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Pharmacokinetics of gastrodin and its metabolite *p*-hydroxybenzyl alcohol in rat blood, brain and bile by microdialysis coupled to LC–MS/MS

Lei-Chwen Lin[a,b], Yen-Fei Chen[a], Wen-Chuan Lee[a], Yu-Tse Wu[a], Tung-Hu Tsai[a,c,*]

[a] *Institute of Traditional Medicine, School of Medicine, National Yang-Ming University, Taipei, Taiwan*
[b] *National Research Institute of Chinese Medicine, Taipei, Taiwan*
[c] *Department of Education and Research, Taipei City Hospital, Renai Branch, Taipei, Taiwan*

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ABSTRACT

Gastrodin is a pharmacologically active substance isolated from *Gastrodia elata* Blume with sedation, anti-convulsion and anti-epilepsy activities. A rapid and sensitive liquid chromatography technique coupled to tandem mass spectrometry (LC–MS/MS) system was developed to determine gastrodin and its metabolite *p*-hydroxybenzyl alcohol (HBA) in rat blood, brain and bile collected using microdialysis technique. The analytes were separated using a reversed phase column (4.6 mm × 150 mm, 5 μm). The mobile phase for column separation was 30% methanol with a flow rate of 0.6 mL/min. As a post-column addition, 1% ammonium hydroxide solution (in methanol) was additionally pumped via a T-connection using a chromatographic pump (BAS PM-80, USA) at a flow rate of 0.2 mL/min after the column separation. A LC–MS/MS system equipped with a negative electrospray ionization (ESI) source in multiple reaction monitoring (MRM) mode was used to monitor *m/z* 285.0 → 122.9 and *m/z* 123.0 → 105.0 transitions for gastrodin and HBA, respectively. The lower limit of quantification (LLoQ) for gastrodin and HBA were 0.5 and 2 ng/mL, respectively. The calibration curves were linear over the range of 0.5–5000 ng/mL and 2–1000 ng/mL for gastrodin and HBA with a coefficient of determination >0.995, respectively. This selective and sensitive method is useful for the determination of gastrodin and HBA and in the pharmacokinetic studies of these compounds.

1. Introduction

Gastrodia elata Blume (Orchidaceae) has been used for anticonvulsant, analgesic, and sedative effective against vertigo, general paralysis, epilepsy, and tetanus since ancient times in China, Japan, Taiwan and eastern Asia [1–3]. Among the components contained in *G. elata* Blume, gastrodin is considered to be a major bioactive component and its pharmacokinetics has been studied [3,4]. Previous studies have demonstrated that gastrodin biotransforms to HBA, which is an aglycone of gastrodin [5]. Both gastrodin and HBA have sedative, anticonvulsive and analgesic actions [6].

Hsieh et al. indicated that acute administration of gastrodin and HBA can improve cycloheximide- and apomorphine-induced amnesia, but not scopolamine-induced acquisition impairment in rats, thus gastrodin and HBA can facilitate memory consolidation and retrieval, but not acquisition [7]. The improvement might be related to the decrease in dopamine concentration or other monoamine concentration and prevent DNA degradation. Moreover, the facilitating effect of gastrodin on learning and memory might be due to its decomposition to HBA.

A previous report has demonstrated that HBA is able to bind benzodiazepine (BZ) receptor in rat brain membrane, and otherwise, gastrodin would have no direct interaction with BZ receptor. Gastrodin might metabolize into HBA in vivo, and then got through the blood–brain barrier and bound to BZ receptor, which mediated the pharmacological effects on central nervous system [8]. You et al. have mentioned that gastrodin could penetrate through the blood–brain barrier into brain, and it was rapidly decomposed to HBA in brain, liver, and blood [9]. Then HBA preserved in the brain and mediated its pharmacological inhibitive effects on the central nervous system.

In our previous report, a HPLC-UV method to measure biological dialysates with gastrodin in a rat has been demonstrated [10], however, its metabolite HBA was not discussed. The analysis of gastrodin and HBA in rats administrated orally with *G. elata* Blume extract through LC–MS had been studied [11], but the HBA is also contained in the herbal extract. This experiment cannot distinguish the HBA from the herbal extract or metabolism. The distribution

* Corresponding author at: Institute of Traditional Medicine, School of Medicine, National Yang-Ming University, 155 Li-Nong Street Section 2, Taipei 112, Taiwan. Tel.: +886 2 2826 7115; fax: +886 2 2822 5044.
E-mail address: thtsai@ym.edu.tw (T.-H. Tsai).

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of gastrodin in the rat brain had been analyzed through HPLC-UV [12]. In this study, we develop a method utilizing the microdialysis technique coupled with LC–MS/MS to monitor gastrodin and its metabolite HBA in biological samples, simultaneously. This study provides a highly sensitive and rapid method in the application of pharmacokinetics of gastrodin and its metabolite HBA.

2. Materials and methods

2.1. Chemicals and materials

Gastrodin was isolated and purified from the dried roots of *G. elata* Blume in our laboratory and the purity is 99% assessed by HPLC method [10]. HBA (97%) was purchased from Acros Organics (Morris Plains, NJ, USA). Liquid chromatographic grade methanol was purchased from Tedia Company Inc. (Fairfield, OH, USA). Ammonia solution 25% and ammonium acetate were purchased from Merck (Darmstadt, Germany). Deionized water (Millipore, Bedford, MA, USA) was used throughout the entire experiment.

