The Determination of 10 Compounds of GuiZhi Decoction in Rat Plasma after Oral Administration by HPLC-MS/MS and its Application to a Pharmacokinetic Study

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Research

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The determination of 10 compounds of GuiZhi Decoction in rat plasma after oral administration by HPLC-MS/MS and its application to a pharmacokinetic study

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Abstract

Background: Guizhi Decoction (GZD), a traditional Chinese medical formula, has been commonly used to treat fever, sweating, and cold in China.

Methods: The high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method was established for the determination of 10 compounds, including cinnamic acid, paeoniflorin, albiflorin, liquiritin, isoliquiritin, liquiritigenin, isoliquiritigenin, glycyrrhizic acid, glycyrrhetic acid, and 6-gingerol. And the specificity, linearity, lower limit of quantification (LLOQ), lower limit of detection (LLOD), precision and accuracy, recovery, matrix effect, and stability were used to verify the HPLC-MS/MS method. This validated method was successfully applied for pharmacokinetic study of the 10 compounds in rat plasma after oral administration of GZD in three doses (40 g crude drug·kg⁻¹, 20 g crude drug·kg⁻¹, 10 g crude drug·kg⁻¹) and intravenous injection of GZD extraction at a dose of 2.0 g crude drug·kg⁻¹. The measurements of pharmacokinetic parameters including AUC_{0-∞}, T_{1/2}, T_{max}, C_{max}, Vz_F, Cl_F, and MRT, were performed using a non-compartmental model with Winnonlin 8.1 software.

Results: The results showed that 10 compounds were detected in plasma after oral administration of GZD. the compounds (except for glycyrrhetic acid) reached the maximum blood concentration quickly, whose T_{max} was about 0.1-0.2 min. And a total of 9 compounds were detected after intravenous injection of GZD. The plasma concentration-time curve of these compounds declines rapidly at the beginning, and then decreased slowly, indicating that the plasma concentration-time curves were double exponential function curves.

Conclusions: In this study, the developed method was suitable for pharmacokinetic analysis of the main compounds of GZD in rat plasma, and may reveal the pharmacodynamic material basis of GZD and provide a reference for the rational use of GZD in the clinic.

Keywords: Guizhi Decoction; HPLC-MS/MS; Multiple Compounds; Pharmacokinetics; Rat Plasma

Background

Guizhi Decoction (GZD) is a classical formula from *Treatise on Febrile Diseases*, a famous ancient Chinese book of traditional Chinese medicine (TCM). Studies have demonstrated that it is mainly used for the treatment of fever, self-sweating, headache, painful stiff nape, and external wind-cold syndrome [1, 2]. GZD is composed of five herbal slices, including 9.0 g of *Cinnamomi Ramulus*, 9.0 g of *Paeoniae Radix Alba*, 9.0 g of *Rhizoma Zingiberis Recens*, 9.0 g of *Fructus Jujubae*, and 6.0 g of *Radix Glycyrrhizae* [3-5]. Modern studies have proven that GZD has bidirectional regulating action on the body temperature, blood pressure, secretion of sweat glands, intestinal peristalsis, and immune function [6, 7].

Before this study, we reviewed a large number of pharmacokinetics (PK) studies of the formulae related to GZD. Some researchers have preliminarily discussed the PK characteristics of GZD [4]. There were some PK studies on other Chinese medicine formulae, such as Gualou-Guizhi Decoction,

