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Short communication

Relative bioavailability of gastrodin and parishin from extract and powder of Gastrodiae rhizoma in rat

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ABSTRACT

A rapid, sensitive and reliable UHPLC-ESI-MS/MS method was developed for simultaneous determination of gastrodin and parishin in rat plasma. The LLOQ of the two analytes were 1.00×10^{-1} and 8.30×10^{-5} μg/mL, respectively. The intra-day and inter-day precision were all less than 10% of the relative standard deviation (RSD), whilst the accuracy were all within ±15% of the relative error (RE). The proposed method was successfully applied for pharmacokinetics study on the two analytes in rats after oral administration of Gastrodiae rhizoma (GR) extract and powder at low, medium and high dosages. Blood samples were collected from the suborbital vein at predetermined time points and were precipitated using methanol. Chromatographic separations were carried out on a Kinetex XB-C18 column (2.1 mm × 150 mm, 1.7 μm) with a gradient mobile phase of acetonitrile-water with 0.1% formic acid as a modifier. The pharmacokinetic parameters of the two analytes in rats were obtained and the relative bioavailability of gastrodin and parishin in two formulations were calculated. The results indicated that higher bioavailability was obtained when low dosage of GR powder was used, whereas, higher bioavailability values were obtained when medium and high dosages of GR extract were used.

1. Introduction

Gastrodiae rhizoma (GR), *Tianma* in Chinese, is the dried rhizomes of *Gastrodia elata* Blume. (Orchidaceae) and is officially listed in Chinese Pharmacopeia (CP) (Edition 2010) [1] as one of the commonly used Chinese medicines for the treatment of febrile convulsion, epileptoid convulsion, tetanus, headache or dizziness and paralysis or numbness of the limbs. Modern pharmacological studies indicated that aqueous extract of GR had anxiolytic-like effects [2] and relatively good favorable effect on prevention and/or treatment of ovariectomy (OVX)-induced osteoporosis [3]. Fifty percent alcohol extract of GR was testified to have a protective effect against neuronal damage in KA-treated rats by reducing nNOS, microglia activation and apoptosis [4] and have anticonvulsive and free radical scavenging activities [5]. Ether fraction of methanol extract of GR was also investigated and the anticonvulsive effect and putative neuroprotective effect against excitotoxicity were reported [6].

Phytochemical studies on GR have revealed the presence of gastrol, phenolic compounds [7], polysaccharides [8], amino acids, nucleosides [9], adenosines [10], vanillyl alcohol [11], gastrodin [10,12,13] and parishins [13,14]. Gastrodin is one of the earliest compounds found in GR and is specified as a marker for quality assessment on the herb in CP (edition 2010). The compound was reported to decrease immune reactivities of gamma-aminobutyric acid shunt enzymes in the hippocampus of seizure sensitive gerbils [15], to play protective action against liver injury induced by CCl_4 in mice [16] and to cure vascular headache with effective rate at 91%. The compound is available in oral formulations and has been reported to be used intravenously and intramuscularly in clinical trials. Parishin, a main highly polar compound from aqueous extract of GR, significantly enhanced the ADCC reaction [17]. The two analytes have been assayed for quality assessment on GR and its related formulas in different analytical approaches [18,19].

GR extract has been manufactured by many Chinese pharmaceutical or biotechnological companies used clinically as therapeutic medicine. However, due to the valuableness of the herb, more and more pharmaceutical companies are inclined to use GR powder. Generally, the herb was smashed into super fine powder, mixed with the extract of other herbs and then manufactured to different formulations with excipients in commercial products on market. In that way, are there any differences on bioactivities

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between GR extract and GR powder? The issue arouses our great interest.

Several studies on pharmacokinetics of gastrodin were reported [20,21], however, no report on pharmacokinetics of parishin has been published so far. In the present study, a reproducible, rapid, sensitive and selective UHPLC-ESI-MS/MS method for simultaneous determination of gastrodin and parishin in rat plasma was developed for the first time, which was then applied to pharmacokinetics of the two analytes after oral administration of GR extract and GR powder. Moreover, on the basis of the pharmacokinetic parameters of the two analytes, bioavailability values of the two formulations were calculated, which will provide useful information for clinical treatments. To the best of our knowledge, this is the first study on bioavailability comparison between two different medication forms of GR, based on the pharmacokinetics of the two analytes.

2. Experimental

2.1. Material and reagents

GR was purchased from Tong-Ren-Tang Pharmacy (Beijing, China) in Guiyang city. Half was ground to fine powder (60 mesh) using a common grinder with a knife blade for extraction. Another half was smashed to superfine powder (less than 10–25 μm in diameter) using micronizer in Guizhou Holy Pharmaceutical Co. Ltd. for intragastric administration directly. They were stored at 4 °C before use.

