# A simplified LC–MS/MS approach for simultaneous quantification and pharmacokinetics of five compounds in rats following oral administration of *Gastrodia elata* extract

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## Abstract

**Background:** To support the multicomponent pharmacokinetics of *Gastrodia elata*, a rapid, simple, and sensitive ultra-performance liquid chromatography tandem with mass spectrometry (LC–MS/MS) approach was established for simultaneous quantification of gastrodin, parishin A, parishin B, parishin C, and parishin E.

**Methods:** Five compounds were extracted from plasma by using one-step protein precipitation. The chromatographic separation was achieved on a $C_{18}$ column with gradient mobile phase comprising acetonitrile and 0.05% formic acid. The detection was performed using negative electrospray ionization in multiple reaction monitoring mode.

**Results:** This new method maximizes assay throughput by using minimal sample clean-up procedures and a shorter analytical run time. The approach exhibited good linearity for the five compounds ($r^2 > 0.995$) in the concentration ranges. The lower limits of quantification (LLOQ) were determined as 1.37 ng/mL for parishin A, parishin B, parishin C, and parishin E and 10 ng/mL for gastrodin. Then the method was fully validated with intra- and inter-day precision, accuracy, matrix effects, extraction recovery, and stability.

**Conclusion:** This validated approach was successfully applied to the pharmacokinetic study following oral administration of *Gastrodia elata* extract to rats. This investigation may provide some guidance for the clinical application and explanation of the pharmacological mechanism of *Gastrodia elata*.

**Keywords:** *Gastrodia elata*, Rat plasma, LC–MS/MS, Pharmacokinetics

## Introduction

*Gastrodia elata* Blume (Tianma in Chinese) is one of the notable and rare medicinal herbs in Traditional Chinese medicine (TCM) and has been officially listed in the Chinese Pharmacopoeia Commission (2015). *Gastrodia elata* Blume (*G. elata*) has properties of neuroprotective (Kim et al. 2003), anti-cardio-cerebral-vascular diseases, anticonvulsive (Hsieh et al. 2001; Kim et al. 2001), antiepileptic (Hsieh et al. 2007; Matias et al. 2016), antipsychotic activities (Chen et al. 2008; Chen & Sheen 2011; Chen et al. 2009), and memory improving (Hsieh et al. 2000; He et al. 2008; Zhan et al. 2016). Gastrodin, a representative and active substance responsible for clinical effects of this botanical drug, has been chosen as a phytochemical marker to evaluate the quality of *G. elata* Blume in Chinese pharmacopeia (Liu

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et al. 2016; Liu et al. 2015; Chen et al. 2015). However, it might be insufficient to control the quality of *G. elata* by only one ingredient as the marker due to its multi-active components and multifarious pharmacological activities.

Until now, most of the pharmacokinetic studies that have been performed on *G. elata* were based on one or several bioactive compounds such as gastrodin in rat plasma after administration of *G. elata*. The quantification of gastrodin and/or its metabolites in plasma by LC–MS/MS was extensively studied in many publications (Jia et al. 2014a; Jia et al. 2014b; Tang et al. 2015c). Relative tissue distribution, excretion studies, and bio-availability of gastrodin and parishin A from powder and extract of *Gastrodiae* Rhizoma were studied in rat by LC–MS/MS (Jiang et al. 2017; Zhao et al. 2014). Tang et al. developed an UPLC-FLD method to study the pharmacokinetics of *Gastrodiae* Rhizoma in rat plasma at LLOQs of 2.5 ng/mL (Tang et al. 2014; Tang et al. 2015a; Tang et al. 2015b). Liu et al. (2017) developed a LC–MS/MS method to determine bioactive compounds in dog plasma within a relatively long chromatographic

analysis time and complex sample pre-processing procedures.

Along with extensive phytochemical studies, more and more new active compounds, especially for parishins, gastrodin conjugates containing a citrate moiety, have been identified and isolated from *G. elata* and reported to exert prominent neuroprotective, anti-inflammatory, anti-oxidant, and many other property pharmacological effects. For example, parishin A and C showed good pharmacological effects on central nervous system diseases, such as Alzheimer's disease (Lin et al. 2016; Liu et al., 2016a, b, c; Huang et al. 2013; Shin et al. 2010). However, to the best of our knowledge, no other published reports are available with regard to the simultaneous determination of gastrodin, parishin A, parishin B, parishin C, and parishin E in rat plasma by LC–MS/MS (Wu et al. 2017).