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Guizhi-Gancao Decoction, Guizhi-Fuling Capsule, Huangqi-Guizhi- Wuwu Decoction and so on. For example, 11 compounds in plasma were detected after the rats were given Gualou-Guizhi Decoction by gavage, including liquiritin, isoliquiritin, liquiritin apioside, isoliquiritin apioside, liquiritigenin, isoliquiritigenin, glycyrrhizic acid, glycyrrhetic acid, paeoniflorin, albiflorin, and paeoniflorin sulfonate. These compounds belonged to *Radix Glycyrrhizae* and *Paeoniae Radix Alba* [8]. In the PK study of Guizhi-Gancao Decoction in vivo, 7 compounds were detected, including cinnamaldehyde, cinnamic acid, 2-methoxy cinnamic acid, glycyrrhizic acid, glycyrrhetic acid, liquiritigenin, and isoliquiritin, from *Cinnamomi Ramulus* and *Radix Glycyrrhizae* [9]. 6 compounds in plasma were quantified in the PK study of Guizhi-Fuling Capsul, involving *Cinnamomi Ramulus* and *Paeoniae Radix Alba* [10]. Moreover, only two compounds (paeoniflorin and astragaloside IV) were analyzed in the PK study of Huangqi-Guizhi-Wuwu decoction [11]. It could be seen that these selected compounds were only from individual herbs in the above studies. These compounds were too few to represent PK activities the whole formula. In addition, after oral administration of TCM formula, some compounds could be transformed into metabolites by enzymes in intestinal flora and liver. These metabolites could be absorbed and have pharmacodynamic effects in vivo. In the previous study, researchers had found that the compounds of GZD absorbed into blood were significantly different from the prototype compounds in decoction by HPLC [12]. We thought only prototype compounds could not represent the real effective ingredients in TCM. There is little doubt that attention should also be paid to metabolites in PK research. In this study, we detected 10 compounds of GZD in rat plasma after intragastric and intravenous administration, including 9 prototype compounds and 1 metabolite.

GZD was composed by five Chinese herbs. In this study, corresponding compounds and their metabolites in each herb were chosen as much as possible, to reveal the overall PK characteristics of GZD. Cinnamic acid was the main compound of *Cinnamomi Ramulus*, It could combat viral infections, protect neural functions, prevent or slow cognitive decline, and support the immune and digestive systems [13, 14]. So, cinnamic acid was selected as the index compounds in *Cinnamomi Ramulus*. Paeoniflorin and albiflorin were the main glycosides in *Paeoniae Radix Alba*, and they were isomers of each other. It had been noted that they could transfer to each other by microorganism [15]. Pharmacological studies have exhibited that they had remarkable effects in the treatment of pain, inflammation, muscle spasms, and neurodegenerative disorder properties [16-19]. So, paeoniflorin and albiflorin were selected. The content of gingerol was high in *Rhizoma Zingiberis* Recens. It has been found to gingerol had a variety of pharmacological effects, such as analgesic, antipyretic, antiemetic, and anti-inflammatory. So, 6-gingerol was selected. Both of liquiritin and isoliquiritin, liquiritigenin and isoliquiritigenin are isomers. These flavonoids compounds had strong therapeutic effects, including anti-inflammation, vasodilation, inhibition of platelet aggregation and regulation of blood lipids. Liquiritin and isoliquiritin were converted into liquiritigenin and isoliquiritigenin in the intestinal tract, which were subsequently metabolized to the glucuronic acid compounds in vivo [20-22]. Glycyrrhetic acid was the glycoside and metabolite of glycyrrhizic acid. Both of them exhibited high activities, such as antibacterial and anti-inflammatory effects [23-25]. So 6 compounds, including 5 prototypes and 1 metabolite, were chosen to be quantitated in *Radix Glycyrrhizae*.

In this study, a selective HPLC-MS/MS method for the simultaneous determination of multiple compounds of GZD in rat plasma was developed. With this method, the PK process after intragastric and intravenous administration were studied. In this study, we expected that the absorption, metabolism and excretion of GZD in vivo could be systematically studied to reveal the pharmacodynamic process of GZD.

Materials and methods

Materials and reagents

Cinnamomi Ramulus, *Paeoniae Radix Alba*, *Rhizoma Zingiberis* Recens, *Fructus Jujubae* and *Radix Glycyrrhizae* were purchased from Beijing Tongrentang pharmacy (Beijing, China), and were authenticated by Chunsheng Liu, the Professor of Beijing University of Chinese Medicine (Beijing, China), in line with the *Pharmacopoeia of the People's Republic of China (Edition 2015)* standards.

