Journal of Chromatography B, 879 (2011) 3407–3414

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

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

Simultaneous determination of asperosaponin VI and its active metabolite hederagenin in rat plasma by liquid chromatography–tandem mass spectrometry with positive/negative ion-switching electrospray ionization and its application in pharmacokinetic study

He Zhu[a], Li Ding[a,*], Shailendra Shakya[a], Xiemin Qi[a], Linlin Hu[a], Xiaolin Yang[b], Zhonglin Yang[b]

[a] Department of Pharmaceutical Analysis, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China
[b] Key Laboratory of Modern Chinese Medicines, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China

ARTICLE INFO

Article history:
Received 17 June 2011
Accepted 7 September 2011
Available online 13 September 2011

Keywords:
Asperosaponin VI
Hederagenin
Liquid chromatography–tandem mass spectrometry
Ion-switching
Pharmacokinetics

ABSTRACT

A new liquid chromatography–tandem mass spectrometry (LC–MS/MS) method operated in the positive/negative electrospray ionization (ESI) switching mode has been developed and validated for the simultaneous determination of asperosaponin VI and its active metabolite hederagenin in rat plasma. After addition of internal standards diazepam (for asperosaponin VI) and glycyrrhetic acid (for hederagenin), the plasma sample was deproteinized with acetonitrile, and separated on a reversed phase C18 column with a mobile phase of methanol (solvent A)–0.05% glacial acetic acid containing 10 mM ammonium acetate and 30 μM sodium acetate (solvent B) using gradient elution. The detection of target compounds was done in multiple reaction monitoring (MRM) mode using a tandem mass spectrometry equipped with positive/negative ion-switching ESI source. At the first segment, the MRM detection was operated in the positive ESI mode using the transitions of m/z 951.5 ($[M+Na]^+$) → 347.1 for asperosaponin VI and m/z 285.1 ($[M+H]^+$) → 193.1 for diazepam for 4 min, then switched to the negative ESI mode using the transitions of m/z 471.3 ($[M-H]^-$) → 471.3 for hederagenin and m/z 469.4 ($[M-H]^-$) → 425.4 for glycyrrhetic acid, respectively. The sodiated molecular ion $[M+Na]^+$ at m/z 951.5 was selected as the precursor ion for asperosaponin VI, since it provided better sensitivity compared to the deprotonated and protonated molecular ions. Sodium acetate was added to the mobile phase to make sure that abundant amount of the sodiated molecular ion of asperosaponin VI could be produced, and more stable and intensive mass response of the product ion could be obtained. For the detection of hederagenin, since all of the mass responses of the fragment ions were very weak, the deprotonated molecular ion $[M-H]^-$ m/z 471.3 was employed as both the precursor ion and the product ion. But the collision energy was still used for the MRM, in order to eliminate the influences induced by the interference substances from the rat plasma. The validated method was successfully applied to study the pharmacokinetics of asperosaponin VI and its active metabolite hederagenin in rat plasma after oral administration of asperosaponin VI at a dose of 90 mg/kg.

1. Introduction

Asperosaponin VI (3-O-α-L-arabinopyranosyl hederagenin-28-β-D-glucopyranoside-(1 → 6)-β-D-glucopyranoside), also named akebia saponin D (Fig. 1A), is a typical bioactive triterpenoid saponin isolated from the rhizome of *Dipsacus asper* Wall (Dipsacaceae) [1]. In the past decades, asperosaponin VI has been used as a tonic, analgesic and anti-inflammatory agent in traditional Chinese medicines. However, recent studies revealed that the root extract of *Dispacus asper* ameliorated the impairment of cognitive dysfunction in a passive avoidance task and suppressed the overexpression of hippocampal Aβ that had been induced by aluminum chloride [2,3]. Moreover, asperosaponin VI, the principle component of *Dispacus asper*, had neuroprotective capacity to antagonize Aβ 25–35 induced cytotoxicity in PC 12 cells by inhibiting excessive Ca^{2+} influx, reducing additional lactate dehydrogenase leakage and preventing the loss of cell viability, thus is pharmacologically effective in the treatment of Alzheimer's disease [4]. Asperosaponin VI was noticed to possess apoptosis-inducing activity via nitric oxide and apoptosis-related p53 and Bax gene expression [5]. It was also observed to exert pro-osteogenic, pro-angiogenic and anti-Nitric Oxide releasing effects in bone fracture treatment [6]. Previous

* Corresponding author. Tel.: +86 25 832712 89; fax: +86 25 832712 89.
E-mail address: dinglidl@hotmail.com (L. Ding).