2.2. Liquid chromatography–tandem mass spectrometry

The LC–MS/MS system consisted of a Waters 2690 Alliance LC with automatic liquid chromatographic sampler and injector; and a Micromass Quattro Ultima tandem quadrupole mass spectrometry (Micromass, Manchester, UK) equipped with an electrospray ionization (ESI) interface. Gastrodin and its metabolite HBA were separated from the dialysate using a LiChrospher® 60 RP-select B column maintained at an ambient temperature. The mobile phase for column separation was 30% methanol with a flow rate of 0.6 mL/min. Ammonia hydroxide solution (1%, v/v in methanol) was additionally pumped via a T-connection using a chromatographic pump (BAS PM-80, USA) at a flow rate of 0.2 mL/min after the column separation as a post-column addition. Data was acquired in the negative-ion mode with an ESI interface. Mass spectrometer conditions were optimized by directly infusing gastrodin or HBA (1 μg/mL, dissolved in 50% methanol–2% ammonia solution with ratio of 7:1, v/v) at a flow rate of 20 μL/min into the Z-spray ion source by a Harvard syringe pump. Multiple reaction monitoring (MRM) analysis was used for quantitation and the samples were quantified using peak area. The MRM transitions were *m*/*z* 285.1 to *m*/*z* 123.0 for gastrodin (Fig. 1) and *m*/*z* 123.1 to *m*/*z* 105.1 for HBA (Fig. 2). The ESI-MS/MS parameters were set as follows: capillary voltage, 2.5 kV; source temperature, 80 °C; desolvation temperature, 250 °C; cone gas flow, 100 L/h; desolvation gas flow, 480 L/h. To obtain optimal responses, the cone voltage was set to 35 and 43 V for gastrodin and HBA, respectively, and the collision energy was adjusted to 10 and 11 eV for gastrodin and HBA. All LC–MS/MS data were processed by the MassLynx version 3.5 NT Quattro data acquisition software.

2.3. Animals

Adult, male Sprague-Dawley rats (280–350 g) were obtained from the Laboratory Animal Center at National Yang-Ming University (Taipei, Taiwan). These animals were specifically pathogen-free and were allowed to acclimate in their environmentally controlled quarters (24 ± 1 °C and 12:12 h light–dark cycle). Food (Laboratory Rodent Diet No. 5001, PMI Feeds Inc., Richmond, IN, USA) and water were available ad libitum.

2.4. Implantation of microdialysis probe and sampling

Microdialysis probes were applied to sample the unbound endogenous or exogenous substances in rat blood, brain, and bile [13]. A dialyzing membrane of 10, 3, and 70 mm were used for blood, brain, and bile sampling, respectively. Under urethane and α-chloralose anesthesia, the flexible blood microdialysis probe was implanted into the jugular vein toward the rat's right atrium. For brain sampling, the rat was mounted on a Kopf stereotaxic frame. An incision was made in the scalp, and drilled a small hole for striatum implantation of a rigid brain microdialysis probe. Stereotaxic coordinates from bregma for striatum implantation were anterior–posterior (AP) 0.2 mm, medial–lateral (ML) −3.0 mm and dorsal–ventral (DV) −7.5 mm according to the Paxinos and Watson atlas [14]. The bile duct microdialysis probes were constructed in

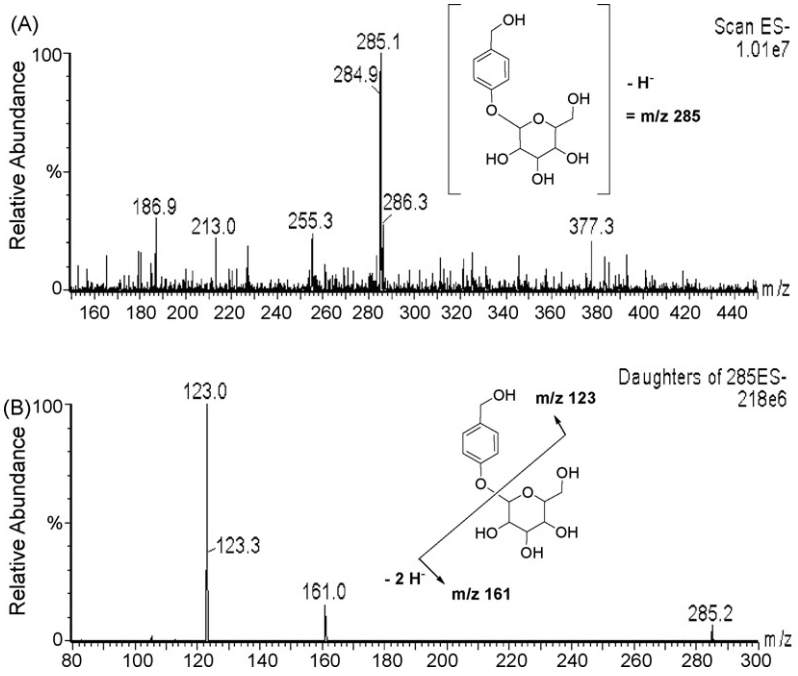

Fig. 1. Full scan of the mass spectrum of gastrodin (A) and MS/MS spectra of *m*/*z* 285, the deprotonated ion of gastrodin (B).

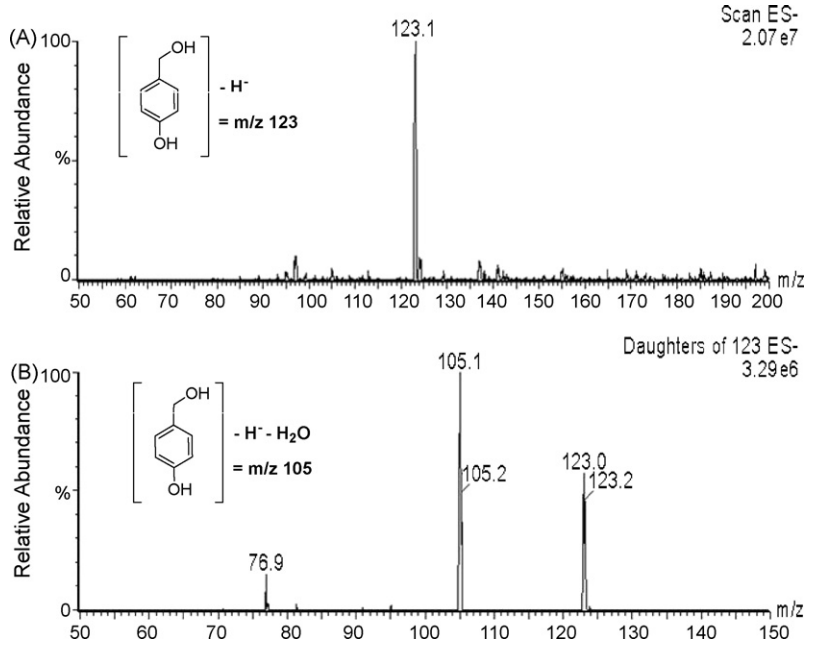

Fig. 2. Full scan of the mass spectrum of HBA (A) and MS/MS spectra of *m*/*z* 123, the deprotonated ion of HBA (B).