Cinnamic acid (110,786–201,604), paeoniflorin (110,736–201,741), liquiritin (111,610–201,106), glycyrrhetic acid (110,723–201,514), icariin (110,737–200,415), and aesculetin

(110,741–200,506) were purchased from National Institutes for Food and Drug Control (Beijing, China). Isoliquiritin (TT102I), liquiritigenin (TT011L), and glycyrrhizic acid (AB291G) were purchased from Epuresino technology (Tianjin, China) Co., Ltd (Tianjin, China). Albiflorin (Y15D8H50784) was purchased from Shanghai yuanye Bio-Technology Co., Ltd (Shanghai, China). 6-Gingerol (ASB-00007164-005) and isoliquiritigenin (ASB-00009265-005) were purchased from ChromaDex, Inc. (Irvine, CA, USA). The purity of all standards was greater than 98%. Acetonitrile was purchased from Fisher Scientific (Hampton, NH, USA). The chemical structures of the analytes and internal standards (ISs) were shown in Fig. 1. Methanol and formic acid were both purchased from Thermo Fisher Scientific-CN (Beijing, China); acetic acid and ammonium acetate were

purchased from Dikma Technologies Inc. (Beijing, China). All reagents were chromatographically pure.

Animals

30 clean male Sprague–Dawley rats (300 ± 20 g) were purchased from SPF (Beijing) Biotechnology Co., Ltd. (Beijing, China quality certification number: SCXK (Jing) 2016–0002). The temperature and humidity of the controlled environmental conditions were $23 \pm 3^\circ\text{C}$ and $50\% \pm 10\%$, respectively. The principles of laboratory animal care and all protocols were in accordance with the relevant national legislation and local guidelines and were approved by Animal Care and

Use Committee of the Institute of Basic Theory for Chinese Medicine, China Academy of Chinese Medical Sciences (Date: 25 March 2019; No.: SYXK (Jing) 2019-008).

Preparation of GZD for oral administration

The volatile oil of *Ramulus Cinnamomi* and *Rhizoma Zingiberis* Recens was extracted by steam distillation with four folds water for 2 h. The residues of the two herbal slices and other three herbal slices were boiled twice with eight folds water for 30 min each time. All those water decoctions were mixed together and concentrated to 1.0 g crude drugs per milliliter. After the volatile oil were added and mixed together, the extraction was stored at 4°C.

Preparation of GZD extraction for injection

GZD solution (1.0 g·mL⁻¹) was centrifuged (15,000 rpm, 15 min, 4°C) and the supernatant was filtered with 0.2 µm filter membranes. Then, 100 mL filtrate was vacuum freeze-dried into powder, which was dissolved with 500 mL sodium chloride injection (0.9%). The solution was filtered with 0.2 µm filter membrane in the super clean bench to remove the bacteria and then packed separately and stored at 4°C. This extraction contained 0.2 g crude drugs per milliliter.

HPLC–MS/MS conditions

Agilent 1260 with the Rapid Resolution Liquid Chromatography (RRLC) system (Agilent Technologies, Santa Clara, CA, USA) was used. The separation of compounds was carried out on the Agilent SB C18 column (2.1 × 50 mm, 1.8 µm) at 35°C.

Agilent 6410 mass spectrometry was equipped with Electron Spray Ionization (ESI), using Multiple Reaction Monitoring (MRM) with negative ion mode detection. The ion source temperature was 350°C; the drying gas was nitrogen (N₂, purity of 99.9%); the flow rate was 10 L·min⁻¹; the nebulizing gas pressure was 40 psi; and the capillary voltage was 4.0 kv. As the analytes and IS had different ionization activities, two optimized mobile phases were used separately to ensure the sensitivity. The MS conditions, including the ion pair, fragmentor voltage, and collision energy were all optimized with standard solutions.

Condition I: the mobile phase was composed of 0.1% aqueous acetic acid (A) and acetonitrile (B) with a gradient program (0.0–1.0 min, 5% B; 1.1–5.0 min, 14% B; 5.1–8.0 min, 20% B; 8.1–11.0 min, 45% B, 11.1–17.0 min, 85% B). Condition II: the mobile phase was composed of 0.2 % acetic acid aqueous solution containing 2 mmol·L⁻¹ ammonium acetate (A) and acetonitrile (B) with a gradient program (0.0–1.0 min, 5%B ; 1.1–8.0 min, 45% B). The flow rate was 0.3 mL·min⁻¹ and the injection volume was 2 µL. The stop time was 8.0 min with the post time of 6.0 min.