To support the multicomponent pharmacokinetics of the extracts of *G. elata*, the aim of the present study was to develop and validate a rapid, simple, and sensitive LC–MS/MS method for the simultaneous determination



**Fig. 1** Chemical structures of five compounds and internal standard. Glc, β-D-glucopyranosyl
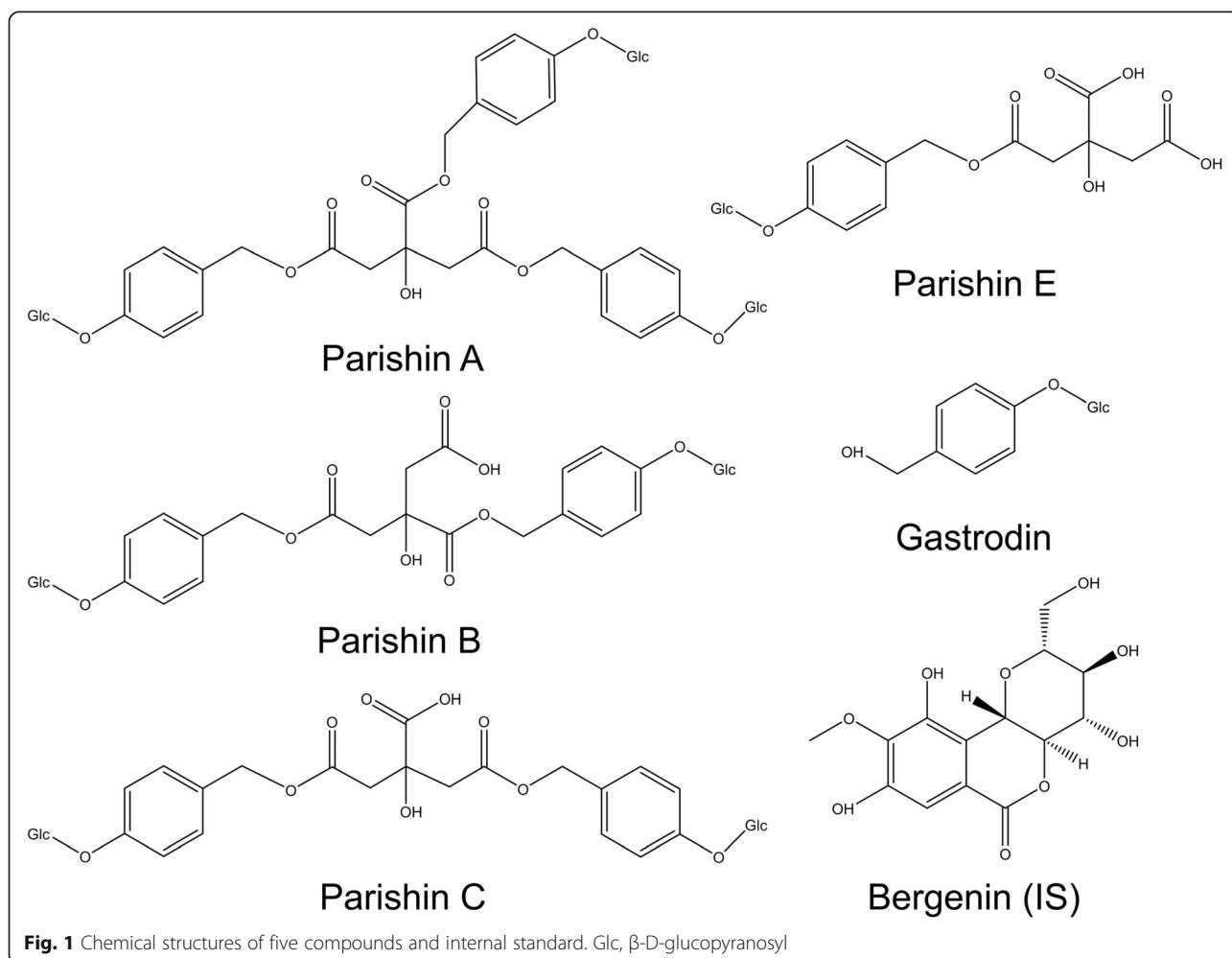
of gastrodin, parishin A, parishin B, parishin C, and parishin E (Fig. 1) in rat plasma and to investigate their pharmacokinetics in rats after the oral administration of the extracts of *Gastrodia elata*.

## Materials and methods
### Reagents and materials
The reference standards gastrodin, parishin A, parishin B, parishin C, and parishin E were obtained from Yuanye biotechnology (Shanghai, China) and their purity exceeded 98%. Bergenin (IS, purity > 98%) was purchased from Biopurify biotechnology (Chengdu, China). Methanol and acetonitrile were purchased from CNW Technologies Gmbh (Dusseldorf, Germany). Formic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA). Water was prepared using a Mill-Q ultrapure water system (Millipore Corp., Billerica, MA, USA). All other chemicals were of analytical grade.