Reference compounds of gastrodin (No. 110807-2002-5) and bergenin (Internal Standard, IS, No. 1532-200202) were purchased from the National Institute for the Control of Biological and Pharmaceutical Drugs (Beijing, China). Parishin (No. 12031) was obtained from Guizhou Dida Technology Co. Ltd (Guiyang, Guizhou, China). Their structures are shown in Fig. 1.

MS-grade methanol and acetonitrile were purchased from Tedia Co. Inc. (Fairfield, OH, U.S.). Formic acid was from ROE Scientific, Inc. (DE, U.S.). Super purified water from Robust Food & Beverage Co. Ltd. was used for all preparations. All other reagents used in the present study were of analytical grades.

2.2. Experimental animals

Adult, male Sprague-Dawley (SD) rats weighed 180 ± 20 g were purchased from Changsha Tianqin Bio-Technology Co., Ltd. (Changsha, China, Certificate No. SCXK2009-0012). These animals were specifically pathogen-free and acclimated for at least a week in their environmentally controlled quarters (24 ± 1 °C and 12/12 h light/dark cycle) with free access to standard chow and water. The rats were fasted overnight but were supplied with water *ad libitum* before the experiments. All experimental protocols were conducted in accordance with the Guide for the Care and Use of Laboratory Animal (National Institutes of Health Publication 85-23, revised edition 1985) and were permitted by Ethic Committee of the University.

2.3. Instrument and conditions

Chromatographic separations were performed on an Accela 1250 UHPLC system (Thermo Fisher Scientific Inc., Waltham, MA, U.S.) equipped with an Accela 1250 photo diode array (PDA) detector, an Accela HTC PAL autosampler, and an Accela 1250 binary pump. Kinetex XB-C18 column (2.1 mm × 150 mm, 1.7 μm) from Phenomenex Inc. (Torrance, California, U.S.) was used for chromatographic separations. The mobile phases consisted of water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B). The gradient elution program was as follows: 0–4 min, 2% B; 4–5 min, 2 → 25% B; 5–10 min, 25% B. The column temperature was maintained at 25 °C. The flow rate was 200 μL/min and the injection volume was 10 μL.

Mass spectrometric analyses were performed on a TSQ quantum ultra triple-quadrupole mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, U.S.) equipped with an electro-spray ionization (ESI) interface in negative mode. The MS instrument parameters were as follows: sheath gas flow rate, 40 (arbitrary units); auxiliary gas flow rate, 15 (arbitrary units); spray voltage, 2500 V; vaporizer temperature, 500 °C; capillary temperature, 350 °C. Helium was used as the collision gas for collision-induced dissociation (CID). Quantification was performed using multiple reactions monitoring (MRM) mode under unit mass-resolution conditions. Data acquisition and processing was performed using Xcalibur 2.1 data system and LC quan 2.6 quantification software.

2.4. Preparation of GR extract

For preparation of GR extract, 15 g of the dried powder (60 mesh) was accurately weighted into a 250 mL-conical flask and extracted with 180 mL of water with ultrasonic extraction at 60 °C for 30 min. The extract was centrifuged at 5000 rpm for 15 min and then the supernatant was evaporated to dryness for intragastric administration.

2.5. Preparation of stock and working solutions

Stock solutions of gastrodin, parishin were prepared in acetonitrile–water (4:96, v/v) and further diluted into 0.31–49 μg/mL, 0.00026–4.18 μg/mL, respectively, as working solutions. All the solutions were stored at 4 °C before analysis.

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

The calibration standard solutions were prepared by freshly spiking 20 μL of the appropriate mixed working solutions into 100 μL of blank plasma to yield the concentrations of 0.10, 0.39, 1.96, 5.23, 7.84, 10.45, 13.07 and 15.68 μg/mL for gastrodin, 0.000083, 0.1670, 0.2783, 0.4448, 0.6688, 0.8896, 1.1136 and 1.3376 μg/mL for parishin, 0.09408 μg/mL for IS, and processed as described in “sample preparation”.

QC samples used for intra- and inter-day accuracy and precision, extraction recovery and stability studies were prepared at concentrations of 1.96, 7.84 and 13.07 μg/mL for gastrodin, 0.00083, 0.6688 and 1.1136 μg/mL for parishin, and 0.09408 μg/mL for IS.