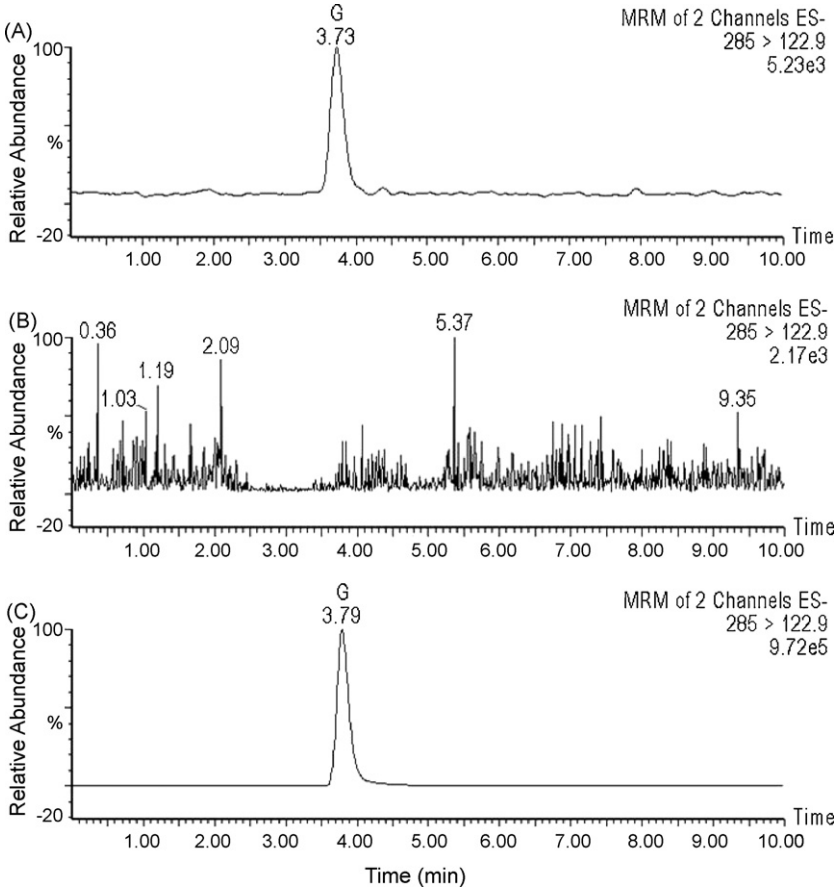

Fig. 3. LC–MS/MS chromatograms of (A) standard gastrodin (1 ng/mL), (B) a blank blood dialysate, and (C) a blood dialysate sample containing gastrodin (191 ng/mL) collected 60 min post-gastrodin administration (50 mg/kg, i.v.). G, gastrodin.

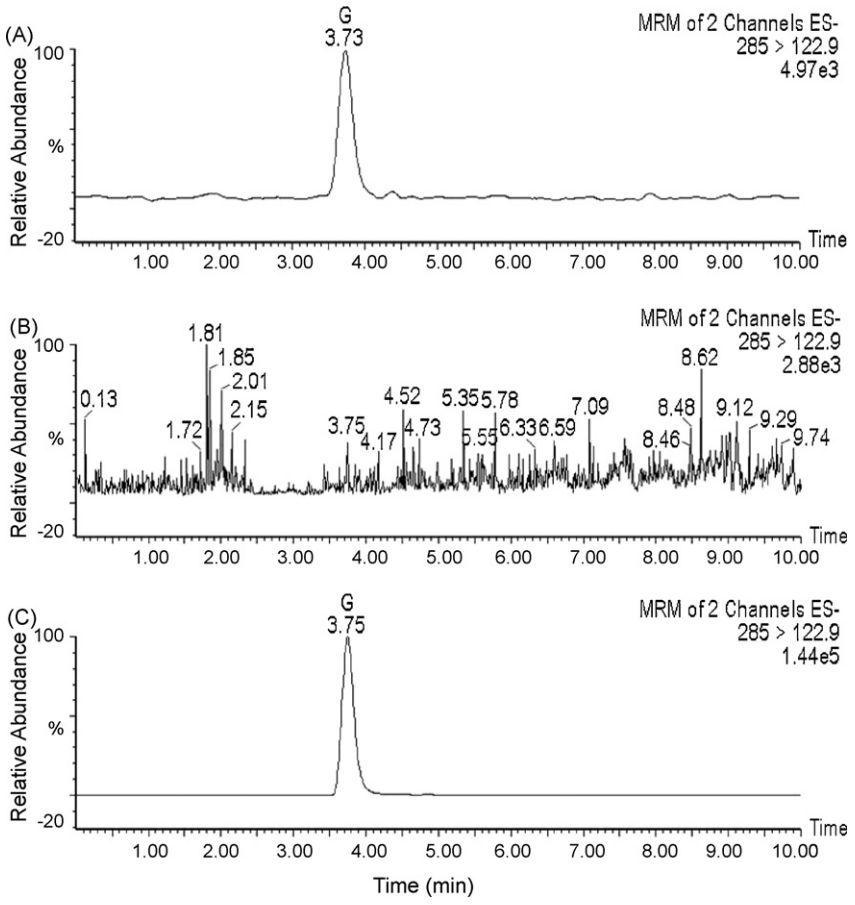

Fig. 4. LC–MS/MS chromatograms of (A) standard gastrodin (1 ng/mL), (B) a blank brain dialysate, and (C) a brain dialysate sample containing gastrodin (29.6 ng/mL) collected 30 min post-gastrodin administration (50 mg/kg, i.v.). G, gastrodin.

house based on a previous design [15,16]. After the implantation of microdialysis probes, the probes were perfused with 2 mM ammonium acetate solution by a microinjection pump (CMA/100) at a flow rate of 2.5 μL/min.