Sample preparation

In this study, the methods between liquid–liquid extraction (LLE) and protein precipitation (PPT) were compared to extract and purify the compounds to be tested in rat plasma. For LLE, ethyl acetate with or without hydrochloric acid were used as the extract liquor. For PPT, methanol and acetonitrile with or without hydrochloric acid were used to precipitate protein. The mixed reference solution was added into the blank plasma samples and prepared with above methods separately and injected into HPLC-MS/MS. The ratio of the peak area of each compound in the plasma sample to that of in the reference solution with same concentration was taken as the extraction rate. The method with a high extraction rate was chosen to prepare the samples.

HPLC–MS/MS method validation

Specificity

The specificity of the method was assessed by comparing chromatograms of blank plasma samples from six individual rats, blank plasma spiked with the 10 analytes and 2 ISs. The plasma samples were obtained at 30 min or 4 h from the rats after oral administration of the GZD or GZD extraction.

Linearity, LLOQ, and LLOD

The standard stock solutions of 10 analytes and 2 ISs were prepared in methanol. The initial concentrations of each compounds were 12,750 ng·mL⁻¹ for cinnamic acid, 3248 ng·mL⁻¹ for paeoniflorin, 3488 ng·mL⁻¹ for albiflorin, 3568 ng·mL⁻¹ for 6-gingerol, 3248 ng·mL⁻¹ for liquiritin, 3248 ng·mL⁻¹ for isoliquiritin, 3728 ng·mL⁻¹ for liquiritigenin, 3328 ng·mL⁻¹ for isoliquiritigenin, 3488 ng·mL⁻¹ for glycyrrhizic acid, and 8360 ng·mL⁻¹ for glycyrrhetic acid. The concentration of 2 ISs were 508 ng·mL⁻¹ for icariin and 638 ng·mL⁻¹ for aesculetin. To prepare the working solutions for calibration samples, 10 series concentrations solutions were prepared by methanol dilution. 20 µL working solution was added to the blank plasma.

Plasma calibration curves were constructed using the peak area ratios of the 10 analytes to the ISs, and applying separate weighted ($1/\chi^2$) least squares linear regression. The lower limit of quantification (LLOQ) and the lower limit of detection (LLOD) were defined by the signal-to-noise ratio method. LLOQ should be ten times the noise level ($S/N \geq 10$), and LLOD should be three times the noise level ($S/N \geq 3$).

Precision and accuracy

The accuracy and precision of the method were evaluated by intra- and inter-day variations. Standard solutions were added to the blank plasma and prepared at three different concentrations (low, medium, and high). Intra-day precision was evaluated with six replicates at one day, and inter-day precision was evaluated at three days. The precision of each compound was evaluated by relative standard deviation (RSD) value, which should not exceed 15.0%, and the accuracy was estimated with the relative error (RE), which should be within $\pm 15.0\%$.

Extraction recovery and matrix Effect

The recoveries of the analytes from plasma samples were determined by comparing the peak areas of the analytes in plasma samples after extraction to those of the same concentration of the analytes spiked into the solution extracted from plasma samples. The matrix effects were measured by comparing the peak areas obtained from samples with the analytes spiked after extraction, at three concentration levels (low, middle, and high), to those obtained from standard solutions at the same concentrations.

Stability

Stability of the analytes from the plasma samples were investigated by determining three different concentrations (low, medium and high samples) in five replicates under different storage conditions. The stability in plasma was assessed by analyzing (i) samples kept at room temperature (25°C) for 24 h, (ii) samples after three freeze-thaw cycles, (iii) samples after stored at -80°C for 15 days.

Pharmacokinetic study

30 clean male Sprague–Dawley rats (300 $\pm$ 20 g) were divided into oral administration (ig) groups (high, medium, and low dose), intravenous injection (iv) group and blank group. There were 6 rats in each ig group and 5 rats in iv group. All animals were fasted for 12 h but with access to water before experiment.