2.7. Preparation of plasma samples

To 100 μL of the plasma sample in a 1.5 mL-Eppendorf tube (EP tube), 20 μL of IS solution (0.294 μg/mL) and 20 μL of 1% formic acid aqueous solution were individually added. After being mixed for 15 s, 300 μL of methanol was added to precipitate protein. Subsequently, the mixture was vortexed for 60 s and then was centrifuged at 12,000 × *g* for 10 min at 4 °C. The supernatant was transferred into another EP tube and evaporated to dryness under the stream of nitrogen in a water bath at 40 °C. The residue was dissolved in 50 μL of acetonitrile–water (4:96, v/v) and then centrifuged at 12,000 × *g* for 10 min. The supernatant was transferred into an auto-sample vial, and a 10 μL aliquot was injected into UHPLC-MS/MS system for analysis.

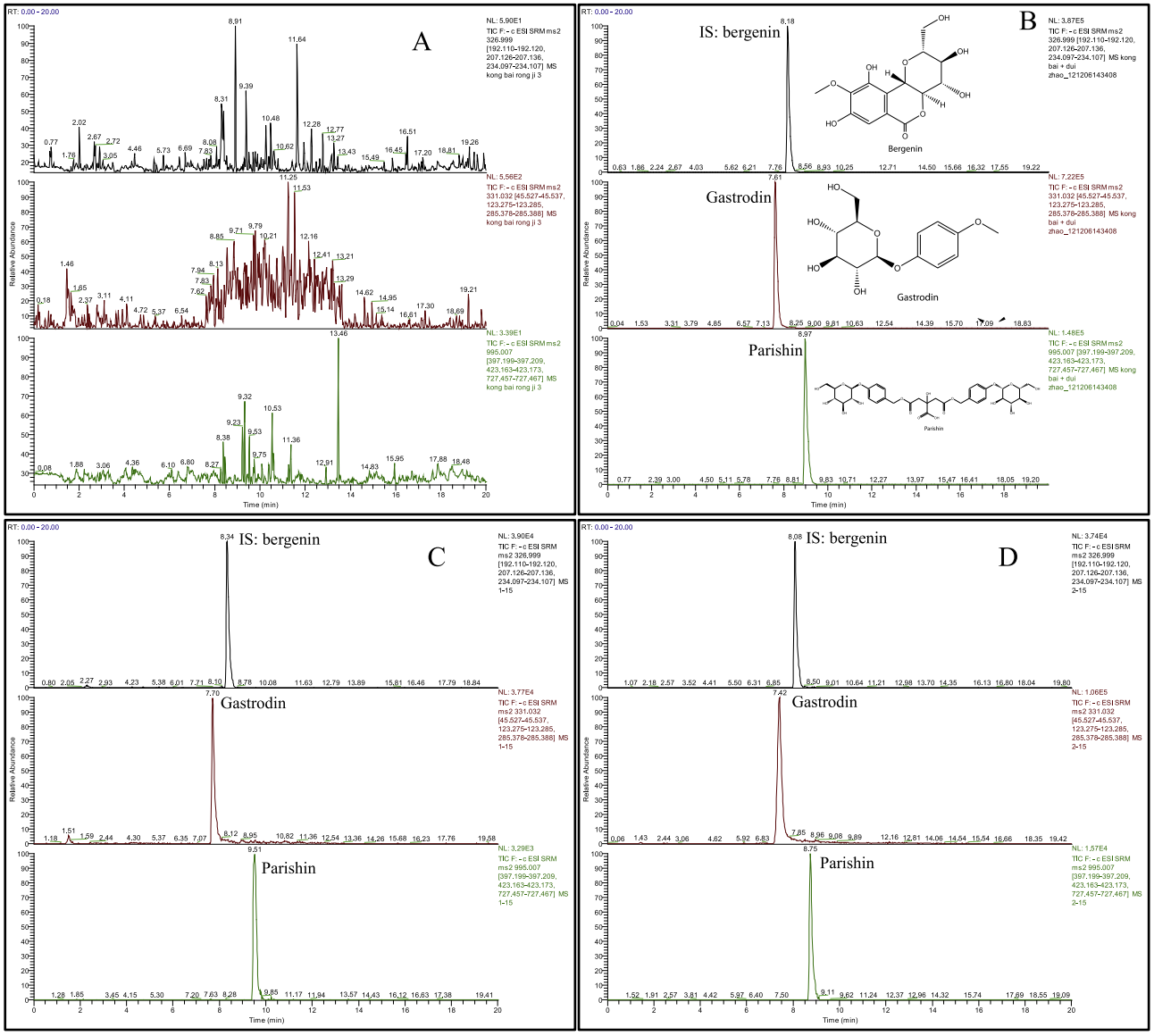

Fig. 1. Representative MRM chromatograms of gastrodin, parishinand IS (bergenin) in rat plasma: (A) blank plasma; (B) blank plasma spiked with gastrodin, parishinand IS at concentration of 7.84, 0.6688, and 0.09408 μg/mL, respectively; (C) plasma at 15 min after oral administration of GR extract; and (D) plasma at 15 min after oral administration of GR powder.