2.5. Method validation

Gastrodin and HBA powders were mixed to dissolve and stock in methanol as a concentration of 50 mg/mL. The stock solutions were then diluted with 2 mM ammonium acetate to give a serial concentration of 0.2, 0.5, 1, 2, 5, 10, 50, 100, 500, 1000, and 5000 ng/mL for gastrodin and 1, 2, 5, 10, 50, 100, 500, 1000 ng/mL for HBA, respectively. An external standard method was used to establish calibration plots of peak area versus concentration for gastrodin and HBA by linear regression analysis of the average of six data point per concentration. All calibration curves were required to have a correlation value of at least 0.995.

For determination of stability of LC–MS/MS method, the accuracy and precision of the analytical method should be evaluated. The inter- and intra-day variabilities were determined by quantitating six replicates at concentrations of 0.5, 1, 2, 5, 10, 50, 100, 500, 1000, and 5000 ng/mL for gastrodin and 2, 5, 10, 50, 100, 500, and 1000 ng/mL for HBA on the same day and 6 successive days, respectively. The accuracy was calculated from the nominal concentration (C_{nom}) and the mean value of observed concentration (C_{obs}) as follows: accuracy (bias, %) = $[(C_{nom} - C_{obs})/C_{nom}] \times 100$. The precision coefficient of variation (CV) was calculated from the standard deviation and observed concentration as follows: precision (% CV) = [standard deviation (S.D.)/C_{obs}] × 100.

Two sets of the samples were prepared to evaluate the matrix effect in the quantitative bioanalytical method.

Set 1. The samples were prepared by mixing 50 μL of the appropriated concentrations of standard solutions of gastodin and HBA with 50 μL mobile phase to the target concentrations of 50, 500, and 5000 ng/mL for gastrodin and 50, 500, and 2500 ng/mL for HBA. After mixing, the solutions were transferred into autosampler vials, and 20 μL was injected into the LC–MS/MS system.

Set 2. The samples were prepared by spiking 50 μL of the appropriated concentrations of standard solutions of gastodin and HBA into 50 μL dialysate collected from microdialysis to the target concentrations of 50, 500, and 5000 ng/mL for gastrodin and 50, 500, and 2500 ng/mL for HBA. After mixing, the solutions were transferred into autosampler vials, and 20 μL was injected into the LC–MS/MS system.

By comparing the peak areas of set 1 and set 2, the ion suppression or enhancement associated was assessed.

In microdialysis experiments, probe calibration is required for accurate estimations of the concentrations in the sampling site. The retrodialysis method was utilized to obtain the in vivo recovery. The blood, brain, and bile microdialysis probes were inserted into the jugular vein, striatum, and bile duct of the rat under anesthesia. Ammonium acetate 2 mM containing gastrodin and HBA (0.5 and 1 μg/mL) was perfused through the probe at a constant flow rate of 2.5 μL/min using an infusing pump (CMA/100). One hour after the probe implantation, the perfusate (C_{perf}) and dialysate (C_{dial}) of gastrdin and HBA were determined by LC–MS/MS. The relative in vivo recovery (R_{dial}) of gastrodin and HBA across the dialysis membrane was defined as $R_{dial} = (C_{perf} - C_{dial})/C_{dial}$. The microdialysate

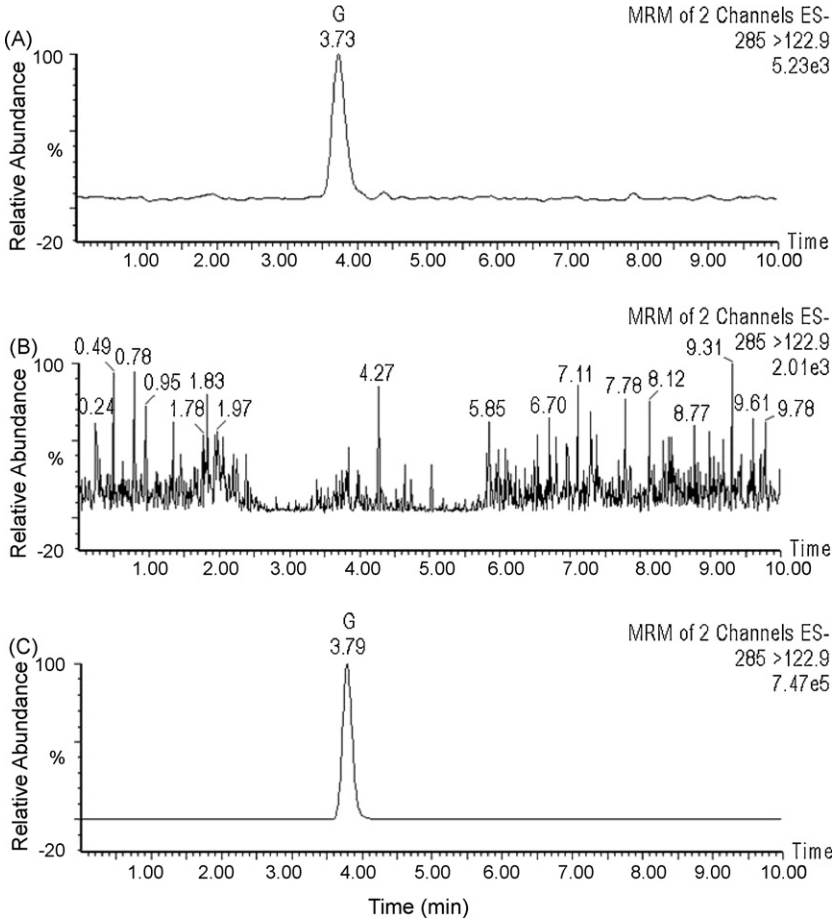

Fig. 5. LC–MS/MS chromatograms of (A) standard gastrodin (1 ng/mL), (B) a blank bile dialysate, and (C) a bile dialysate sample containing gastrodin (184 ng/mL) collected 60 min post-gastrodin administration (50 mg/kg, i.v.). G, gastrodin.

concentrations (C_m) of gastrodin were converted to unbound concentrations (C_u) as follows: $C_m = C_m/R_{dial}$.