1570-0232/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2011.09.014

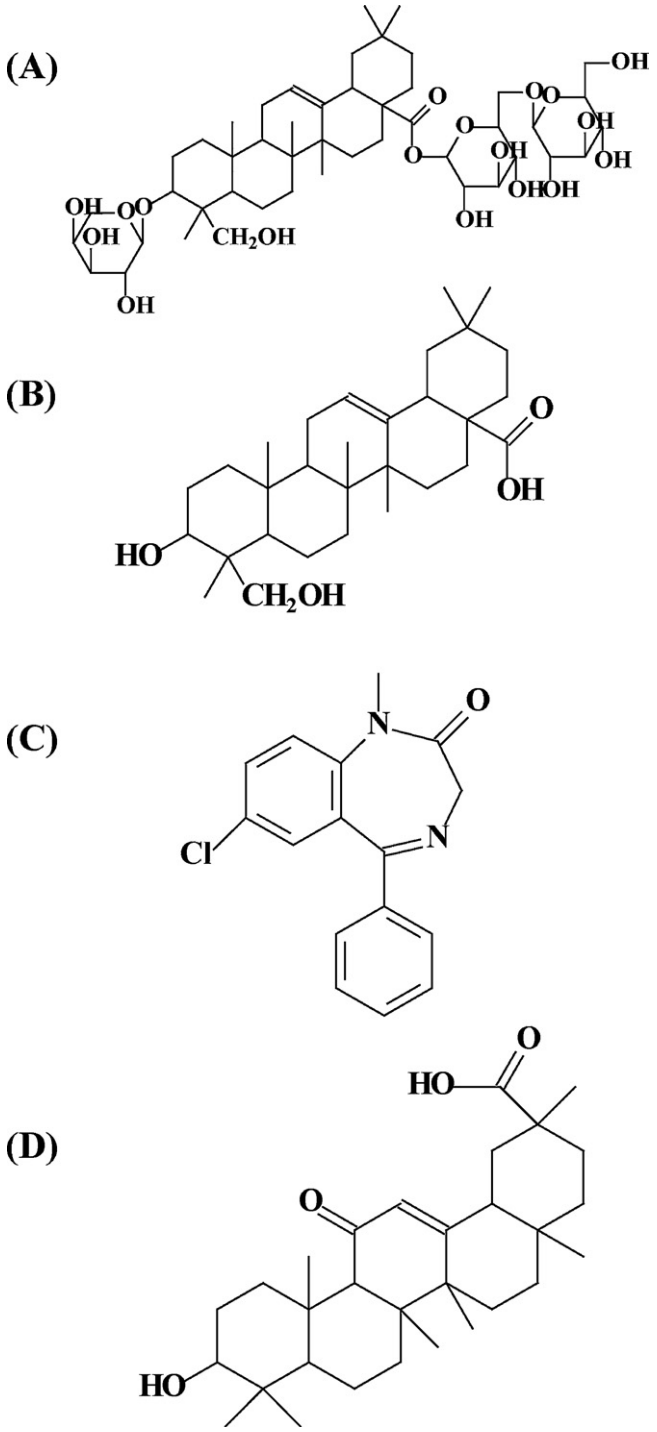

Fig. 1. Chemical structure of asperosaponin VI (A), hederagenin (B), diazepam (C) and glycyrrhetic acid (D).

studies indicated that asperosaponin VI is metabolized into five main metabolites in rats [7]. One of the main metabolites is hederagenin (Fig. 1B), the aglycone moiety of asperosaponin VI, which has been found to exhibit anti-depressant effect [8], anti-nociceptive and anti-inflammatory effect [9]. Several literatures are available on the pharmacological effects of asperosaponin VI and hederagenin, but little information was found on its analytical method and pharmacokinetic study. Two LC–MS methods for the analysis of asperosaponin VI in rat [10] and dog [11] plasma were developed for the pharmacokinetic studies. However, the quantitative method for the simultaneous determination of asperosaponin VI and its metabolite hederagenin has not been reported up to now.

In this paper, we present a simple LC–MS/MS method using positive/negative ion-switching ESI mode for the simultaneous quantification of asperosaponin VI and hederagenin in rat plasma using diazepam (IS-1, Fig. 1C) and glycyrrhetic acid (IS-2, Fig. 1D) as the internal standards for asperosaponin VI and hederagenin, respectively. This method was successfully applied for the evaluation of pharmacokinetic profiles of asperosaponin VI and its metabolite hederagenin in rats after oral administration asperosaponin VI at a dose of 90 mg/kg.

2. Experimental

2.1. Chemicals and reagents

The reference standard of asperosaponin VI (>98.0% purity) and hederagenin (>98.0% purity) were provided by Key Laboratory of Modern Chinese Medicine, China Pharmaceutical University. The internal standards IS-1 and IS-2 were purchased from National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). Methanol (HPLC grade) was purchased from Merck KGaA (Darmstadt, Germany). Ammonium acetate and glacial acetic acid (analytical grade) were purchased from Nanjing Chemical Reagents Co., Ltd. (Nanjing, China). Sodium acetate was purchased from Nanjing Chemical Reagent No.1 Factory (Nanjing, China). Water was prepared with double distillation.

2.2. Instrumentation

The liquid chromatography was performed on an Agilent 1200 Series liquid chromatography (Agilent Technologies, Palo Alto, CA, USA), which included an Agilent 1200 binary pump (model G1312B), vacuum degasser (model G1322A), Agilent 1200 autosampler (model G1367C), temperature controlled column compartment (model G1330B). The LC system was coupled with an Agilent 6410B triple quadrupole mass spectrometer (USA) equipped with an electrospray source (model G1956B). The signal acquisition and peak integration were performed using the Masshunter Qualitative Analysis Software (B.03.01Build 346) supplied by Agilent Technologies.

2.3. Chromatographic conditions

Chromatographic separations were performed on a Hedera ODS-2 analytical column (2.1 mm × 150 mm, 5 μm, Hanbon Sci&Tech, China) with a security Guard-C_{18} column (4 mm × 2.0 mm, 5 μm, Phenomenex). The samples on the column were eluted with a gradient mixture of methanol (solvent A) and 0.05% glacial acetic acid with 10 mM ammonium acetate and 30 μM sodium acetate (solvent B). The gradient program was as follow: 0–1.6 min: 73% A; 1.6–1.7 min: from 73% to 89% A; 1.7–5.6 min: 89% A; 5.6–5.8 min: from 89% to 73% A and followed by re-equilibration at 73% A until 9 min. The flow rate was at 0.39 mL/min with column temperature at 30 °C. Autosampler temperature was maintained at 8 °C and the injection volume was set at 6 μL.

2.4. MS/MS conditions

The ESI source was operated with polarity switching between positive and negative modes in a single run. The first segment, with positive ion mode, was designed between 0 and 4 min. It allowed detection for asperosaponin VI and IS-1. The second, with negative ion mode, was used for detection of hederagenin and IS-2 at the time period of 4–9 min. The operational parameters of ESI source

Table 1
HPLC–MS/MS conditions for compound analysis.

Analytes	ESI mode	Precursor ion (m/z)	Product ion (m/z)	Fragmentor voltage (V)	Collision energy (eV)
Asperosaponin VI	Positive	951.5	347.1	158	68
Diazepam (IS-1)	Positive	285.1	193.1	165	32
Hederagenin	Negative	471.3	471.3	255	55
Glycyrrhetic acid (IS-2)	Negative	469.4	425.4	158	50

were as follows: vaporizing temperature 350 °C; nebulizing gas pressure 60 psig; drying gas flow 12 L/min and capillary potential 4000 V. The precursor ions, product ions, and MS/MS parameters are displayed in Table 1.

2.5. Preparation of standard solutions

The standard stock solutions of asperosaponin VI, hederagenin, the IS-1 and IS-2 were prepared in acetonitrile at the concentration of 1 mg/mL, respectively. The working standards were prepared by dilution of the stock solution in acetonitrile to obtain the desired concentrations. All of the standard solutions were stored at −20 °C for further use.