### LC–MS/MS
The LC–MS/MS system consisted of a Applied Biosystems-Sciex 5500 triple-quadrupole mass spectrometer (Applied Biosystems, Toronto, Canada) interfaced by an electrospray ionization (ESI) probe with a Shimadzu ultra-performance liquid chromatography (Kyoto, Japan). The ionspray voltage was set at − 45000 eV, and curtain, collision, and ion source gases were 35, 7.0, and 55 arbitrary units, respectively. The entrance potential and collision cell exit potential were − 10 V and − 14 V, respectively. The capillary temperature was 550 °C. The declustering potential and collision energies in negative ion mode used for the measurement of the five compounds and I.S. were provided in Table 1. Chromatographic separation for quantification assay was achieved on an Agilent Extend-C$_{18}$ column (100 mm × 2.1 mm, 1.8 μm) using a mobile phases consisting of 0.05% *v/v* formic acid in water (A) and acetonitrile (B) at a flow rate of 0.3 mL/min. A gradient program was used as follows: 0–2.0 min at 2% solvent B, 2.0–5.0 min from 2% B to 15% B, and 5.0–7.0 min from 15% B to 35% B.

### Preparation of calibration standard and quality control samples
The stock solutions of gastrodin, parishin A, parishin B, parishin C, and parishin E were separately prepared by dissolving accurately weighed analytes in DMSO to yield a final concentration of 5 mg/mL. The stock solutions were first diluted to 100 μg/mL mixed solution of gastrodin, parishin A, parishin B, parishin C and parishin E with 50% methanol water. Working solutions were prepared by serial dilution of 100 μg/mL mixed solution with 50% methanol water to obtain concentrations of 27.4–6660 ng/mL for parishin A, parishin B, and parishin C, 27.4–60,000 ng/mL for parishin E, and 200–60,000 ng/mL for gastrodin, respectively. The standard plasma samples of the nominal concentration were prepared by adding serial working solution to blank rat plasma. The calibration curves with non-zero standard levels consisted of gastrodin/parishin A/parishin B/parishin C/parishin E in the concentration of 1.37–333.0 ng/mL for parishin A, parishin B, and parishin C, 1.37–3000.0 ng/mL for parishin E and 10.0–3000.0 ng/mL for gastrodin. The internal standard solution at the 300 ng/mL was prepared in precipitant. The nominal plasma concentrations of quality control (QCs) samples, at concentrations of parishin A, parishin B, parishin C, (5.0, 100.0, 300.0 ng/mL), parishin E (5.0, 900.0, 2700.0 ng/mL), and gastrodin (20.0, 900.0, 2700.0 ng/mL) were prepared from independent weighing of the five compounds. All solutions were stored at − 70 °C.

### Preparation of G. elata extract
The dried roots of *G. elata* (Tianma) were obtained from Shanghai Dehua Chinese Pharmaceutical Products Co., Ltd. (Shanghai, China). For preparation of the extracts, 100 g of Tianma was pulverized, mixed, and steeped in 1000 mL of ethanol water (70:30) for 0.5 h at room temperature before a sonication-enhanced extraction for 3 times at 50 °C, 20 min each time. The extract was separated by filtration, and the residue was re-extracted with 800 mL of ethanol water (70:30). The pooled extract was concentrated under reduced pressure at 50 °C. The extract was stored at − 20 °C pending use.

*G. elata* extract (40 mg) was added in 1 mL of 50% methanol in water to obtain a concentration of 40 mg/

**Table 1** Ion pair and MS parameter for the analysis of the five analytes and the internal standard in negative ion ESI mode

|  | Ion pair(*m/z*) | Declustering potential (V) | Collision energy (V) | Retention time (min) |
| --- | --- | --- | --- | --- |
| Gastrodin | 331.2→123.0 | − 23.0 | − 19.0 | 1.96 |
| Parishin A | 995.5→727.0 | − 26.9 | − 25.0 | 7.10 |
| Parishin B | 727.4→160.9 | − 5.07 | − 41.7 | 6.61 |
| Parishin C | 727.0→161.1 | − 20.0 | − 42.9 | 6.81 |
| Parishin E | 459.2→110.8 | − 48.8 | − 29.3 | 5.43 |
| Bergenin (IS) | 327.0→192.0 | − 115.0 | − 27.9 | 5.31 |

mL. Then, the sample was diluted with initial mobile phase to obtain solutions with concentration at 4 μg/mL. All the samples were filtered through 0.22 μm membrane filter before analysis. The content of gastrodin, parishin A, parishin B, parishin C, and parishin E in the extracts was 1.03%, 0.42%, 0.30%, 0.06%, and 0.26 %, respectively. The quantitative analysis of these compounds in the extracts was based on the previous method with some modification (Li et al. 2018).

## Pharmacokinetic study

Rat experiments were in compliance with Ethical Treatment of Laboratory Animals (The Ministry of Science and Technology of China, 2006) and were reviewed and approved by the University Committee on Use and Care of Animals, Nanjing University of Chinese Medicine (Nanjing, China). Male Sprague–Dawley rats (0.24–0.27 kg; the animal breeding farm of Qinglong mountain in Jiangning District, Nanjing, China; license approval number: SYXK (Su) 2018-0049)) were kept in rat cages at 20 ± 2 °C and relative humidity of 50 ± 20% with a 12-h light/dark cycle. Filtered tap water and commercial rat chow ad libitum were given to the rodents. Rats were allowed to acclimate to the facilities and environment for 7 days before the experiments. Six rats were fasted overnight with free access to water prior to the experiment. Rats were orally administered with the suspension of extracts of *G. elata* at 5 mL/kg (calculated as the total amount of extracts, equivalent to gastrodin 103.3 mg/kg, parishin A 42.5 mg/kg, parishin B 29.5 mg/kg, parishin C 5.30 mg/kg, and parishin E 26.9 mg/kg). Serial blood samples (400 μL) were collected from the orbital vein into EDTA-tubes at pre-dosing and 5, 15, 30, and 45 min and 1, 2, 4, 6, 8, 10, and 24 h post-dosing. Blood samples were immediately centrifuged at 12,000 rpm for 5 min, and the plasma fractions were decanted and frozen at − 70 °C until analysis.