2.6. Drug administration

During a 2-h post-implantation period, 25 μL of drug-free dialysates of blood, brain, and bile were collected every 10 min, respectively, by a microfraction collector for specificity study. Gastrodin (50 mg/kg) was then intravenously administered via the femoral vein in a normal saline solution. Next, 25 μL of dialysate was collected every 10 min, and then 10 μL of dialysate was injected into LC–MS/MS for analysis.

3. Results and discussion

3.1. Optimization of LC–MS/MS conditions

To determine the concentration of gastrodin and HBA in rat blood, brain, and bile, we optimized the LC–MS/MS conditions. Analysis was tested with both positive and negative ion mode, but the sensitivity obtained with a negative ion mode was much higher than that of positive ion mode. Different mobile phases containing typical buffer (ammonium hydroxide solution, 25%) and organic solvents (acetonitrile and methanol) were tested for sensitivity. Methanol gave higher MS signals for both gastrodin and HBA than acetonitrile probably due to its more favorable ionization properties [17]. As we know, ESI is attained by a gas-phase reaction and, in the case of small molecules, by ion evaporation. Low surface tension and dielectric constant of the solvent can promote ion evaporation. In addition, the gas-phase basicity or proton affinity and gas-phase acidity are important solvent properties for ionization [18]. Hence, we used methanol as the composition of mobile phase.

The signal intensity variations of gastrodin and HBA using various ratios of ammonium hydroxide solution as elute solution were also evaluated. The ratio of ammonium hydroxide solution was investigated by directly infusing 50% methanol–ammonia solution (7:1, v/v) containing 1 μg/mL of gastrodin and HBA to MS to obtain best MS signal. We investigated the signal intensities of gastrodin and HBA resulted from ammonium hydroxide solution in the range of 0–1.25% of the total. The results show that 0.25% ammonium hydroxide solution of the total gave the best signals. From the results, we found that the signal intensities of gastrodin and HBA remained the same as the concentration of ammonium hydroxide solution gradually increased. On the contrary, too much ammonium solution will inhibit the signal intensities of both compounds and induce unwanted noise ions.

From the previous report, the post-column base addition is proposed to raise effluent pH, helping the ionization process of the weakly acidic compounds with higher pK_a values [16]. ESI detection of weakly acidic phenols has been suggested using a mobile phase with a post-column addition of ammonia. Deprotonation of phenol is better achieved by adding ammonia post-column to the LC mobile phase [19]. The basic character of ammonia favors the formation of negatively charged ions and therefore the intensity of the signal. The pK_a values of gastrodin and HBA have been determined to be about 9.3 and 9.9, respectively, by capillary electrophoresis

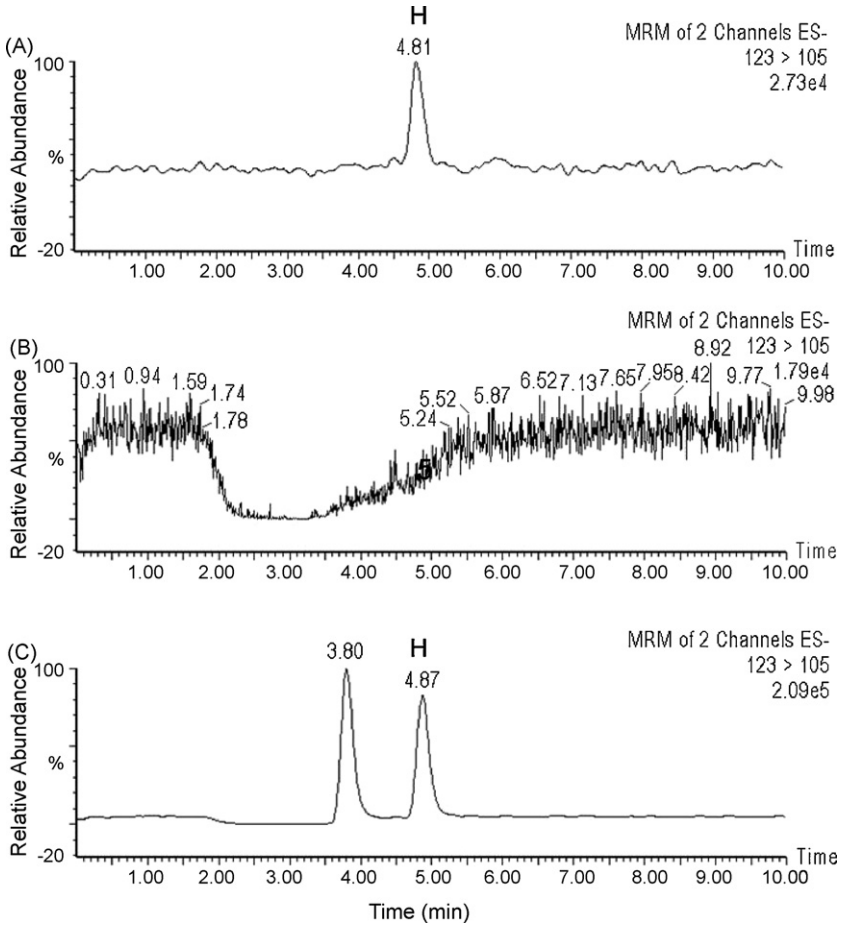

Fig. 6. LC–MS/MS chromatograms of (A) standard HBA (5 ng/mL), (B) a blank blood dialysate, and (C) a blood dialysate sample containing HBA (45.1 ng/mL) collected 20 min post-gastrodin administration (50 mg/kg, i.v.). H, HBA.