2.6. Sample preparation

All of the frozen standards and samples were allowed to thaw at room temperature. The protein precipitation procedure was applied in the sample preparation. After adding 5 μL of the mixed ISs solution (800 ng/mL of the IS-1, 1600 ng/mL of the IS-2), aliquot of 30 μL rat plasma sample was treated with 90 μL acetonitrile solution, followed by vortex mix for 5 min and centrifugation for 10 min at 15,600 rpm. The supernatant was transferred into an injection vial.

2.7. Assay validation

The method was validated for selectivity, linearity, precision, accuracy, extraction recovery and stability according to FDA guidance for validation of bioanalytical methods.

The selectivity was assessed by comparing the chromatograms of six different sources of blank rat plasma with the corresponding spiked plasma. Each blank plasma sample was tested using the proposed extraction procedure and LC–MS/MS conditions to ensure no interference of asperosaponin VI, hederagenin and the ISs from plasma.

Calibration standard samples were prepared by adding different volume of working standards at different concentrations. The solutions were dried under nitrogen gas flow and then, aliquot of 30 μL plasma was added. The final concentrations of 2, 8, 30, 100, 300, 600, 1200 and 2000 ng/mL for asperosaponin VI and 2, 10, 25, 50, 100, 200 and 300 ng/mL for hederagenin were prepared. The calibration curve was constructed by plotting the each respective peak area ratios of asperosaponin VI to the IS-1 and hederagenin to the IS-2 v/s the concentrations of asperosaponin VI and hederagenin, respectively, using the weighting factor of $1/C^2$.

Quality control (QC) samples were prepared in blank plasma at the concentrations of 5, 80, 1700 ng/mL for asperosaponin VI and 5, 40, 260 ng/mL for hederagenin. All QC samples were prepared independently and analyzed in each analytical batch along with the unknown samples. Qualified results of the QC samples validated the reliability of the analytical batches.

The lower limit of quantification (LLOQ) was the lowest concentration on the calibration curve that can be measured with acceptable accuracy and precision. The LLOQ was established using five samples independent of standards. The precision and accuracy at LLOQ should fall within the range of 80–120%.

The accuracy, intra-batch and inter-batch precisions of the method were determined by analyzing five replicates at QC concentrations along with one calibration curve on three different batches. Assay precision was calculated using the relative standard deviation (RSD %) and using one-way analysis of variance (ANOVA). The accuracy of an analytical method describes the closeness of mean test results obtained by the method as the relative deviation in the calculated value (E) of a standard from that of its true value (T) expressed as a percentage (RE %). It was calculated using the formula: RE % = $(E - T)/T \times 100\%$. The intra- and inter-batch precisions were required to be less than 15%, and the accuracy to be within ±15%.

The matrix effect (ME) was defined as the ion suppression/enhancement on the ionization of analytes, which was evaluated by comparing the responses of the deproteinized sample of blank plasma from five spiked QC samples ($n = 5$) with those of the standard samples at equivalent concentrations.

Each respective extraction recovery of asperosaponin VI and hederagenin was determined at three different concentration levels of QC samples by comparing peak areas of analytes extracted from plasma samples with peak areas obtained from the pure standard without the procedure of extraction. Five replicates of extracted samples and three replicates of unextracted samples were run at each concentration.

The stability of each analyte in blank rat plasma was assessed by analyzing three concentration levels of QC samples at different conditions, including kept at room temperature for 7 h (short-term stability), stored at −20 °C for 3 months (long-term stability) and after three freeze–thaw cycles. The autosampler stability was conducted by reanalyzing the extracted QC samples kept under autosampler conditions (8 °C) for 11 h.

2.8. Application to pharmacokinetic study

Twelve Sprague-Dawley rats (200–220 g) were obtained from the Experimental Animal Center of Zhejiang (Hangzhou, China) and were kept in an environmentally controlled room (temperature: 25 ± 2 °C, humidity: 50 ± 20%, 12 h dark–light cycle) for at least 1 week before the experiment. All animal experiments were carried out according to the Guidelines for the Care and Use of Laboratory Animals of Southeast University (Nanjing, China).

The rats were fasted overnight before drug administration. Blood samples (0.1 mL) were collected from the ocular vein into heparinized tubes before and 10 min, 20 min, 30 min, 1 h, 2 h, 3 h, 5 h, 7 h, 9 h, 11 h, 14 h, 18 h, 24 h and 30 h after oral administration (90 mg/kg), and then immediately centrifuged at 4000 rpm for 10 min. The obtained plasma was stored frozen at −80 °C until analysis.

2.9. Statistical analysis

Pharmacokinetic parameters were determined by using the plasma concentration–time data and calculated by Drug And Statistic (DAS) 2.0 pharmacokinetic software (Chinese Pharmacological Association, Anhui, China). The maximum plasma concentration (C_{max}) and the time to attain it (T_{max}) were noted directly. The elimination rate constant (k_{el}) was calculated by linear regression

of the terminal points of the semi-log plot of plasma concentration against time. Elimination half-life ($t_{1/2}$) was calculated using the formula: $t_{1/2} = 0.693/k_{el}$. The area under the plasma concentration–time curve to the last measurable plasma concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule. The area under the concentration–time curve to time infinity ($AUC_{0-\infty}$) was calculated as: $AUC_{0-\infty} = AUC_{0-t} + C_t/k_{el}$, where C_t was the last measurable plasma concentration and k_{el} was the elimination rate constant.

3. Results and discussion

3.1. Conditions for ESI-MS/MS

In this experiment, MS/MS operation parameters were carefully optimized for determination of asperosaponin VI and hederagenin. It was found that asperosaponin VI could be ionized under both positive and negative ESI modes, whereas hederagenin could be ionized only in the negative mode. But when we used the negative ion as the detection mode, the mass response of asperosaponin VI was not sensitive enough to perform the pharmacokinetic studies. So, the positive ion mode was selected for the detection of asperosaponin VI, whereas the negative ion mode was selected for hederagenin.