Rats in ig group were given GZD by gavage according to their body weight (4.0 mL·100 g⁻¹). The high dose group was given GZD in a concentration of 1.0 g crude drug·mL⁻¹, which was about 10 times as much as the clinical dosage (40.0 g crude drug·kg⁻¹). The medium dose group was in a concentration of 0.5 g crude drug·mL⁻¹ (20.0 g crude drug·kg⁻¹), and the low dose group was in a concentration of 0.25 g crude drug·mL⁻¹ (10.0 g crude drug·kg⁻¹). 300 µL blood samples were collected in Eppendorf tubes from the postorbital venous plexus veins of each rat by capillary tubes before dose (0 h) and after doses at 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 12 h, and 24 h.

Rats in iv group were injected with GZD extraction via the caudal vein according to their body weight (1.0 mL·100 g⁻¹), which was equivalent to 0.5 times as much as the clinical dosage (2.0 g crude drug·kg⁻¹). Blood samples were collected at 0 min, 2 min, 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 12 h, and 24 h after injecting GZD extraction.

Rats in the blank group were used to collect blank blood samples from the abdominal aorta after anesthetized by intraperitoneal injection of 3% sodium pentobarbital in the dosage of 0.5 mL·100 g⁻¹.

The blood samples were centrifuged at 12,000 rpm for 10 min at 4°C, and stored at –80°C until analysis.

Data analysis

The validated HPLC-MS/MS methods were applied to analyze the concentrations of 10 compounds in rat plasma after oral or parenteral administration GZD at different times. The pharmacokinetic parameters were obtained by the non-compartmental analysis of plasma concentration versus time data using a non-compartmental model of the Phoenix Winnonlin 8.1 software (Certara, USA).

Results

Optimization of chromatographic and Mass conditions

In order to obtain high detection sensitivity and good peak symmetry of the analytes, different ratios of formic acid, acetic acid, or ammonium acetate were chosen as the mobile phase annexing additive. Finally, we found that the separation and detection of the analytes were determined with these two phase conditions. The mobile phase used acetonitrile and 0.1% formic acid was suitable for paeoniflorin, albiflorin, liquiritin, isoliquiritin, liquiritigenin, isoliquiritigenin, glycyrrhizic acid, glycyrrhetic acid, and icariin (IS1), while the mobile phase condition of acetonitrile and 0.2% acetic acid containing 2 mmol·L^{–1} ammonium acetate was suitable for cinnamic acid, 6-gingerol, and aesculetin (IS2). The ionization conditions of those compounds, such as capillary voltage, nebulizer pressure, fragmentor voltage, and collision energy, were optimized by using the standard solutions. The optimized ionization conditions for the 10 compounds and 2 ISs were summarized in Table 1.

Table 1 MS/MS parameters of the analytes and internal standards

Analytes	Quantification Transition (<i>m/z</i>)	Dwell Time (ms)	Fragmentor Voltage (V)	Collision Energy (V)
Cinnamic acid	147.0 → 103.0	60	62	6
Paeoniflorin	525.2 → 121.0	70	157	37
Albiflorin	525.2 → 121.0	70	157	37
Liquiritin	417.2 → 255.2	70	152	15
Isoliquiritin	417.2 → 255.2	70	152	15
Liquiritigenin	255.1 → 119.0	70	105	24
Isoliquiritigenin	255.1 → 119.0	70	105	24
Glycyrrhizic acid	821.2 → 350.9	70	181	40
Glycyrrhetic acid	469.4 → 425.5	70	190	41
6-Gingerol	293.1 → 99.1	60	82	6
Icariin (IS1)	721.3 → 367.2	60	120	35
Aesculetin (IS2)	177.1 → 133.0	60	110	15

Sample pretreatment

The absolute recoveries of 10 analytes and 2 ISs after different sample pretreatments could be found in Table 2. The final sample treatment method was as follows: 20 µL IS solution (500 ng·mL^{–1} of icariin; 600 ng·mL^{–1} of aesculetin) and 10 µL hydrochloric acid (20 mmol·L^{–1}) were added into each 100 µL plasma sample. The sample was mixed for 1 min and 200 µL methanol was added. Then the mixture was vortex for 3 min. The sample was centrifuged at 12,000 rpm for 15 min at 4°C. 300 µL of the supernatant was collected and evaporated to dryness by vacuum freeze-drying. Finally, the residue was dissolved by 100 µL mobile phase (ACN: H₂O = 1:1) and vortexed for 3 min. After centrifuged at 12,000 rpm for 15 min at 4°C, 2 µL aliquot was injected into HPLC-MS/MS system.