Table 1
Intra-day and inter-day precision (% R.S.D.) and accuracy (% bias) of LC–MS/MS method for the determination of gastrodin

Nominal concentration (ng/mL)	Observed concentration (ng/mL)	R.S.D. (%)	Bias (%)
Intra-day			
0.5	0.58 ± 0.04	6.9	16
1	1.04 ± 0.16	15	4
2	2.01 ± 0.22	11	0.5
5	4.86 ± 0.33	6.8	−2.8
10	10.1 ± 0.65	6.4	1
50	50.9 ± 1.48	2.9	1.8
100	100 ± 6.22	6.2	0
500	524 ± 27	5.2	4.8
1000	1030 ± 44	4.2	3
5000	4975 ± 10.7	0.2	−0.5
Inter-day			
0.5	0.56 ± 0.03	5.4	12
1	1.10 ± 0.10	9.1	10
2	2.17 ± 0.04	1.8	8.5
5	4.89 ± 0.26	5.3	−2.2
10	9.62 ± 0.21	2.2	−3.8
50	48.7 ± 2.04	4.2	−2.6
100	102 ± 6.05	5.9	2
500	498 ± 12.5	2.5	−0.4
1000	1013 ± 25.5	2.5	1.3
5000	4981 ± 14.0	0.3	−0.4

Data expressed as mean ± S.D. ($n = 6$).

method [20,21]. For these reasons, ammonium hydroxide solution was added post-column to enhance the signals of both gastrodin and HBA.

3.2. Specificity and selectivity

The specificity of the method was evaluated as lack of endogenous interference by the analysis of drug-free dialysates from blood, brain, and bile of rats. To investigate potential endogenous interference, blank dialysate were analyzed before gastrodin administration. Under the given condition, gastrodin eluted at a retention time of 3.7 min. LC–MS/MS chromatograms of gastrodin in blood, brain, and bile dialysates are shown in Figs. 3–5. No detectable interfering peak was found with the retention time close to that of gastrodin in blood, brain, and bile dialysates post-gastrodin administration (50 mg/kg, i.v.). The metabolite HBA was eluted at retention time of 4.8 min. The result demonstrated that there is little interfere with the determination of the gastrodin and its metabolite, granting good method selectivity. Figs. 6–8 show the LC–MS/MS chromatograms of HBA in blood, brain and bile dialysates after gastrodin administration (50 mg/kg, i.v.). No detectable interference peak was found with retention time close to that of HBA in blood, brain, and bile dialysates.

3.3. Linearity, precision, and accuracy

Standard curves for gastrodin and HBA were shown to be linear using weighted ($1/x$) linear regression in the concentration range

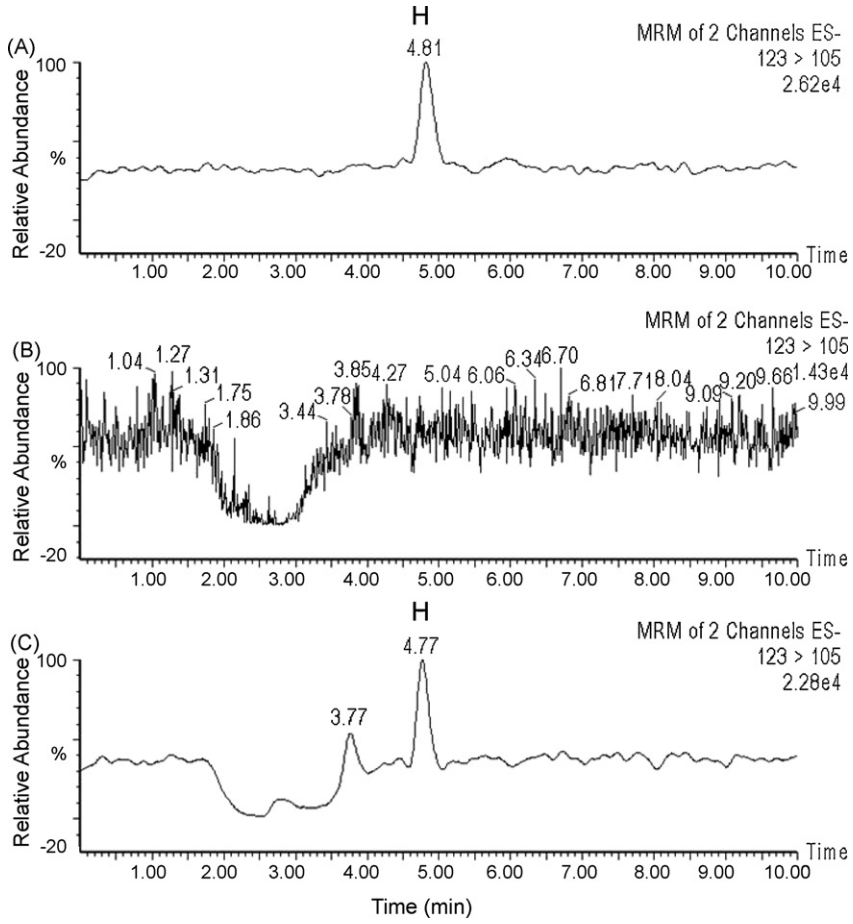

Fig. 7. LC–MS/MS chromatograms of (A) standard HBA (5 ng/mL), (B) a blank brain dialysate, and (C) a brain dialysate sample containing HBA (4.13 ng/mL) collected 20 min post-gastrodin administration (50 mg/kg, i.v.). H, HBA.