## Plasma sample preparation

In order to increase sample throughput and simplify sample preparation, the rat plasma sample was prepared using the protein precipitation procedure without any intermediary solvent evaporation step. Sample preparation for rat samples was performed using methanol and acetonitrile (50:50, *v/v*) as precipitant with a volumetric precipitant-to-sample ratio of 1:3. Rat plasma samples (30 μL) were precipitated with 90 μL methanol/acetonitrile (50:50, *v/v*, containing 0.05% formic acid and 300 ng/mL internal standard solution) followed by centrifugation at 10,600*g* for 5 min. Then, 3 μL of the supernatant was injected into LC–MS/MS-based analysis.

## Method validation

Validation procedures were fully accomplished in terms of selectivity, linearity, extraction recovery and matrix effect, accuracy and precision, and stability according to FDA guidelines for bioanalytical method validation (https://www.fda.gov/media/70858/download).

### Selectivity and specificity

Selectivity was investigated by comparing chromatograms of blank rat plasma from six different batches with the corresponding blank plasma samples spiked with the five compounds and I.S., and the plasma samples from the rats after oral administration of the *G. elata* extracts were obtained. Specificity is the ability of analytical methods to distinguish and quantify analytes in the presence of other components.

### Linearity and LLOQ

Calibration curves were constructed using weighted (1/$X^2$) least square analysis by plotting the peak area ratios of five analytes to I.S. (Y) versus the corresponding nominal concentration of calibration standards (X). The signal-to-noise (S/N) was measured to be greater than 10 at the lowest concentrations of the standard curve.

### Precision and accuracy

The intra- and inter-day accuracy and precision were determined by analyzing quality control plasma samples in quintuplicate at three concentration levels (low, medium, and high) over three separate days.

### Recovery and matrix effect

The matrix effect (ME) of the five analytes and I.S. were evaluated by comparing the mean peak areas of QC levels spiked after extraction to the mean peak areas of the unextracted samples (*n* = 5). The extraction recoveries of the five analytes were determined by comparing the mean peak areas of the regularly pretreated QC samples at low and high concentrations with the mean peak areas of spike-after-extraction samples (*n* = 3).

### Stability

Under different conditions, the stability of rat plasma samples was studied by analyzing triplicate samples at low and high QC levels. These conditions included short-term stability (the spiked samples were exposed to room temperature for 2 h), long-term stability (storage at − 20 °C for 30 days), and freeze–thaw stability (three complete freeze/thaw cycles) post-preparation stability (the ready-to-inject samples in an autosampler were exposed to 4 °C for 12 h).

## Data processing and statistical analysis

The following pharmacokinetic parameters of five compounds in rats were processed by non-compartmental analysis using the Kinetica software package (version 5.0; InnaPhase Corporation, Philadelphia, PA, USA), maximum plasma concentration ($C_{max}$) and time to reach $C_{max}$ ($T_{max}$), terminal elimination half-life ($t_{1/2}$), area under the plasma concentration-time curve from zero to last sampling time ($AUC_{0-t}$), and to infinity ($AUC_{0-\infty}$). All data were expressed as means ± SD.