Table 2 Absolute recoveries of the analytes and internal standards after different sample pretreatments methods (n = 3, mean ± SD).

Analytes	Ethylacetate (%)	Ethylacetate + 50 mmol·L ⁻¹ HCl (%)	MeOH (%)	MeOH + 50 mmol·L ⁻¹ HCl (%)	MeOH + 20 mmol·L ⁻¹ HCl (%)	MeOH + 10 mmol·L ⁻¹ HCl (%)	MeOH + 5 mmol·L ⁻¹ HCl (%)	ACN (%)	ACN + 50 mmol·L ⁻¹ HCl (%)	ACN + 20 mmol·L ⁻¹ HCl (%)	ACN + 10 mmol·L ⁻¹ HCl (%)	ACN + 5 mmol·L ⁻¹ HCl (%)
Cinnamic acid	8.0 ± 0.5	107.4 ± 8.5	102.2 ± 8.6	108.0 ± 11.7	106.8 ± 2.4	101.0 ± 2.7	103.6 ± 8.7	108.3 ± 11.9	100.3 ± 12.9	108.7 ± 17.1	106.9 ± 15.1	105.3 ± 12.4
Paeoniflorin	30.0 ± 3.3	25.7 ± 7.2	64.8 ± 7.4	72.4 ± 4.2	81.8 ± 7.6	85.8 ± 3.1	70.0 ± 9.7	64.7 ± 9.5	71.6 ± 8.9	84.9 ± 3.8	85.6 ± 6.3	94.9 ± 7.9
Albiflorin	46.5 ± 0.7	42.5 ± 8.9	62.9 ± 13.2	106.6 ± 12.1	111.5 ± 2.3	117.9 ± 1.1	100.6 ± 7.4	73.0 ± 3.5	96.4 ± 16.7	120.1 ± 10.6	109.5 ± 10.8	118.8 ± 2.1
Liquiritin	40.9 ± 8.3	91.2 ± 12.6	97.4 ± 13.7	92.9 ± 8.7	108.2 ± 2.5	111.3 ± 2.2	105.5 ± 14.4	116.4 ± 17.6	93.2 ± 13.9	108.1 ± 16.5	113.7 ± 15.4	115.2 ± 15.0
Isoliquiritin	77.3 ± 6.7	102.8 ± 8.6	61.3 ± 10.7	94.5 ± 10.4	100.2 ± 1.9	102.1 ± 1.8	95.8 ± 6.6	62.7 ± 16.8	96.6 ± 10.0	106.8 ± 19.6	82.7 ± 12.8	103.5 ± 10.4
Liquiritigenin	103.9 ± 7.7	98.2 ± 8.6	76.2 ± 12.3	97.3 ± 0.1	107.3 ± 1.3	110.9 ± 1.0	102.5 ± 11.8	75.6 ± 15.6	98.1 ± 9.4	112.0 ± 8.3	108.7 ± 11.7	114.5 ± 16.5
Isoliquiritigenin	105.4 ± 6.3	75.9 ± 6.3	49.3 ± 10.5	106.3 ± 0.7	109.9 ± 2.2	115.4 ± 1.9	99.9 ± 7.7	58.6 ± 11.2	115.8 ± 10.5	126.6 ± 17.6	109.5 ± 7.6	113.8 ± 2.5
Glycyrrhizic acid	0.0 ± 0.0	0.0 ± 0.0	101.8 ± 17.2	12.3 ± 0.6	52.6 ± 5.0	59.9 ± 0.9	63.5 ± 1.3	77.9 ± 1.9	17.2 ± 23.6	44.1 ± 9.6	64.4 ± 14.6	63.2 ± 4.1
Glycyrrhetinic acid	36.0 ± 7.1	48.7 ± 4.4	36.2 ± 6.9	55.2 ± 7.7	59.4 ± 1.2	58.6 ± 1.5	59.0 ± 9.6	39.3 ± 9.4	63.2 ± 14.3	64.6 ± 3.6	61.7 ± 4.3	65.6 ± 15.1
6-Gingerol	130.0 ± 17.6	6.0 ± 4.2	84.2 ± 12.4	102.3 ± 8.7	100.4 ± 14.8	111.7 ± 1.9	101.4 ± 7.8	89.2 ± 7.7	100.3 ± 11.7	108.5 ± 12.9	100.0 ± 13.5	108.7 ± 12.4
IS1	94.2 ± 9.6	89.2 ± 10.8	72.1 ± 12.1	94.1 ± 6.0	114.4 ± 12.8	120.2 ± 2.6	105.9 ± 15.0	82.2 ± 13.1	104.0 ± 8.5	120.2 ± 17.5	114.1 ± 10.0	124.4 ± 7.6
IS2	80.1 ± 10.2	2.5 ± 0.0	51.3 ± 4.0	98.3 ± 5.7	82.0 ± 1.2	62.6 ± 0.3	50.0 ± 2.7	47.0 ± 6.8	75.9 ± 10.2	61.9 ± 0.2	42.3 ± 5.4	40.6 ± 2.6