of 0.5–5000 and 2–1000 ng/mL, respectively, with a coefficient of determination (r^2) greater than 0.995 for all curves. The limit of detection (LoD) for gastrodin and HBA defined, as signal-to-noise ratio of 3, were 0.2 and 1 ng/mL, respectively. The lower limit of quantitation (LLoQ) for gastrodin was established at 0.5 ng/mL with an intra-day precision (% R.S.D.) and accuracy (% bias) of 6.9% and 16%, respectively, and an inter-day precision and accuracy of 5.4% and 12%, respectively. The lower limit of quantitation (LLoQ) for HBA was established at 2 ng/mL with an intra-day precision (% R.S.D.) and accuracy (% bias) of 12% and −0.5%, respectively, and an inter-day precision and accuracy of 7.3% and −4.5%, respectively. The overall precision ranged from 0.2% to 15% and accuracy ranged from −2.8% to 16% for gastrodin (Table 1). The precision ranged from 1.8% to 12% and accuracy ranged from −4.5% to 5% for HBA (Table 2).

3.4. *In vivo recovery of gastrodin and HBA from microdialysis probe*

Average in vivo recovery levels of gastrodin and HBA were 57.8 ± 9.1% and 48.3 ± 5.2% in blood, 10.1 ± 2.2% and 7.6 ± 2.4% in brain, and 92.3 ± 2.8% and 93.5 ± 4.0% in bile, respectively, at gastrodin and HBA concentrations of 500 and 1000 ng/mL with six individual experiments for each concentration (Table 3).

3.5. *Matrix effect of dialysate*

The peak area ratio of the standard solutions mixed with mobile phase compared to that spiking into dialysates expresses the matrix effect in the sample matrix. When the ratio value equals 1 represented the response in the mobile phase and in the dialysate was the same and no matrix effect was observed. The results listed in Table 4 showed that the mean matrix effect of gastrodin and HBA were 0.93 ± 0.02 to 1.00 ± 0.06 and 1.02 ± 0.08 to 0.95 ± 0.06, respectively. The value is close to 1, the matrix effect is low and in the quantitative analysis the matrix effect could be ignored.

Table 2
Intra-day and inter-day precision (% R.S.D.) and accuracy (% bias) of LC–MS/MS method for the determination of HBA

Nominal concentration (ng/mL)	Observed concentration (ng/mL)	R.S.D. (%)	Bias (%)
Intra-day			
2	1.99 ± 0.23	12	−0.5
5	4.96 ± 0.36	7.3	−0.8
10	9.96 ± 0.68	6.8	−0.4
50	50.2 ± 2.69	5.4	0.4
100	103 ± 5.58	5.4	3
500	494 ± 15.5	3.1	−1.2
1000	1005 ± 18.0	1.8	0.5
Inter-day			
2	1.91 ± 0.14	7.3	−4.5
5	5.12 ± 0.23	4.5	2.4
10	10.2 ± 0.74	7.3	2
50	48.5 ± 1.74	3.6	−3
100	105 ± 9.85	9.4	5
500	507 ± 18.5	3.6	1.4
1000	1003 ± 21.9	2.2	0.3

Data expressed as mean ± S.D. (n = 6).

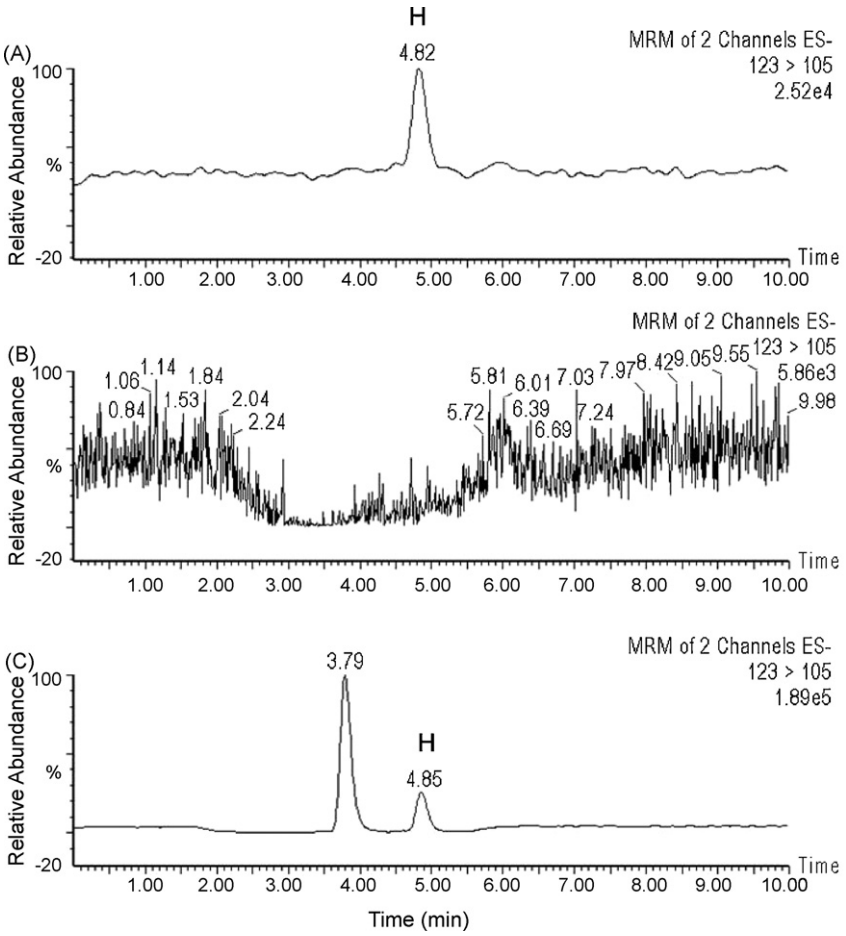

Fig. 8. LC–MS/MS chromatograms of (A) standard HBA (5 ng/mL), (B) a blank bile dialysate, and (C) a bile dialysate sample containing HBA (15.2 ng/mL) collected 20 min post-gastrodin administration (50 mg/kg, i.v.). H, HBA.