2.8. Method validation

The method validation was conducted according to the accepted FDA Guidance for Industry, Bioanalytical Method Validation (US-FDA, 2001).

2.8.1. Selectivity and matrix effect

The selectivity of the method was evaluated by analyzing blank plasma samples from six rats using the proposed preparation procedure and instrument conditions to determine the potential interferences of the biological matrix at retention times of the analytes and the IS. The matrix effects of gastrodin and parishin at three QC levels and the IS at 0.09408 μg/mL were measured by comparing peak areas of the analytes and the IS dissolved in blank plasma samples after extraction with those of the reference compounds dissolved in acetonitrile–water (4:96, v/v) at the same concentration level.

2.9. Extraction recovery

The extraction recoveries of gastrodin and parishin at three QC levels and the IS at 0.09408 μg/mL were measured by comparing the peak areas of the analytes and the IS added to and extracted from the blank plasma with those of the extracted blank plasma samples added with the standards at the same concentration level. Five determinations were conducted at each concentration level.

2.9.1. Calibration curves and sensitivity

Calibration curves were generated by plotting the peak area ratios of the analytes to IS vs. the nominal concentrations of the calibration standards by linear regression with the weighting factor of $1/x^2$. The lower limit of quantification (LLOQ) was defined as the lowest concentrations of the analytes with precision not exceeding 20% of the relative standard deviation and accuracy within the range of 80–120%. The signal-to-noise ratio of the analyte was at least 5.

2.10. Precision and accuracy

Intra- and inter- batch precision and accuracy were assessed using five batches of QC samples at three concentration levels as described above and calculated using the calibration curves constructed, ensuring that the precision was within 15% of the RSD, and accuracy was within ±15% of the relative error (RE).

2.11. Stability

To determine freeze–thaw stability, QC samples at three concentration levels were stored at −20 °C for 24 h and thawed at room temperature. When completely thawed, the samples were refrozen for 24 h under the same conditions. The freeze–thaw cycles were repeated three times and then the samples were analyzed. To determine short-term stability, QC samples at three concentration levels were kept at room temperature for 12 h. The post-preparative stability was determined using the prepared QC samples kept in the autosampler at 4 °C for 48 h.

2.12. Pharmacokinetic study

Before pharmacokinetic study, the contents of gastrodin and parishin in different medication forms were quantified using the same chromatographic conditions as described above. The contents of gastrodin were 30.22 and 6.35, and the contents of parishin were 33.68 and 5.49 mg/g, respectively, in GR extract and GR superfine powder. GR extract and superfine powder were dissolved in saline to give suspension at concentration of 50 mg/mL. Six male SD rats in different groups were fasted for 12 h prior to administration. The rats in low-, medium- and high-dose groups were given two different medication forms at three levels equivalent to 315, 630 and 1260 mg GR raw material/kg weight of rat, respectively. The animals had free access to water during the experiment. 0.3 mL of the blood was collected from the suborbital vein before administration and 5, 10, 15, 20, 35, 50, 60, 70, 90, 120, 150, 240, 360 and 480 min after administration. The plasma was immediately separated for analysis. The pharmacokinetic parameters maximum plasma concentration (C_{max}) and the time of maximum plasma concentration (T_{max}) were observed directly from the measured data. The elimination rate constant (K_e) was calculated by linear regression of the terminal points in a semi-log plot of the plasma concentration against time. The elimination half-life ($t_{1/2}$) was calculated using the formula $t_{1/2} = 0.693/K_e$. The area under plasma concentration–time curve (AUC_{0-t}) to the last measurable plasma concentration (C_t) was estimated by using the linear trapezoidal rule. The area under the plasma concentration time–curve from time zero to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-\infty} = AUC_{0-t} + C_t/K_e$.

The relative bioactivities (F_{re}) of gastrodin and parishin in plasma were calculated as the ratio of $AUC_{g.e}/AUC_{g.p}$ and $AUC_{p.e}/AUC_{p.p}$, respectively. ("g.e" and "g.p" means administration of gastrodin from GR extract and GR powder; "p.e" and "p.p" means administration of parishin from GR extract and GR powder).

Data are presented as mean ± SD. Comparisons of the pharmacokinetic data were performed by Student's *t*-test and the statistically significant difference was set at a value of $P < 0.05$ (SPSS statistical software package, Version 17.0, SPSS Inc., Chicago, IL, U.S.).