## Results and discussion

### Method development

As mentioned in previous studies, we also found the acidity may be an important factor affecting the sensitivity of parishin E (Liu et al. 2017). The relatively high
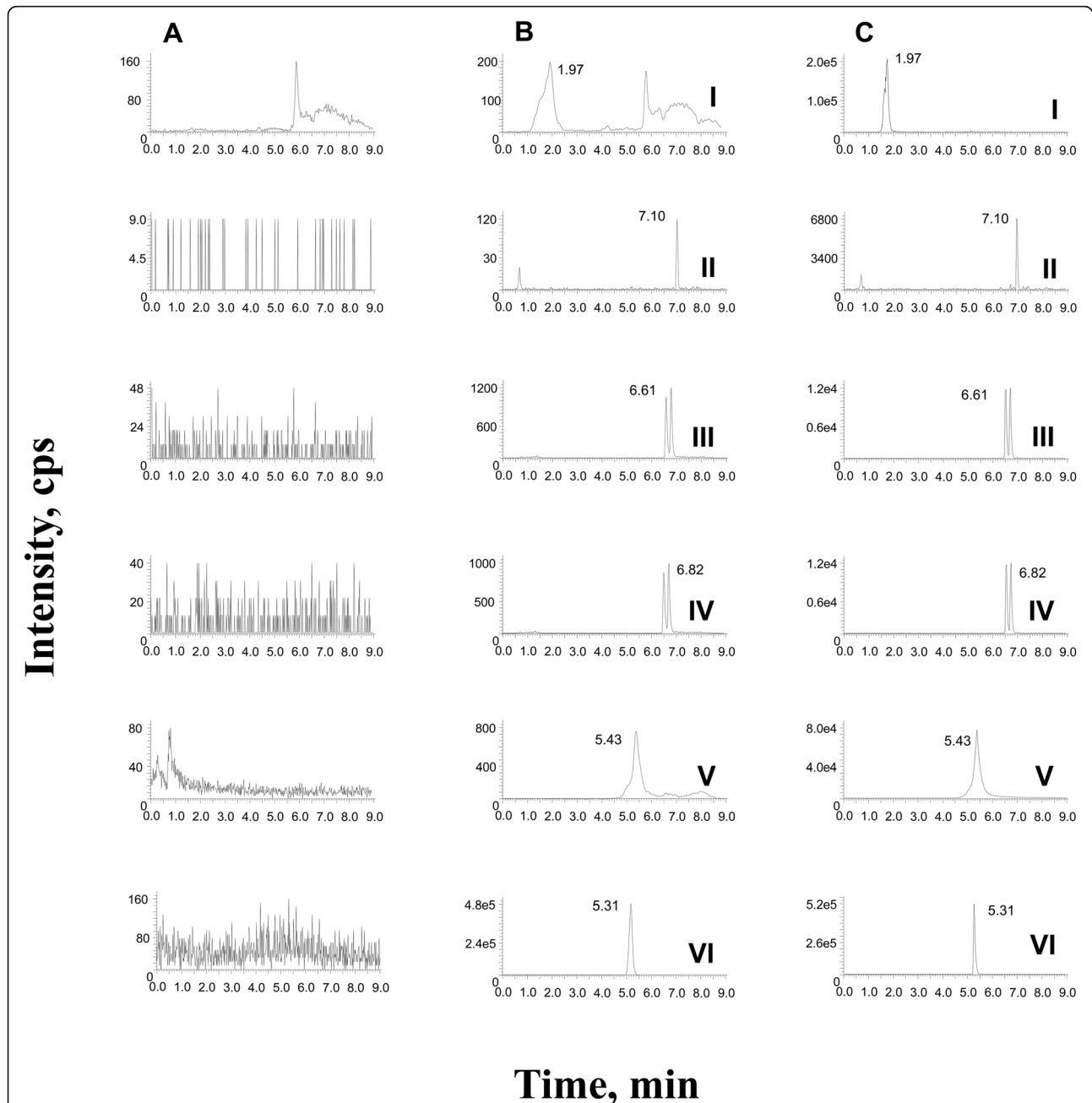


**Fig. 2** Typical MRM chromatograms of gastrodin (I), parishin A (II), parishin B (III), parishin C (IV), parishin E (V), and bergenin (I.S., VI) in rat plasma. **a** A blank rat plasma sample. **b** A spiked plasma sample with the analytes at LLOQ QC level and I.S. **c** A plasma sample collected at 1 h from a rat after oral *G. elata* extract administration

acidity of mobile phase can improve the peak shape and sensitivity of parishin E. Finally, 0.05% formic acid was chosen as the mobile phase condition. On the other hand, the peak time of water-soluble compound, gastrodin, is affected by the percentage of aqueous phase in the initial mobile phase, which may in turn affect matrix effects. For assays based on LC–MS/MS, coeluting undetected matrix components may affect detection responses of target analytes. Such matrix effects can be overcome by changing chromatographic conditions. Finally, 2% acetonitrile water was chosen as the optimal initial mobile phase condition.

All measurements had stronger responses in negative ion mode than in positive mode. The analyte detection and quantification using MRM mode was performed for achieving higher selectivity and sensitivity. After optimization, the transitions of 331.2→123.0, 995.5→727.0, 727.4→160.9, 727.0→161.1, 459.2→110.8, 327.0→192.0 were applied for analysis of gastrodin, parishin A, parishin B, parishin C, parishin E, and bergenin, respectively.

## Method validation
### Selectivity and chromatography
Figure 2 showed the typical MRM chromatograms of (A) a blank rat plasma sample, (B) a spiked plasma sample with the analytes at LLOQ QC level and I.S., (C) a plasma sample collected at 1 h from a rat after oral *G. elata* extract administration. No interference from the endogenous substances with the analytes and the I.S. in the rat plasma samples at the retention times was observed. Under the LC–MS/MS conditions described

above, compounds I–V and I.S. were eluted at 1.97, 7.10, 6.61, 6.81, 5.43, and 5.31 min, respectively. The analytes can be differentiated from the rat plasma matrix and quantitatively determined at the lowest concentration of the standard curve.