The base peak (the highest peak) in the positive ESI mass spectrum of asperosaponin VI was its sodiated molecular ion $[M+Na]^+$ m/z 951.5. So, it was selected as the precursor ion for the MRM of asperosaponin VI. The product ion, which was produced by the predominant fragmentation occurred at the glucose portion of asperosaponin VI (Fig. 2A), chosen for the MRM was the ion m/z 347.1. Sodium acetate was added to the aqueous portion of the mobile phase to make sure that abundant amount of the sodiated molecular ion of asperosaponin VI could be produced [12–14], and more stable and intensive mass response of the product ion m/z 347.1 could be achieved. In order to select the optimal concentration of sodium acetate in the aqueous portion, the QC samples of three different concentrations of asperosaponin VI at 5, 80 and 1700 ng/mL were injected for 5 times into LC–MS/MS to assess under each of the four different concentration levels of sodium acetate (0, 10, 30 and 50 μM) in the aqueous portion of the mobile phase. The addition of 30 μM and 50 μM of sodium acetate in the aqueous portion of the mobile phase could give the most intense signal with acceptable RSD values (RSD % < 6%, Fig. 4). Use of less amount of nonvolatile salt in the mobile phase was generally advised. So, finally, a concentration of 30 μM sodium acetate was added into the aqueous portion of the mobile phase.

The base peak in the negative ESI mass spectrum of hederagenin was its deprotonated molecular ion $[M-H]^-$ m/z 471.3. So, it was selected as the precursor ion for the MRM of hederagenin. However, even when the collision energy was used as high as 90 eV, the product ion m/z 393.1 was very weak; but when the collision energy was higher than 95 eV, the precursor ion was broken into small fragment ions, but the ion m/z 393.1 was not increased. At first, the MRM mode was used, and the product ion m/z 393.1 was adopted for the MRM. However, under this condition, even an LLOQ of 30 ng/mL hederagenin in the plasma sample could not be reached. The MS2 SIM mode was also tried, but under this condition, lots of endogenous interferences from the rat plasma were encountered at the retention time of hederagenin, only an LLOQ of 10 ng/mL hederagenin in the plasma sample was reached, and this was not sensitive enough to perform the pharmacokinetic study of hederagenin in rats. Finally, the MRM mode using lower collision energy, and the deprotonated molecular ion $[M-H]^-$ m/z 471.3 (Fig. 2B) was employed as both the precursor ion and the product ion. Under this condition, less endogenous interferences

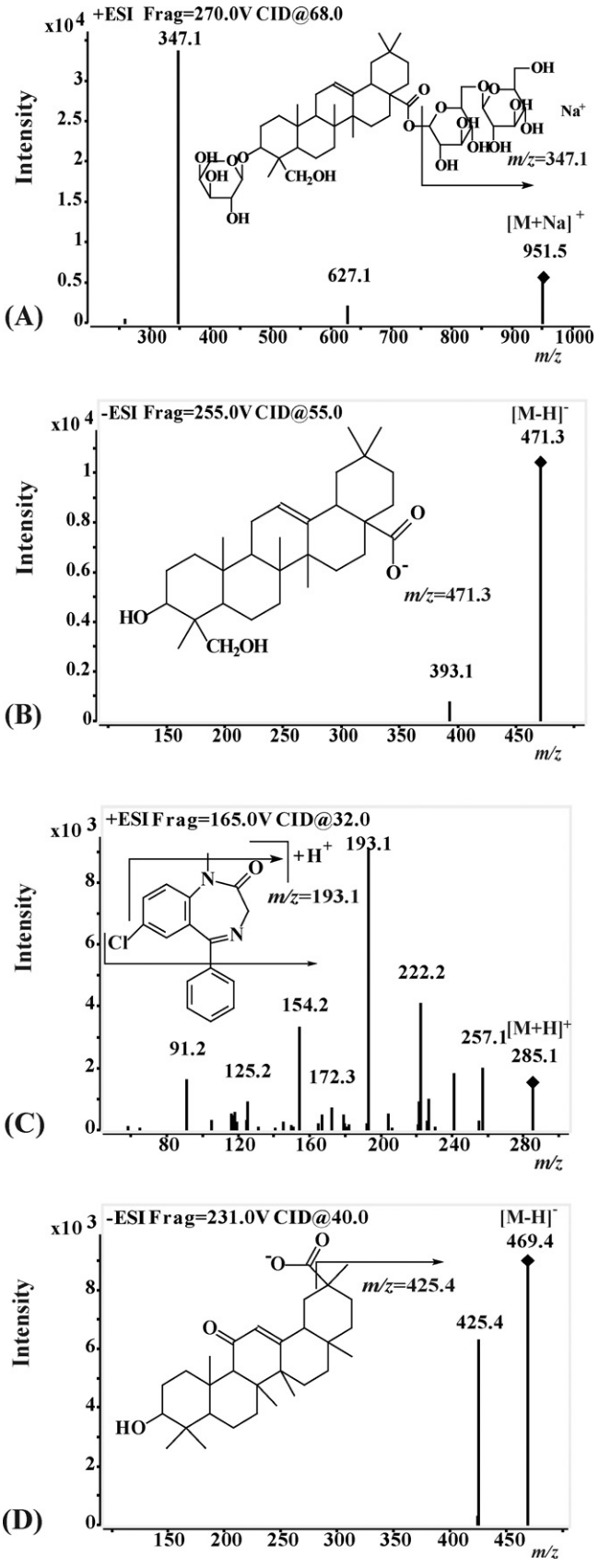

Fig. 2. Mass spectra of asperosaponin VI (A), hederagenin (B), diazepam (C) and glycyrrhetic acid (D).