3. Results and discussion

3.1. Optimization of UHPLC–MS/MS conditions

The selection of the UHPLC–MS/MS conditions was guided with the requirement for obtaining chromatograms with satisfactory resolutions of the analytes and the IS with other endogenous compounds in rat plasma. A few columns were screened before Kinetex XB-C18 column (2.1 mm × 150 mm, 1.7 μm) was finally selected as the column of choice. Different mobile phase were tested. As a result, satisfactory resolution values, sharp and symmetrical peaks were obtained by using acetonitrile–water (containing 0.5% formic acid, v/v) system. It was worth noting that the high chromatographic resolution of UHPLC system enabled to increase both analysis speed and peak capacity. Meanwhile, the mobile phase composition, flow rate and column temperature had been adjusted to obtain satisfactory resolutions with no cross-talk observed. The best chromatographic separations were achieved under the optimized instrument conditions with an acceptable run time within 10 min. The representative MRM chromatograms of blank plasma, plasma spiked with the two analytes and the IS, and the plasma samples from rats after oral administration of GR extract and GR powder were shown in Fig. 1, showing that the peaks of the analytes and the IS were detected with excellent resolutions and peak shapes.

3.2. Method validation

3.2.1. Selectivity and matrix effect

Under the UHPLC–MS/MS conditions described above, gastrodin, parishin and the IS were eluted at 6.8, 8.7, and 8.0 min, respectively (Fig. 1). No interfering endogenous substances were observed at the retention times of the two analytes and the IS in blank plasma sample. All of them had high ionization efficiencies. The matrix effects for gastrodin at concentrations of 1.96, 7.84 and 13.07 μg/mL were measured to be 106.35 ± 2.8, 104.28 ± 1.8 and 105.28 ± 0.8 ($n = 3$), respectively. The values for parishin at concentrations of 0.00083, 0.6688 and 1.1136 μg/mL were 104.28 ± 3.8, 97.28 ± 1.2 and 95.22 ± 3.4 ($n = 3$), respectively. The value for the IS at concentration of 0.09408 μg/mL was 103.27 ± 2.1 ($n = 3$). The results indicated that the matrix effects on the ionization of the two analytes and the IS from plasma were negligible in the developed method.

3.3. Extraction recovery

Methanol used in the experiment showed good extraction efficiency. Mean absolute recoveries of gastrodin at three QC levels were 86.26 ± 3.1, 69.46 ± 4.55 and 78.75 ± 6.29 ($n = 3$), respectively. The values of parishin at three QC levels were 62.65 ± 7.6, 57.50 ± 5.24 and 64.59 ± 2.84 ($n = 3$), respectively. The value of the IS at concentration of 0.09408 μg/mL from rat plasma were 87.77 ± 3.1.

3.3.1. Calibration curves and sensitivity

The linear regression equations for gastrodin and parishin are $y = 0.9428x - 0.1583$ (0.1–15.7 μg/mL, 0.9965) and $y = 0.008x + 0.0447$ (0.000083–1.34 μg/mL, 0.9973), respectively. The LLOQs for gastrodin and parishin were 0.1 and 0.000083 μg/mL, respectively, indicating that the developed method is sensitive for the quantitative evaluation of the two analytes. The residuals (difference between the back-calculated concentration of the calibration standard and its nominal concentration) were no more than ±15% at all concentrations except the values obtained at LLOQ level (no more than 20%), demonstrating that the values were all within the acceptable range.

3.4. Precision and accuracy

The intra- and inter-day precisions of gastrodin were within the ranges from 4.27 to 5.30% and 3.81 to 7.52%, respectively. The intra- and inter-day precisions of parishin were within the ranges from