### Linearity and LLOQs
The linear regressions were fitted well over a concentration range of 10.0–3000.0 ng/mL for gastrodin, 1.37–333.0 ng/mL for parishin A/B/C, and 1.37–3000.0 ng/mL for parishin E in rat plasma with the correlation coefficient ($r^2$) exceeded 0.995. The lower limit of quantification (LLOQ) values were 10.0 ng/mL for gastrodin, 1.37 ng/mL for parishin A, parishin B, parishin C, and parishin E, which were sufficiently sensitive to determine the low concentration of five analytes after oral *G. elata* extract of in the rat plasma.

### Precision and accuracy
Table 2 summarizes the intra- and inter-day precision and accuracy for the five analytes at three concentration levels (i.e., LQC, MQC, and HQC) on three different batches. At each level, the intra- and inter-day accuracy ranged from 86.4 to 114.5%. The precisions (% CV) of these analytes were all within ± 14.1%. All the values indicated that the precision and accuracy values were within the accepted limits.

### Matrix effect and recovery
As shown in Table 3, the matrix effects of five analytes and I.S. at the low, middle, and high QC levels were

**Table 2** Intra-batch (*n* = 5) and inter-batch (*n* = 15) accuracy and precision for the analysis of the five analytes in rat plasma

| Compounds | Conc. spiked (ng/mL) | Intra-batch data | | Inter-batch data | |
|---|---|---|---|---|---|
| | | Accuracy (%) | Precision (%CV) | Accuracy (%) | Precision (%CV) |
| Gastrodin | 20 | 114.5 | 4.6 | 111.9 | 5.9 |
| | 900 | 93.6 | 9.1 | 93.8 | 0.4 |
| | 2700 | 86.4 | 3.5 | 91.5 | 7.9 |
| Parishin A | 5 | 107.4 | 9.6 | 111.9 | 5.8 |
| | 100 | 89.2 | 8.1 | 93.9 | 9.6 |
| | 300 | 90.5 | 5.9 | 88.8 | 0.4 |
| Parishin B | 5 | 108.6 | 4.8 | 106.3 | 11.3 |
| | 100 | 105.9 | 4.7 | 102.6 | 6.1 |
| | 300 | 107.7 | 3.5 | 100.4 | 1.5 |
| Parishin C | 5 | 97.1 | 3.7 | 95.0 | 12.9 |
| | 100 | 99.8 | 2.2 | 95.4 | 1.0 |
| | 300 | 98.6 | 5.1 | 99.9 | 1.8 |
| Parishin E | 5 | 102.2 | 14.1 | 101.0 | 7.1 |
| | 900 | 87.5 | 13.4 | 90.4 | 0.7 |
| | 2700 | 100.3 | 2.5 | 96.3 | 5.8 |

**Table 3** Matrix effects (*n* = 5) and extraction recovery (*n* = 3) results for analysis of the five analytes and I.S. in rat plasma

| Compounds | Spiked conc. (ng/mL) | Matrix effect (%) | | Extraction recovery (%) | |
|---|---|---|---|---|---|
| | | Mean (%) | RSD (%) | Mean (%) | RSD (%) |
| Gastrodin | 20 | 104.10 | 8.44 | 113.95 | 6.50 |
| | 2700 | 89.87 | 10.72 | 100.59 | 10.59 |
| Parishin A | 5 | 84.33 | 4.56 | 114.26 | 3.49 |
| | 300 | 92.48 | 2.52 | 101.12 | 0.23 |
| Parishin B | 5 | 94.09 | 1.77 | 118.70 | 1.26 |
| | 300 | 106.36 | 1.71 | 117.48 | 1.24 |
| Parishin C | 5 | 103.70 | 2.91 | 100.71 | 2.87 |
| | 300 | 105.41 | 3.57 | 114.63 | 2.72 |
| Parishin E | 5 | 115.25 | 11.67 | 85.97 | 13.58 |
| | 2700 | 111.13 | 3.32 | 108.02 | 2.84 |

between 84.33 and 115.25%, and the RSD values were within 11.67%. The results showed that the matrix effects ion suppression or plasma matrix enhancement of the five analytes and the I.S. from the rat plasma were negligible under the current conditions. The extraction recoveries were in the range of 85.97–120.12%, with a RSD of < 13.58%. The recovery rate was within the acceptable standards.