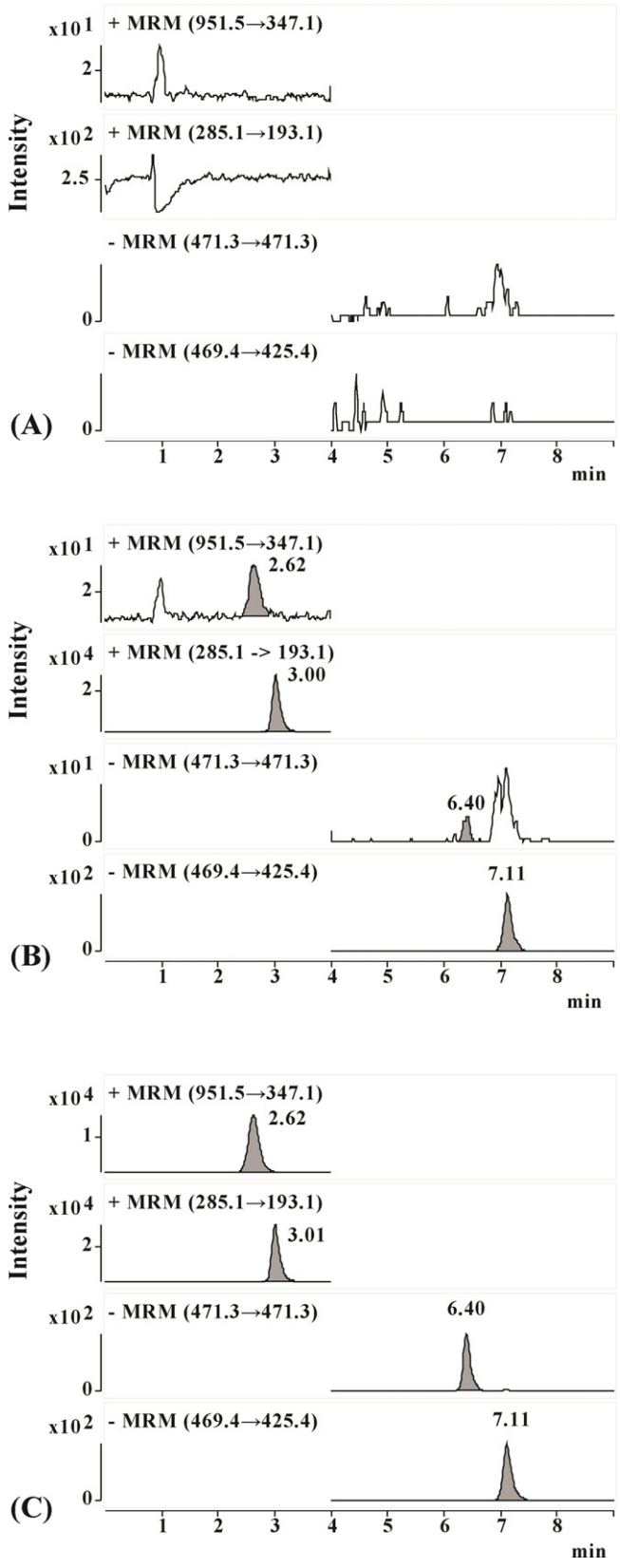

Fig. 3. MRM chromatograms of (A) blank, (B) plasma spiked with asperosaponin VI and hederagenin at 2 ng/mL (LLOQ) and the ISs, and (C) a plasma sample of rat collected after 10 h of single dose of oral administration.

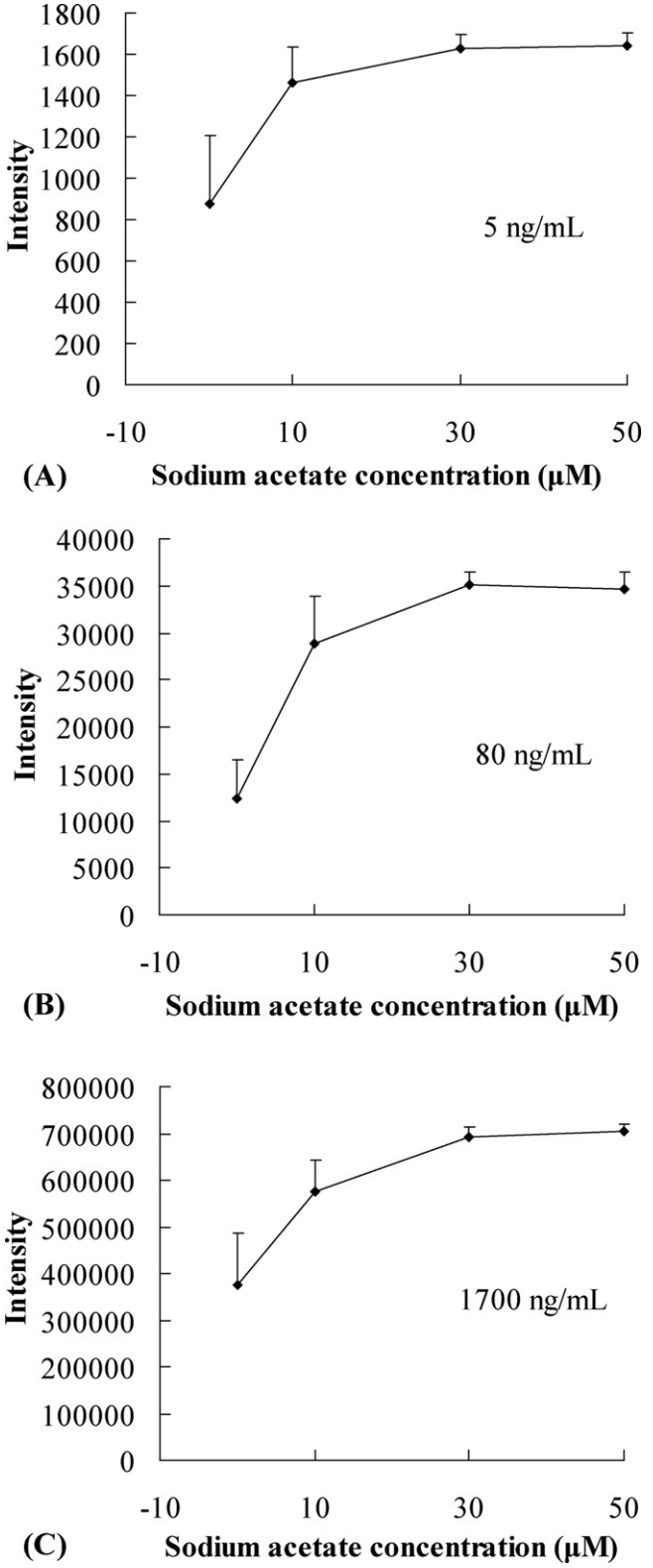

Fig. 4. Influence of the concentration of sodium acetate on the signal intensity and stability of asperosaponin VI.

from the rat plasma were observed. In order to optimize the best collision energy for the MRM, the QC samples of hederagenin at three different concentration levels (5, 40 and 250 ng/mL) in rat plasma were injected ($n = 5$) to assess at different collision energy (10, 30 and 55 eV). At the collision energy of 55 eV, no endogenous

