 2.4 | 9.8 | −9.0 |

**Table 2**
Extraction recovery and matrix effect for the hederagenin determination in rat plasma and CSF ($n = 5$).

| Assay | Con. (ng/mL) | Recovery (%) mean ± SD | RSD (%) | Matrix effect (%) mean ± SD | RSD (%) |
|---|---|---|---|---|---|
| Plasma | 1.02 (low) | 87.9 ± 2.5 | 2.8 | 101.8 ± 6.2 | 6.1 |
| | 10.2 (middle) | 85.3 ± 3.7 | 4.3 | 98.3 ± 4.9 | 5.0 |
| | 102 (high) | 87.2 ± 2.3 | 2.6 | 95.2 ± 2.6 | 2.7 |
| IS | 10.0 | 86.8 ± 1.7 | 2.0 | 99.1 ± 5.4 | 5.4 |
| CSF | 1.02 (low) | 85.2 ± 5.1 | 6.0 | 102.0 ± 7.0 | 6.8 |
| | 10.2 (middle) | 87.9 ± 4.3 | 4.9 | 102.5 ± 4.1 | 4.0 |
| | 102 (high) | 89.3 ± 3.1 | 3.5 | 99.6 ± 3.7 | 3.7 |
| IS | 10.0 | 88.5 ± 2.9 | 3.3 | 102.8 ± 2.3 | 2.2 |

**Table 3**
Stability of hederagenin in plasma and CSF at three QC levels ($n = 5$).

| Assay | Stability | Accuracy (%) (mean ± SD) | | |
|---|---|---|---|---|
| | | 1.02 (ng/mL) | 10.2 (ng/mL) | 102 (ng/mL) |
| Plasma | Short-term stability | 99.0 ± 5.9 | 108.4 ± 4.6 | 99.6 ± 5.6 |
| | Long-term stability (7D) | 95.1 ± 8.0 | 93.8 ± 6.5 | 98.6 ± 6.8 |
| | Freeze–thaw stability ($n = 3$) | 99.0 ± 5.4 | 101.5 ± 3.7 | 99.5 ± 2.2 |
| | Post-preparative stability | 105.9 ± 5.8 | 98.7 ± 5.7 | 101.6 ± 4.6 |
| CSF | Short-term stability | 106.9 ± 6.5 | 109.4 ± 3.9 | 102.3 ± 4.1 |
| | Long-term stability (7D) | 102.9 ± 3.4 | 97.1 ± 3.1 | 100.9 ± 5.5 |
| | Freeze–thaw stability ($n = 3$) | 94.1 ± 8.4 | 97.5 ± 1.9 | 96.7 ± 5.9 |
| | Post-preparative stability | 101.0 ± 4.8 | 98.9 ± 2.5 | 104.0 ± 7.5 |

matrix effect on the estimation of hederagenin and amobarbital concentrations.

### 3.4.5. Stability

Stock solutions of hederagenin and amobarbital were stable at room temperature for 4 h and at −20 °C for 7 days. As shown in Table 3, results from all stability tests demonstrated good stability of hederagenin over all steps for determination, indicating that hederagenin exhibited no significant degradation under these experimental conditions. Therefore, the method is proven to be applicable for routine analysis.

## 3.5. Pharmacokinetic application

The present method was successfully applied to the pharmacokinetic study of hederagenin after oral administration in rats.

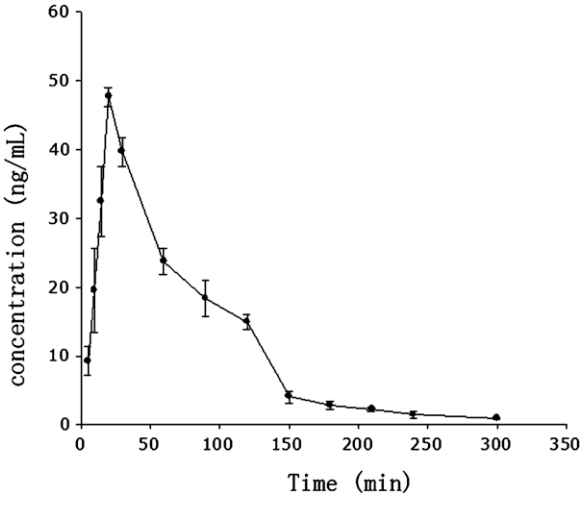


**Fig. 4.** Mean plasma concentration–time curves of hederagenin in rat after a single oral dose of FAE (about 280 mg/kg hederagenin) ($n = 6$).

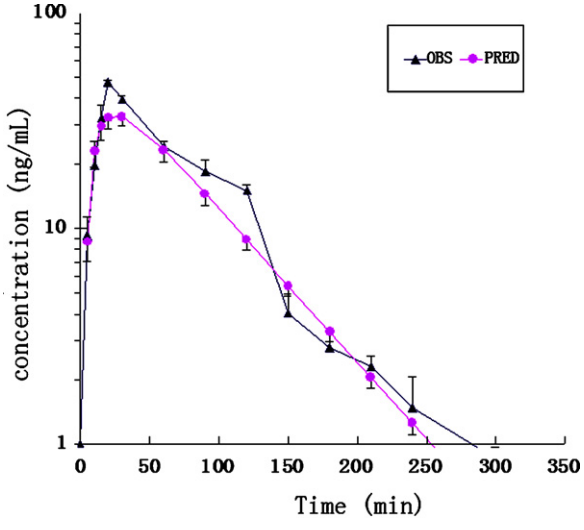


**Fig. 5.** Semi-log of time course of hederagenin in rat plasma after a single oral dose of FAE (about 280 mg/kg hederagenin) ($n = 6$).

**Table 4**
Pharmacokinetic parameters of hederagenin in rat plasma after a single oral dose of FAE ($n = 6$).

| Parameters | In plasma |
|---|---|
| $T_{max}$ (min) | 18.33 ± 2.58 |
| $C_{max}$ (μg/L) | 47.73 ± 1.39 |
| $t_{1/2}$ (min) | 44.06 ± 2.98 |
| $AUC_{0-t}$ (μg/L min) | 3066.46 ± 243.07 |
| $AUC_{0-\infty}$ (μg/L min) | 3131.47 ± 236.83 |
| CL (L/(min/kg)) | 128.36 ± 9.90 |
| $K_e$ (1/min) | 0.016 ± 0.001 |

The time-concentration curves and semi-log of time plot of hederagenin at dosages of 400 mg/kg FAE in rat plasma was illustrated in Figs. 4 and 5 and the parameters obtained are shown in Table 4. Results of this study show that hederagenin can be detected immediately in plasma within 5 min and reach its peak concentration of 47.73 ng/mL about 20 min after oral administration, which indicated that hederagenin could be quickly gastrointestinal absorbed.

In addition, at 20 min, 6.17 ± 0.22 ng/mL (RSD, 3.5%) of hederagenin was detected in rat CSF after administration of a single dose of 400 mg/kg FAE (about 280 mg/kg hederagenin). Thus, this result indicated that hederagenin can pass through the blood–brain barrier and distribute into the CSF quickly, as well as rouses interest in the further study of hederagenin from *Fructus akebiae* with regard to its effects on antidepressant functions.

## 4. Conclusion

A quantitative method for the determination of hederagenin in rat CSF and plasma by UFLC–MS/MS was developed and fully validated. The features such as high sensitivity, selectivity and quick analysis (4 min) make this method particularly attractive for high throughput bioanalysis of hederagenin. The method has been successfully applied in a pharmacokinetic study of hederagenin in rat plasma and CSF.

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