3.6. LC–MS/MS analysis of gastrodin and its metabolite HBA in rat biological samples

The concentration versus time curves for gastrodin and its metabolite HBA in rat blood, brain, and bile after gastrodin (50 mg/kg, i.v.) administration are shown in Fig. 9. Gastrodin quickly distributed to the bile and brain and quickly transformed into HBA after IV administration. HBA was found in the bile and brain at the first sampling point (10 min), and declined rapidly after gastrodin IV administration. The concentration of gastrodin in blood reached a peak concentration (C_{max}) of 24.1 μg/mL whereas HBA reached a peak concentration of 109 ng/mL at 15 min. The concentration of gastrodin in brain reached a peak concentration of 0.68 μg/mL whereas HBA reached a peak concentration of 77.7 ng/mL at 15 min. The concentration of gastrodin in bile reached a peak concentration of 2.6 μg/mL at 15 min whereas HBA reached a peak concentration of 34.7 ng/mL at 15 min. The results of the pharmacokinetics parameters were consistent to our previous study through an HPLC-UV-microdialysis sampling technique [10].

Table 3
In vivo microdialysis recovery (%) of gastrodin and HBA in rat blood, brain, and bile using 2 mM ammonium acetate as perfusate

Concentration (ng/mL)	Recovery (%) of gastrodin	Recovery (%) of HBA
Blood		
500	52.8 ± 9.91	51.8 ± 3.7
1000	62.8 ± 4.4	47.1 ± 5.1
Average	57.8 ± 9.1	48.3 ± 5.2
Brain		
500	–	–
1000	10.1 ± 2.2	7.6 ± 4.3
Average	10.1 ± 2.2	7.6 ± 4.3
Bile		
500	90.3 ± 2.2	92.4 ± 5.3
1000	94.4 ± 1.7	94.6 ± 1.6
Average	92.3 ± 2.8	93.5 ± 4.0

Data expressed as means ± S.D. ($n = 3$).

Table 4
Matrix effect (%) data for gastrodin and HBA in three different lots of dialysate

Nominal concentration (ng/mL)	Blood	Brain	Bile
Gastrodin			
50	91.40 ± 13.15	105.00 ± 7.68	94.86 ± 8.93
500	92.23 ± 3.21	93.41 ± 5.65	92.07 ± 1.82
5000	94.93 ± 4.09	101.98 ± 10.51	91.41 ± 0.85
Mean	92.85 ± 1.85	100.13 ± 6.01	92.78 ± 1.83
HBA			
50	111.29 ± 6.83	94.04 ± 8.56	93.77 ± 6.90
500	98.63 ± 2.05	90.28 ± 4.03	97.32 ± 5.60
2500	97.26 ± 3.82	101.83 ± 4.04	99.18 ± 5.69
Mean	102.39 ± 7.74	95.38 ± 5.89	96.76 ± 2.74

Data are expressed as the ratio (%) of the mean peak area of a dialysate spiked with standard solution to the mean peak area of standard solution prepared in mobile solution, and are as expressed means ± S.D. ($n = 3$).

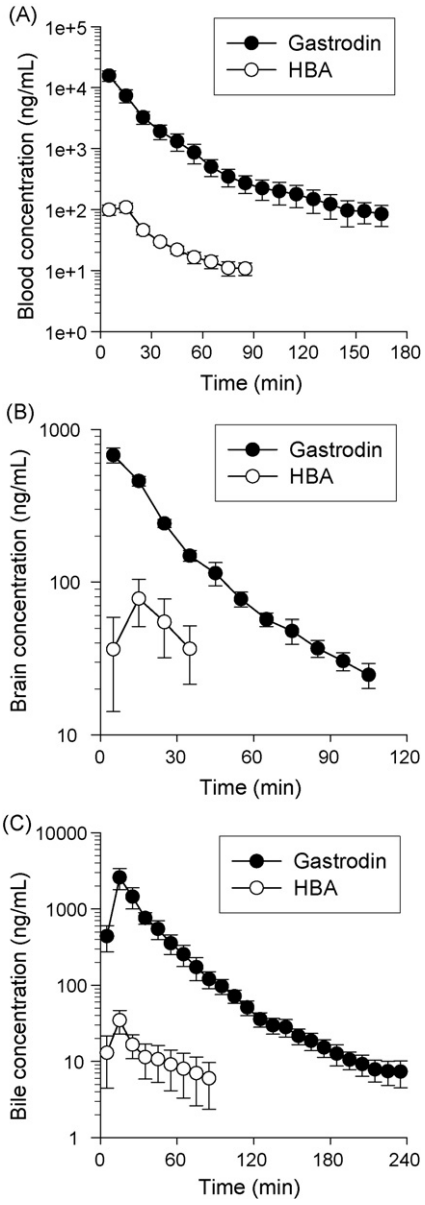

Fig. 9. Mean gastrodin and HBA level in rat blood (A), brain (B), and bile (C) after gastrodin (50 mg/kg, i.v.) administration ($n = 6$).

After gastrodin administration (50 mg/kg, i.v.), an unknown peak was observed in blood, brain, and bile dialysate at a retention time of 3.8 min under the MRM mode of HBA (Figs. 6–8). The unknown substance also decreased with time. It means the unknown substance has the same molecular weight and a product ion of m/z 105. To investigate the fragment ion pattern of the unknown substance, we performed a product ion scanning on it. It displayed an identical fragment pattern with HBA. Therefore, we proposed that the unknown substance might be an isomer of HBA. But more evidence is required to prove it.

4. Conclusions

We use microdialysis to monitor the concentration–time profiles of gastrodin and its metabolite HBA. A liquid chromatographic electrospray tandem mass spectrometric method for quantitative determination of gastrodin and its metabolite HBA in biological dialysates was developed and validated. This experimental method could potentially yield more complete metabolic and pharmacokinetic data.

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