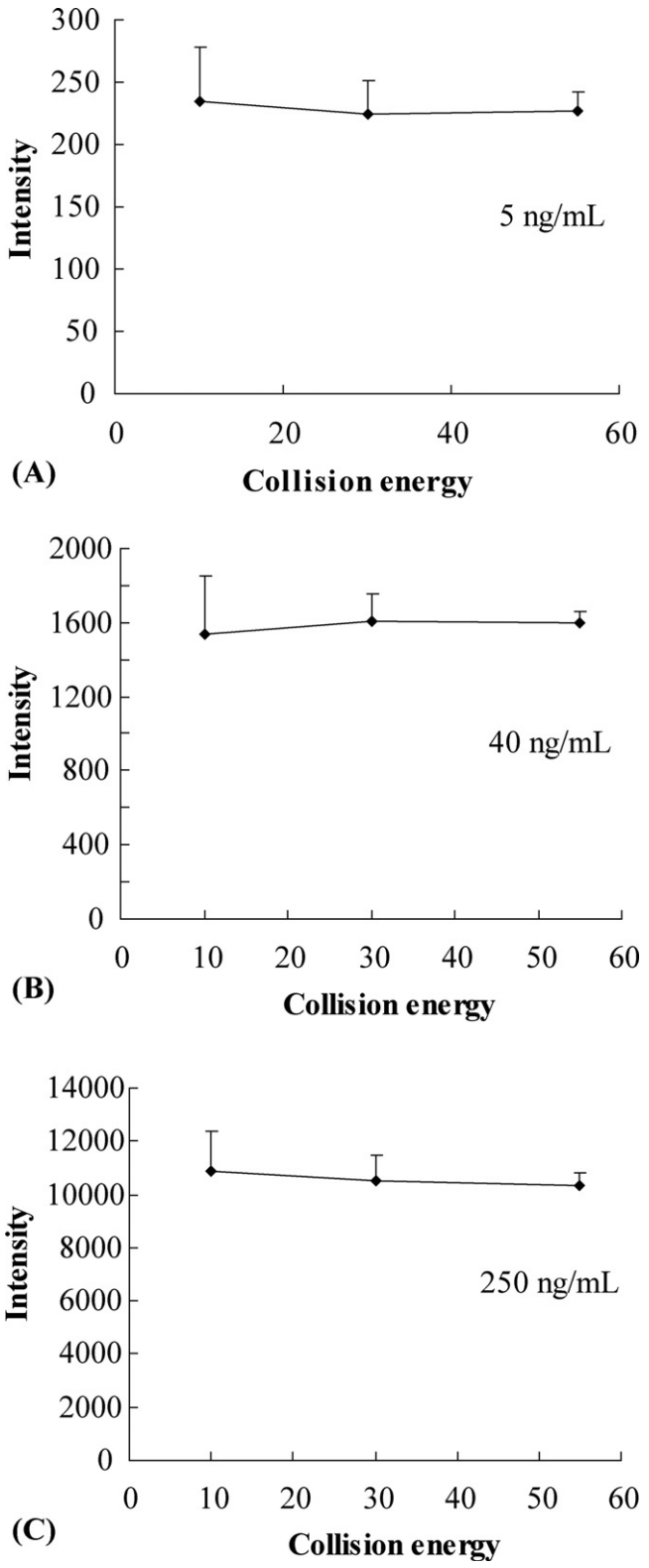

Fig. 5. Influence of collision energy on the stability of hederagenin.

interferences from the rat plasma were observed, and the most stable and intensive mass response of the hederagenin was achieved with the acceptable RSD values less than 7% (see Fig. 5). Finally, MRM mode at the collision energy of 55 eV was adopted for the assay of hederagenin, and under this condition an LLOQ of 2 ng/mL hederagenin in rat plasma was achieved.

3.2. Conditions of LC

The selection of mobile phase components was a critical factor for achieving good chromatographic peak shapes and resolution. Methanol produced a higher mass spectrometric response than acetonitrile and thus was chosen as an organic phase. The addition of 0.05% glacial acetic acid in the aqueous portion of the mobile phase produced more symmetrical peaks for hederagenin and the IS-2. The peak shape was also improved by using 10 mM ammonium acetate to adjust the mobile phase pH. In order to obtain good retention time, peak shape and separation, several trials to screen the ratio of methanol to water were carried out. Finally, a gradient elution system (methanol–0.05% glacial acetic acid containing 10 mM ammonium acetate and 30 μM sodium acetate) as previously described was chosen. Under this chromatographic condition, the effective separation was achieved for asperosaponin VI, hederagenin, the IS-1 and IS-2 from the endogenous interferences.

3.3. Sample preparation

One of the important aspects concerning the sample preparation for pharmacokinetic study is the sample volume. It is recommended to reduce the plasma sample volume collected from the animals as much as possible. The method described here presents an advantage from this point of view, since the plasma volume needed to perform the analysis is 30 μL.

The most widely employed biological sample preparation techniques are liquid–liquid extraction (LLE), protein precipitation (PPT), and solid-phase extraction (SPE). SPE is not suitable for the small volume of 30 μL plasma sample because the recovery would be very low. The high polar character of asperosaponin VI makes it difficulty to extract it from plasma samples by LLE method. Thus, the PPT method was applied to prepare the plasma sample. Acetonitrile and methanol were tested to use as the precipitation solvents. The test results showed that acetonitrile gave much higher recoveries of the analytes than methanol. Thus, acetonitrile was finally chosen as the protein precipitating reagent in the experiment.

3.4. Assay validation

The specificity of this method was confirmed. Fig. 3 shows that chromatographic profiles of blank plasma, blank plasma spiked with asperosaponin VI, hederagenin, IS-1 and IS-2, and plasma sample obtained at 10 h after oral administration of asperosaponin VI. Under the described chromatographic conditions, asperosaponin VI, hederagenin, IS-1 and IS-2 were well separated, the retention times were 2.67, 3.06 min and 6.46, 7.16 min, respectively, and no interfering endogenous peaks around their retention times were observed.