Note: MeOH: protein precipitation with methanol; ACN: protein sediment by acetonitrile; Ethyl acetate: extracting with ethyl acetate; HCl: adding hydrochloric acid; MeOH + 50 mmol/L HCl: protein precipitation with methanol under acidification.

Method validation

specificity

Under the developed chromatographic and mass conditions, the sample chromatograms of blank plasma, the standard sample solution, the drug-containing plasma samples after oral administration of GZD, and the solution of GZD were presented in Fig. 2. The method had high specificity and could be used for the qualitative determination of these compounds in plasma.

Linearity, LLOQ, and LLOD

The results showed that the 10 compounds had good linearity in the corresponding concentration range. The regression equation, linear range and correlation coefficient (r), LLOQ, and lower limit of detection (LLOD) of each compound were listed in Table 3.

Precision and accuracy

The results of precision and accuracy at the three different concentration levels were presented in Table 4. The intra- and inter-day RSD values were below 6.9% and 9.6% respectively, while the corresponding RE values ranged from -10.8% to 5.3%. All the assay values were within the acceptable criteria.

Matrix effect and extraction recovery

Average recoveries of investigated targets ranged from 85.4% to 113.9%. The RSD values of all analytes were lower than 15.3%, which indicated that there was no significant loss of the compounds among the process of the protein precipitation in the plasma samples. The matrix effect of each compound ranged from 85.4% to 116.8%, indicating that there was no obvious matrix interference. The results showed that the method was accurate and acceptable. The data of extraction recovery and matrix effect were listed in Table 5.

Stability

After storage at -80°C for 15 days and three freeze-thaw cycles, the stability RSD values of 10 compounds in plasma were all less than 9.9% (Table 6). The results showed that the detected analytes were all satisfied with the criteria under all conditions, so the samples were stable during the test process.

Fig. 2 Multiple reaction monitoring mode (MRM) chromatograms of cinnamic acid (1), paeoniflorin (2), albiflorin (3), liquiritin (4), isoliquiritin (5), liquiritigenin (6), isoliquiritigenin (7), glycyrrhizic acid (8), glycyrrhetic acid (9), 6-gingerol (10), icariin (11; IS1), and aesculetin (12; IS2). blank rat serum (A), blank rat serum spiked with the analytes and ISs (B), rat serum samples at 5 min, 1 h, or 8 h after oral administration of GZD (C), and GZD after 1000 times dilution with methanol (D).

Table 3 Calibration curve, linear range, lower limit of detection (LLOD), and lower limit of quantification (LLOQ) of 10 analytes ($n = 3$)