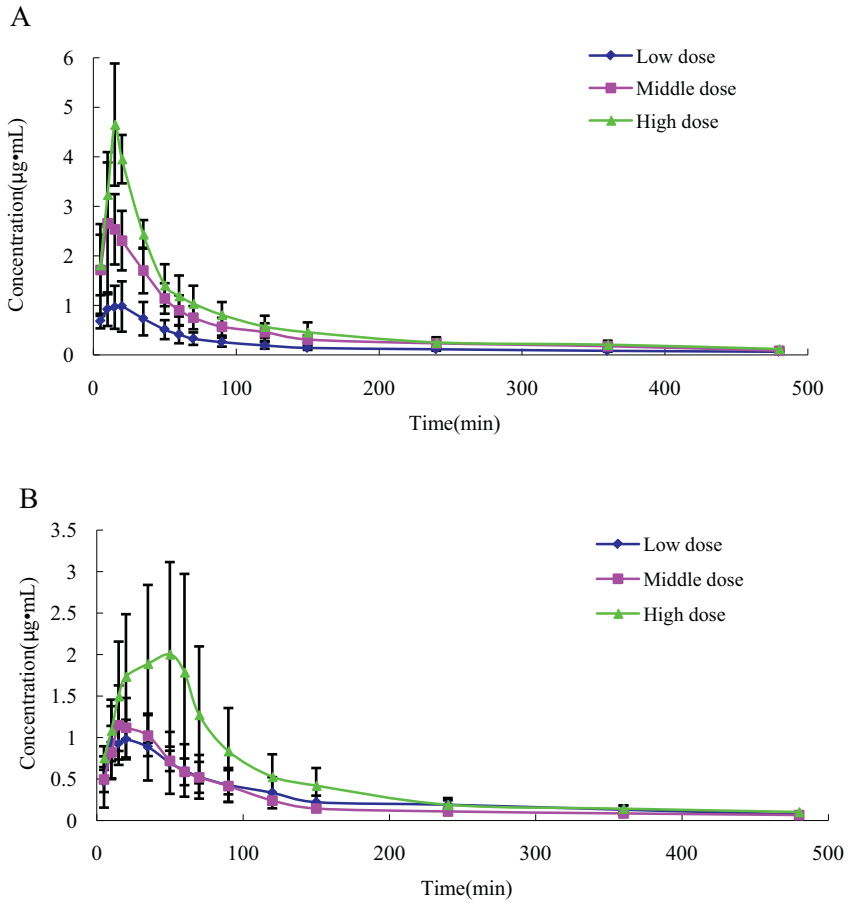

Fig. 2. Mean ± SD ($n = 6$) plasma concentration–time curves of gastrodin in rats ($n = 6$) after oral administration of GR extract (A) and GR powder (B).

1.98 to 8.75% and 0.69 to 3.57%, respectively. The accuracy values obtained from QC samples were within ±15% for gastrodin and within ±10% for parishin, respectively. The assay values on both intra- and inter-day were all within the acceptable range.

3.5. Stability

The stability of gastrodin and parishin was assessed under various conditions. The results indicate that the two analytes were stable in plasma after three freeze–thaw cycles (%RE, ±7%) and at room temperature for 12 h (%RE, ±20%). Post-preparative stability of the analytes also showed that no significant degradation occurred when the extracted samples were kept in the auto-sampler at 4 °C for 48 h (%RE, ±8%).

3.6. Application to pharmacokinetic studies

The developed UHPLC–ESI–MS/MS method was successfully applied to pharmacokinetic studies on gastrodin and parishin in rat plasma after oral administration of GR extract and GR powder. The mean plasma concentration-time curves ($n = 6$) of the two analytes are shown in Figs. 2 and 3, and the pharmacokinetic parameters are presented in Tables 1 and 2.

Table 1
Pharmacokinetic parameters of gastrodin in rats after oral administrations of GR extract and GR powder. Differences in means were detected using one-way ANOVA. Values in the same row marked by the same letter are not significantly different ($P \leq 0.05$).

Analyte	Parameter	Unit	Low dose	Middle dose	High dose
Gastrodin in GR extract	$T_{1/2}$	min	326.35 ± 153.93[a]	176.51 ± 34.16[b]	158.82 ± 35.00[b]
	T_{max}	min	18.33 ± 9.31[a]	12.50 ± 4.18[a]	14.17 ± 2.04[a]
	C_{max}	μg/mL	1.05 ± 0.47[a]	2.97 ± 1.12[b]	4.66 ± 1.23[c]
	AUC_{0-t}	μg min/mL	95.65 ± 22.42[a]	215.61 ± 41.01[b]	293.46 ± 55.43[c]
	$AUC_{0-\infty}$	μg min/mL	125.48 ± 20.58[a]	237.24 ± 41.29[b]	321.46 ± 58.35[c]
	V_d	(mg/kg)/(μg/mL)	1196.58 ± 530.78[a]	701.13 ± 208.64[a]	920.85 ± 244.12[a]
	CL	(mg/kg)/(μg/mL)/min	2.57 ± 0.47[a]	2.72 ± 0.43[a]	4.03 ± 0.76[b]
Gastrodin in GR powder	$T_{1/2}$	min	177.70 ± 55.47[a]	345.07 ± 102.03[b]	260.50 ± 115.99[c]
	T_{max}	min	18.33 ± 12.91[a]	25.00 ± 10.95[ab]	40.83 ± 15.94[b]
	C_{max}	μg/mL	1.25 ± 0.23[a]	1.25 ± 0.38[a]	2.29 ± 1.12[a]
	AUC_{0-t}	μg min/mL	131.31 ± 33.19[a]	113.41 ± 21.68[a]	229.44 ± 96.62[b]
	$AUC_{0-\infty}$	μg min/mL	149.77 ± 31.7[a]	146.83 ± 23.59[a]	265.78 ± 86.67[b]
	V_d	(mg/kg)/(μg/mL)	574.84 ± 269.93[a]	2152.71 ± 578.57[b]	2164.03 ± 1482.07[b]
	CL	(mg/kg)/(μg/mL)/min	2.18 ± 0.42[a]	4.38 ± 0.71[b]	5.28 ± 2.02[b]