The calibration curves of the analytes showed good linearity over the studied concentration range (2–2000 ng/mL for asperosaponin VI and 2–300 ng/mL for hederagenin), with correlation coefficients (r^2) >0.996. The typical standard curves were $f = 0.001130C - 0.0007674$ for asperosaponin VI and $f = 0.003465C - 0.0002304$ for hederagenin, where f represents the peak area ratio of asperosaponin VI to the IS-1 and hederagenin to the IS-2 and C represents the plasma concentration of asperosaponin VI and hederagenin. The lowest concentrations in the calibration curves for both asperosaponin VI and hederagenin with signal to noise ratio greater than 10 was taken as LLOQ and was determined to be 2 ng/mL which was sufficient to perform pharmacokinetic studies of asperosaponin VI and hederagenin in rats. Table 2 summarizes the intra- and inter-batch precision and accuracy for asperosaponin VI and hederagenin evaluated by assaying the QC samples. The precision was calculated by using one-way ANOVA. In this assay, the intra-batch precision was 7.0%

Table 2
Precision and accuracy data for the analysis of asperosaponin VI and hederagenin in rat plasma (five replicates per run).

Analyte	Concentration levels (ng/mL)		RSD %		RE %
	Added	Found	Intra-batch	Inter-batch	Accuracy
Asperosaponin VI	5.200	5.216	5.7	12.3	0.3
	83.20	85.82	4.1	3.9	3.1
	1768	1820	4.7	5.6	2.9
Hederagenin	5.250	5.215	6.4	6.6	−0.7
	42.00	41.17	7.4	8.8	−2.0
	262.5	266.9	4.4	11.2	1.7

RSD, relative standard deviation; RE %, [(found − added)/added] × 100.

or less, and the inter-batch precision was 12.3% or less for each QC level of asperosaponin VI and hederagenin. The results above demonstrated the acceptable accuracy and precision of the present method.

The matrix effect of five different batches of rat plasma were 101.0 ± 6.7%, 101.8 ± 1.8% and 101.2 ± 4.1% for asperosaponin VI and 96.0 ± 8.6%, 107.0 ± 5.8% and 104.2 ± 6.5% for hederagenin, at low, medium and high QC levels, respectively. The matrix effect of the IS-1 was 99.7 ± 2.4% and the IS-2 was 97.8 ± 3.0%. No significant matrix effect for asperosaponin VI, hederagenin and ISs were observed indicating that no co-eluting substance influenced the ionization of the analytes. The recoveries in rat plasma were 89.8 ± 5.2%, 83.4 ± 4.1% and 91.2 ± 3.4% for asperosaponin VI and 91.4 ± 5.4%, 92.4 ± 6.0% and 87.4 ± 3.7% for a hederagenin at low, medium and high QC levels, respectively. The extraction recovery of the IS-1 was 96.8 ± 4.4%, and that for the IS-2 was 88.9 ± 4.9%.

The stability results of asperosaponin VI and hederagenin in rat plasma are summarized in Table 3. Asperosaponin VI and hederagenin in rat plasma were found to be stable after being placed at room temperature for 7 h, stored at −20 °C for 3 months and through three freeze–thaw cycles. Furthermore, samples after treatment were stable at 8 °C in autosampler for a period of 11 h, which indicated that a large number of samples could be determined in each analytical run.

Table 4
Pharmacokinetic parameters of asperosaponin VI and hederagenin in rat.

Parameter	Asperosaponin VI	Hederagenin
AUC_{0-30} (ng/mL h)	131.6 ± 97.9	273.9 ± 170.6
$AUC_{0-\infty}$ (ng/mL h)	144.7 ± 97.5	324.7 ± 149.9
$C_{max}1$ (ng/mL)	29.9 ± 25.9	25.5 ± 11.8
$C_{max}2$ (ng/mL)	28.0 ± 27.6	
$T_{max}1$ (h)	0.28 ± 0.16	13.0 ± 3.6
$T_{max}2$ (h)	6.8 ± 2.0	
$t_{1/2}$ (h)	3.4 ± 2.2	5.6 ± 3.4

AUC, area under the drug concentration–time cure; C_{max}, maximum concentration; T_{max}, time to reach peak concentration; $t_{1/2}$, elimination half life.

3.5. Pharmacokinetic study

The pharmacokinetics of asperosaponin VI and its metabolite hederagenin after oral administration in Sprague-Dawley rats was studied. The mean plasma concentration–time profiles of asperosaponin VI and hederagenin are shown in Fig. 6, and pharmacokinetic parameters are shown in Table 4. The bimodal phenomenon was observed in the plasma concentration–time curve of asperosaponin VI. The first peak at 0.28 ± 0.08 h (C_{max}: 29.9 ± 25.9 ng/mL) and the second peak at 6.8 ± 2.0 h (C_{max}: 28.0 ± 27.6 ng/mL) were observed. Previous

Table 3
Stability of asperosaponin VI and hederagenin in rat plasma at three QC levels (n = 3).

Storage conditions	Analyte	Concentration levels (ng/mL)		RSD %	RE %
		Added	Found		
Short-term stability (7 h, room temperature)	Asperosaponin VI	5.200	5.487	5.5	5.5
		83.20	87.18	4.8	4.8
		1768	1797	1.6	1.6
	Hederagenin	5.250	5.091	−3.0	−3.0
		42.00	39.70	−5.5	−5.5
		262.5	250.9	−4.4	−4.4
3 Months long-term stability	Asperosaponin VI	5.200	5.656	8.8	8.8
		83.20	77.92	−6.3	−6.3
		1768	1755	−0.7	−0.7
	Hederagenin	5.250	5.240	−0.2	−0.2
		42.00	42.69	1.7	1.7
		262.5	270.0	2.9	2.9
Freeze/thaw stability (3 cycles)	Asperosaponin VI	5.200	5.139	−1.2	−1.2
		83.20	84.58	1.7	1.7
		1768	1789	1.2	1.2
	Hederagenin	5.250	5.160	−1.7	−1.7
		42.00	42.21	0.5	0.5
		262.5	287.9	9.7	9.7
Pre-preparative stability at 8 °C for 11 h (Autosampler)	Asperosaponin VI	5.200	5.114	−1.7	−1.7
		83.20	88.28	6.1	6.1
		1768	1694	−4.2	−4.2
	Hederagenin	5.250	5.441	3.6	3.6
		42.00	40.84	−2.8	−2.8
		262.5	250.0	−4.8	−4.8

RSD: relative standard deviation; RE %: [(found–added)/added] × 100.