**Stability**

Five analytes were stable in rat plasma on bench top at room temperature for 2 h, freezing at − 70 °C for 30 days, after three freeze/thaw cycles and in autosampler rack at 4 °C for 12 h except parishin A for three freeze–thaw cycles and the results were summarized in Table 4. In addition, we found that parishin A was unstable in rat plasma at 4 h (accuracy; 81.7%). Thus, we supposed that

three freeze–thaw cycles and long-term storage at room temperature might influence quantitative analysis of parishin A in rat plasma. So the samples of parishin A are best not to be repeatedly frozen–thawed and long-term stored at room temperature. Processing the samples in an ice water was applied to minimize possible degradation of parishin A.
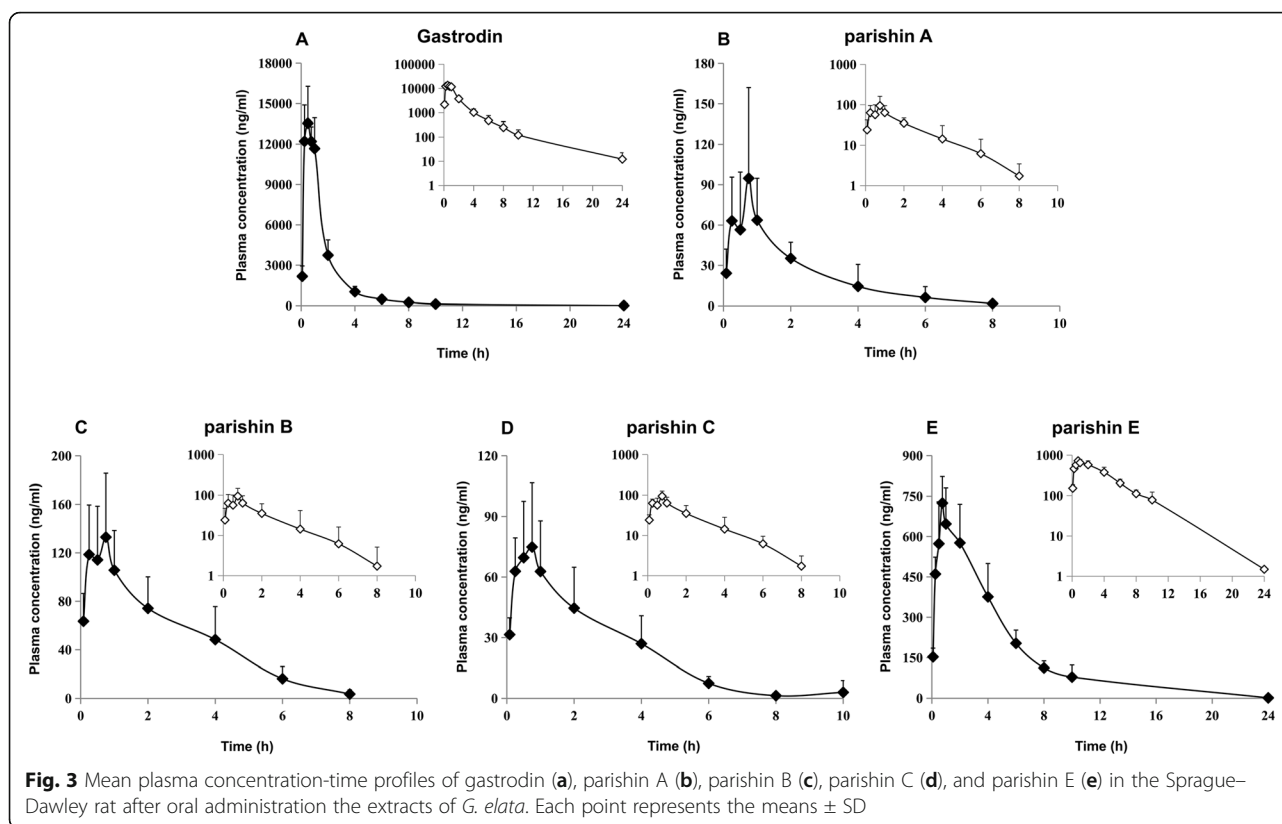
**Pharmacokinetic study**

In this study, a rapid and validated LC–MS/MS method was successfully developed for the pharmacokinetic study and simultaneous determination of the five analytes in rat after oral *G. elata* extract administration. The mean concentration-time curves of five analytes are presented in Fig. 3. The corresponding pharmacokinetic parameters including maximum plasma concentration ($C_{max}$), time to reach the $C_{max}$ ($t_{max}$), area under concentration-time curve (AUC), and terminal half-life ($t_{1/2}$) were shown in Table 5.

As shown in Table 5, all of the analytes could achieve the maximum concentration in 0.8 h, suggesting that all components were absorbed rapidly from the stomach into the blood. Comprehensive area under plasma concentration-time curve (AUC) analysis showed that systemic exposure to gastrodin was the highest. After dosing, the systemic exposure level (indicated by $AUC_{0-\infty}$) of parishin E was only one-eighth of gastrodin, and the other three compounds, especially parishin A exhibited lower systemic exposure levels < 200 ng/mL h and ranked in the order as follows: gastrodin (mean $AUC_{0-\infty}$, 24,899 ng/mL h) > parishin E (3390 ng/mL h) > parishin B (407 ng/mL h) > parishin C (227 ng/mL h) ≈ parishin A (181 ng/mL h). It showed that all analytes were eliminated from rats with a relatively high speed ($t_{1/2}$, 1.23 to 2.61 h) and the elimination rate of parishins