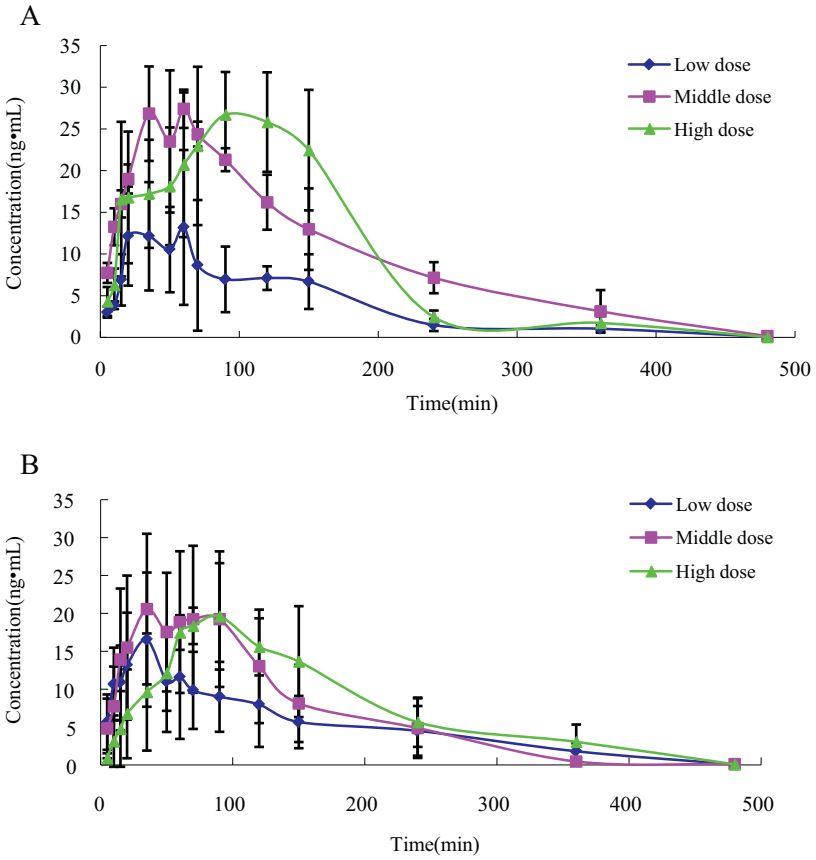

Fig. 3. Mean ± SD ($n = 6$) plasma concentration–time curves of parishin in rats ($n = 6$) after oral administration of GR extract (A) and GR powder (B).

We found that gastrodin was detected in rat plasma at 5 min after oral administration of both GR extract and GR powder. On one hand, it reached the T_{max} at 18.33, 12.50 and 14.17 min, respectively, after oral administration of GR extract at low, middle and high levels of doses and eliminated rapidly from the plasma. The data were found to be similar to those that were reported in publications [22,23]. On the other hand, the compound reached the T_{max} at 18.33, 25.00 and 40.83 min, respectively, after oral administration of GR powder at low, middle and high levels of doses. The values of T_{max} obtained in middle and high levels of doses after oral administrations of GR extract were shorter than that obtained from GR powder, indicating that gastrodin was absorbed faster when GR extract was used. However, the values of T_{max} obtained in low level of dose after oral administrations of GR extract and GR powder were found to be similar, which displayed the same absorption behavior. C_{max} of gastrodin were 1.05, 2.97 and 4.66 μg/mL, 1.25, 1.25 and 2.29 μg/mL after oral administration of GR extract and GR powder at three levels of doses, respectively. AUC_{0-t} were 95.65, 215.61 and 293.46 μg min/mL, 131.31, 113.41 and 229.44 μg min/mL after oral administration of GR extract and GR powder at three levels of doses, respectively. It was worth noting that gastrodin was detected up to 480 min after oral administration of both GR extract and GR powder, suggesting that the compound could not be easily eliminated in rat plasma.

Few publications were found to report the pharmacokinetic study on parishin. When GR extract was used as formulation, parishin reached the T_{max} at 38.33, 56.67 and 113.33 min, respectively, at low, middle and high levels of doses, whereas,

Table 2
Pharmacokinetic parameters of parishin in rats after oral administrations of GR extract and GR powder. Differences in means were detected using one-way ANOVA. Values in the same row marked by the same letter are not significantly different ($P \leq 0.05$).