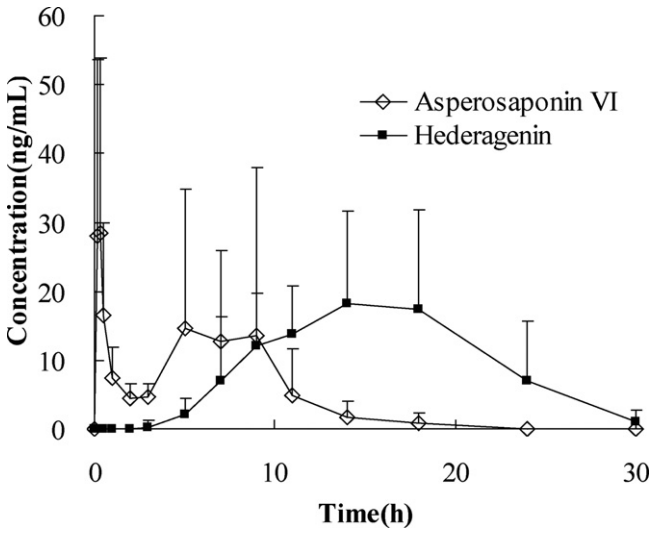

Fig. 6. Plasma concentration–time profiles of asperosaponin VI and hederagenin after oral administration of asperosaponin VI (90 mg/kg).

pharmacokinetic studies had also revealed the bimodal phenomenon in several saponin-containing analytes [15,16]. Some factors that may affect the absorption process include presystemic metabolism/efflux, "absorption window" along the gastrointestinal tract, enterohepatic recirculation, variable gastric emptying and drug–drug interactions [17]. Further detailed absorption studies are needed to elucidate the mechanism of the bimodal phenomenon in pharmacokinetics.

After oral administration of asperosaponin VI, the metabolite hederagenin was detected in all rat plasma samples. Hederagenin was first occurred at 3 h after oral administration of asperosaponin VI in some rats, but the largest concentration was occurred at 9–18 h. This phenomenon was also observed in previous saponin studies [18]. The reason could be because hederagenin was first metabolized by intestinal microflora and then absorbed by gastrointestinal tract.

4. Conclusion

For the first time, a sensitive and selective method for the simultaneous determination of asperosaponin VI and its active metabolite hederagenin in rat plasma was developed by using LC–MS/MS technique operated in positive and negative ionization switching mode. This developed method was used to perform pharmacokinetic study in rat plasma after oral administration of 90 mg/kg asperosaponin VI. Sodium acetate (30 μM) was added to the aqueous portion of the mobile phase to make sure that much more amount of the sodiated molecular ion of asperosaponin VI could be produced, and more stable and intense mass response of the product ion could be achieved. For the detection of hederagenin, the MRM mode, with the deprotonated molecular ion as both the precursor ion and the product ion, was employed, in which collision energy of 55 eV was used to eliminate the interferences from the rat plasma. It made the method specific and sensitive enough to detect lower concentrations of hederagenin. This validated method is an excellent analytical option for simultaneous determination of asperosaponin VI and its active metabolite hederagenin in rat plasma.

Acknowledgements

This study was supported by the National Science & Technology Major Project "Key New Drug Creation and Manufacturing Program", China (Project No. 2009ZX09102-118), and Key Project of the National Natural Science Foundation of China (Project No.30730113).

References

[1] K.Y. Jung, K.H. Son, J.C. Do, Arch. Pharm. Res. 16 (1993) 32–35.
[2] T.S. Anekonda, P.H. Reddy, Brain Res. Rev. 50 (2005) 361–376.
[3] Z.J. Zhang, Y.H. Qian, H.T. Hu, J. Yang, G.D. Yang, Life Sci. 73 (2003) 2443–2454.
[4] Y.Q. Zhou, Z.L. Yang, L. Xu, P. Li, Y.Z. Hu, Cell Biol. Int. 33 (2009) 1102–1110.
[5] S.I. Jeong, B. Zhou, J.B. Bae, N.S. Kim, S.G. Kim, J. Kwon, D.K. Kim, T.Y. Shin, J.P. Lim, H. Kim, H.K. Kim, C.H. Oh, Arch. Pharm. Res. 31 (2008) 1399–1404.
[6] L.H. Peng, C.H. Ko, S.W. Siu, C.M. Koon, G.L. Yue, W.H. Cheng, T.W. Lau, Q.B. Han, K.M. Ng, K.P. Fung, C.B.S. Lau, P.C. Leung, J. Ethnopharmacol. 131 (2010) 282–289.
[7] K. Li, X.L. Yang, C.F. Zhang, Z.L. Yang, Chin. J. Nat. Med. 7 (2009) 440–443.
[8] D. Zhou, H. Jin, H.B. Lin, X.M. Yang, Y.F. Cheng, F.J. Deng, J.P. Xu, Pharmacol. Biochem. Behav. 94 (2010) 488–495.
[9] J. Choi, H.J. Jung, K.T. Lee, H.J. Park, J. Med. Food 8 (2005) 78–85.
[10] K. Li, L. Ding, Z.L. Yang, E.H. Liu, L.W. Qi, P. Li, Y.Z. Hu, Biomed. Chromatogr. 24 (2010) 550–555.
[11] S. Shakya, H. Zhu, L. Ding, X.L. Du, X.M. Qi, X.L. Yang, Z.L. Yang, Biomed. Chromatogr. (2011), doi:10.1002/bmc.1634.
[12] B. Li, Z. Abliz, G. Fu, M. Tang, S. Yu, Rapid Commun. Mass Spectrom. 19 (2005) 381–390.
[13] K. Fouberta, F. Cuyckensb, K. Vleeschouwera, M. Theunisa, A. Vlietincka, L. Pietersa, S. Apersa, Talanta 81 (2010) 1258–1263.
[14] X. Huang, L. Ding, J.P. Sheng, L.H. Jian, Z.X. Zhang, Y.D. Zhang, J. Chin. Pharm. Univ. 34 (2003) 569–572.
[15] F. Cai, L. Sun, S. Gao, Y. Yang, Q. Yang, W. Chen, J. Pharm. Biomed. Anal. 48 (2008) 1411–1416.
[16] C.Y. Chen, L.W. Qi, L. Yi, P. Li, X.D. Wen, J. Chromatogr. B 877 (2009) 159–165.
[17] X. Zhang, D. Zhang, J. Xu, J. Gu, Y. Zhao, J. Chromatogr. B 858 (2007) 65–70.
[18] T. Fang, Y. Wang, Y. Ma, W. Su, Y. Bai, P. Zhao, J. Pharm. Biomed. Anal. 40 (2006) 454–459.