**Table 4** Stability data of the five analytes and I.S. in rat plasma under different conditions

| Compounds | Nominal concentration (ng/mL) | On bench top at room temperature for 2 h | | Freezing at − 70 °C for 30 days | | After three freeze/thaw cycles (− 70 °C to 25 °C) | | In autosampler rack at 4 °C for 12 h | |
|---|---|---|---|---|---|---|---|---|---|
| | | Accuracy (%) | Precision (% CV) | Accuracy (%) | Precision (% CV) | Accuracy (%) | Precision (% CV) | Accuracy (%) | Precision (% CV) |
| Gastrodin | 20.0 | 94.62 | 1.48 | 95.21 | 10.17 | 106.26 | 6.07 | 102.31 | 1.30 |
| | 2700 | 109.86 | 15.47 | 111.85 | 4.98 | 97.53 | 2.04 | 103.61 | 0.09 |
| Parishin A | 5.0 | 91.97 | 4.53 | 87.79 | 12.01 | 84.98 | 1.52 | 89.52 | 1.13 |
| | 300 | 85.09 | 7.20 | 101.42 | 9.89 | 85.25 | 2.56 | 91.38 | 0.10 |
| Parishin B | 5.0 | 110.51 | 4.21 | 102.78 | 4.85 | 113.57 | 4.50 | 102.78 | 4.85 |
| | 300 | 113.26 | 5.49 | 99.16 | 2.42 | 102.96 | 4.89 | 98.94 | 0.72 |
| Parishin C | 5.0 | 115.42 | 5.36 | 103.81 | 3.68 | 109.40 | 12.45 | 115.42 | 5.36 |
| | 300 | 103.93 | 8.00 | 102.05 | 1.37 | 93.55 | 4.86 | 103.93 | 8.00 |
| Parishin E | 5.0 | 106.28 | 2.50 | 100.69 | 1.58 | 110.3 | 5.97 | 103.56 | 1.81 |
| | 2700 | 108.35 | 2.55 | 104.45 | 5.61 | 86.20 | 6.18 | 103.55 | 9.97 |
| IS | 300 | 100.09 | 7.45 | 99.12 | 0.65 | 109.15 | 12.59 | 98.32 | 3.22 |

**Fig. 3** Mean plasma concentration-time profiles of gastrodin (**a**), parishin A (**b**), parishin B (**c**), parishin C (**d**), and parishin E (**e**) in the Sprague–Dawley rat after oral administration the extracts of *G. elata*. Each point represents the means ± SD

in vivo is faster than that of gastrodin. Compared to relatively high concentration in the *G. elata* extract, the low concentration of parishin A in rat plasma was largely due to the rapid metabolism or the low bioavailability. Parishin A might be biotransformed to gastrodin, parishin E, parishin B, and parishin C in gastrointestinal tract (Tang et al. 2014; Tang et al. 2015a; Tang et al. 2015c; Liu et al. 2017).

## Conclusions

In summary, a rapid, sensitive, and validated LC–MS/MS method was developed for simultaneous determination of gastrodin, parishin A, parishin B, parishin C, and parishin E in rat plasma and successfully applied to the pharmacokinetic studies of active components of *G.*

*elata* extract. This method maximizes assay throughput with a short time for the preparation step and a short analytical run time. Except for gastrodin, parishin A, parishin B, parishin C, and parishin E were effective components absorbed into plasma of rats from *G. elata* and might be as the potential markers to control the quality of *G. elata*. Furthermore, the obtained knowledge will provide some guidance for the clinical application and explanation of the pharmacological mechanism of *G. elata*.

**Table 5** Pharmacokinetic parameters for gastrodin, parishin A, parishin B, parishin C, and parishin E in rats after the oral administration of the extracts of *G. elata* at 10 g/kg

| Compounds | $C_{max}$ (ng/mL) | $T_{max}$ (h) | $AUC_{0-t}$ (ng/mL h) | $AUC_{0-\infty}$ (ng/mL h) | $t_{1/2}$ (h) |
|---|---|---|---|---|---|
| Gastrodin | 14,398 ± 1897 | 0.50 ± 0.18 | 24,880 ± 3384 | 24,899 ± 3399 | 2.37 ± 0.24 |
| Parishin A | 107 ± 61 | 0.75 ± 0.18 | 176 ± 85 | 181 ± 49 | 1.23 ± 0.33 |
| Parishin B | 136 ± 51 | 0.75 ± 0.18 | 389 ± 147 | 407 ± 147 | 1.68 ± 0.41 |
| Parishin C | 79 ± 29 | 0.65 ± 0.29 | 220 ± 91 | 227 ± 91 | 1.45 ± 0.27 |
| Parishin E | 728 ± 13 | 0.80 ± 0.11 | 3384 ± 717 | 3390 ± 717 | 2.61 ± 0.13 |

$C_{max}$ maximum concentration; $T_{max}$ time to maximum concentration, $AUC_{0-t}$ area under the concentration-time curve from zero to last sampling time, $AUC_{0-\infty}$ area under the concentration-time curve from zero to infinity;$t_{1/2}$ elimination half-life

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