Analyte	Parameter	Unit	Low dose	Middle dose	High dose
Parishin in GR extract	$T_{1/2}$	min	53.24 ± 10.42^{ab}	62.42 ± 4.8^{a}	38.49 ± 5.7^{b}
	T_{max}	min	38.33 ± 20.21^{a}	56.67 ± 5.77^{ab}	113.33 ± 40.41^{b}
	C_{max}	ng/mL	19.99 ± 2.2^{a}	30.22 ± 2.59^{b}	31.33 ± 2.65^{b}
	AUC_{0-t}	ng min/mL	1836.96 ± 565.63^{a}	4644.92 ± 816.21^{b}	4596.29 ± 311.6^{b}
	$AUC_{0-\infty}$	ng min/mL	1841.74 ± 571.82^{a}	4658.11 ± 818.08^{b}	4598.03 ± 311.2^{b}
	V_d	(mg/kg)/(ng/mL)	13.44 ± 1.53^{a}	12.32 ± 1.3^{a}	15.29 ± 2.83^{a}
	CL	(mg/kg)/(ng/mL)/min	0.18 ± 0.05^{a}	0.14 ± 0.02^{a}	0.27 ± 0.02^{b}
Parishin in GR powder	$T_{1/2}$	min	62.48 ± 19.19^{a}	54.25 ± 16.29^{a}	54.28 ± 8.58^{a}
	T_{max}	min	26.67 ± 11.79^{a}	75.00 ± 42.72^{b}	83.33 ± 11.55^{b}
	C_{max}	ng/mL	17.43 ± 8.06^{a}	21.53 ± 9.31^{a}	21.54 ± 3.69^{a}
	AUC_{0-t}	ng min/mL	2415.04 ± 1311.38^{a}	3223.83 ± 1703.26^{a}	3597.91 ± 1252.77^{a}
	$AUC_{0-\infty}$	ng min/mL	2421.83 ± 1307.8^{a}	3233 ± 1700.91^{a}	3605.12 ± 1249.65^{a}
	V_d	(mg/kg)/(ng/mL)	16.35 ± 13.1^{a}	21.28 ± 18.7^{a}	31.2 ± 16.69^{a}
	CL	(mg/kg)/(ng/mL)/min	0.16 ± 0.09^{a}	0.24 ± 0.14^{a}	0.38 ± 0.15^{a}

the compound reached the T_{max} at 26.67, 75.00 and 83.33 min, respectively, when GR powder was orally administered. C_{max} of parishin were much lower than that of gastrodin which reached 19.99, 30.22 and 31.33 ng/mL, 17.43, 21.53 and 21.54 ng/mL after oral administration of GR extract and GR powder at three levels of doses, respectively. AUC_{0-t} were 1836.96, 4644.92 and 4596.29 ng min/mL, 2415.04, 3223.83 and 3597.91 ng min/mL after oral administration of GR extract and GR powder at three levels of doses, respectively

On the basis of the AUC_{0-t} data, the relative bioavailability rations of gastrodin from GR extract to that from GR powder at low, medium and high dosages were 72.84, 190.12 and 127.90%, respectively. Meanwhile, the relative bioavailability ratios of parishin from GR extract to that from GR powder at low, medium and high dosages were 76.06, 144.08 and 127.75, respectively. The results indicated that the relative oral bioavailability of the two analytes were higher when administrated with GR powder at low dosage. In contrast, the relative oral bioavailability of the two analytes was higher when administrated with GR extracts at medium and high dosages.

4. Conclusion

Firstly, in the present study, an UHPLC–ESI–MS/MS method was developed and validated to determine simultaneously the active compounds gastrodin and parishin in rat plasma for the first time. The described method was sensitive and efficient with a short run time of 10 min and high accuracy that met all requirements in bioanalysis. Sample preparation was simple and reliable. Secondly, the validated method was successfully applied for pharmacokinetics study on the two analytes in rats after oral administration of GR powder and GR extract at low, medium and high dosages. Thirdly, the relative bioavailability of gastrodin and parishin in two formulations were calculated. The results indicated that the relative bioavailability of gastrodin and parishin from GR extract compared with that from GR powder at low dosage were 72.84 and 76.06%, respectively. Whereas, the relative bioavailability of the two analytes from GR extract compared with that from GR powder at medium and high dosages increased to190.12 and 144.08%, 127.90 and 127.75%, respectively.

This is the first attempt to reveal the relative bioavailability of gastrodin and parishin after oral administration of two different GR formulations. The powder formulation maximizes the systemic exposure to gastrodin and parishin and is more suitable to be administered than the extract at low dosage. However, the extract formulation maximizes the systemic exposure to the two analytes and fits for administration than the powder at medium and high dosages. Therefore, to ensure optimal absorption, GR should be administered with different formulations according to doses. Powder could not be considered as the best formulation just because of its preciousness.

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