Analytes	Regression equation	Liner range (ng·mL ⁻¹)	r	LLOQ (ng·mL ⁻¹)	LLOD (ng·mL ⁻¹)
Cinnamic acid	$y = 0.9385x + 0.0344$	6-6120	0.9995	6	2
Paeoniflorin	$y = 0.3622x + 0.0136$	6-1624	0.9994	6	2
Albiflorin	$y = 0.3895x - 0.0395$	13-1635	0.9996	13	3
Liquiritin	$y = 2.5403x + 0.0443$	1-1624	0.9996	1	1
Isoliquiritin	$y = 2.2788x + 0.0393$	3-1624	0.9997	3	1
Liquiritigenin	$y = 1.8926x - 0.0016$	4-1864	0.9993	4	1
Isoliquiritigenin	$y = 3.0726x + 0.0336$	3-812	0.9991	3	1
Glycyrrhizic acid	$y = 0.4481x + 0.0487$	7-1744	0.9993	7	3
Glycyrrhetic acid	$y = 0.4370x + 0.0427$	4-2090	0.9993	4	2
6-Gingerol	$y = 1.0994x + 0.0392$	7-1784	0.9983	7	3

Table 4 Intra-day and inter-day precision and accuracy data of the 10 compounds spiked in rat plasma (mean $\pm$ SD; 3 replicates per day for 3 days)

Analytes	Spiked conc. (ng·mL ⁻¹)	Intra-Day ($n = 5$)			Inter-Day ($n = 5$)		
		Conc. (ng·mL ⁻¹)	RSD %	RE %	Conc. (ng·mL ⁻¹)	RSD %	RE %
Cinnamic acid	51	51.3 $\pm$ 0.9	2.0	0.6	47.8 $\pm$ 4.1	8.8	-6.2
	204	209.4 $\pm$ 9.6	5.1	2.7	203.0 $\pm$ 17.2	8.8	-0.5
	812	818.6 $\pm$ 31.3	4.3	0.3	815.6 $\pm$ 22.7	2.9	-0.1
Paeoniflorin	51	50.8 $\pm$ 1.9	4.2	0.1	53.1 $\pm$ 3.5	6.8	4.6
	203	202.9 $\pm$ 10.0	5.5	-0.1	200.1 $\pm$ 13.1	6.8	-1.4
	812	807.6 $\pm$ 38.5	5.3	-0.6	812.6 $\pm$ 21.4	2.7	0.1
Albiflorin	55	54.5 $\pm$ 1.1	2.2	-0.1	51.9 $\pm$ 4.5	8.9	-4.8
	218	218.0 $\pm$ 7.6	3.9	0.0	220.6 $\pm$ 12.0	5.6	1.2
	872	886.5 $\pm$ 39.0	4.9	1.7	870.6 $\pm$ 20.4	2.4	-0.2
Liquiritin	51	53.2 $\pm$ 2.4	5.0	4.8	48.5 $\pm$ 4.5	9.6	-4.5
	203	198.2 $\pm$ 6.4	3.6	-2.4	205.4 $\pm$ 10.6	5.3	1.2
	812	812.9 $\pm$ 22.1	3.0	0.1	811.5 $\pm$ 21.1	2.7	-0.1
Isoliquiritin	51	49.4 $\pm$ 1.0	2.3	-2.6	46.7 $\pm$ 3.9	8.7	-8.1
	203	208.1 $\pm$ 12.8	6.9	2.5	208.1 $\pm$ 17.1	8.5	2.5
	812	810.6 $\pm$ 44.0	6.1	-0.2	802.7 $\pm$ 40.5	5.2	-1.2
Liquiritigenin	58	55.6 $\pm$ 2.0	4.0	-4.5	54.3 $\pm$ 4.3	8.2	-6.7
	233	232.5 $\pm$ 5.6	2.7	-0.2	242.8 $\pm$ 20.5	8.7	4.2
	932	850.1 $\pm$ 52.5	6.9	-8.8	888.0 $\pm$ 41.2	4.8	-4.7
Isoliquiritigenin	51	47.3 $\pm$ 1.7	4.1	-6.9	45.3 $\pm$ 3.8	8.8	-10.8
	203	207.4 $\pm$ 9.5	5.1	2.2	207.5 $\pm$ 15.7	7.8	2.2
	812	793.3 $\pm$ 44.8	6.3	-2.3	794.9 $\pm$ 32.1	4.2	-2.1
Glycyrrhizic acid	55	57.4 $\pm$ 1.3	2.6	5.3	52.0 $\pm$ 3.8	7.5	-4.7
	218	213.3 $\pm$ 7.2	3.8	-2.2	223.2 $\pm$ 13.9	6.4	2.4
	872	808.5 $\pm$ 22.5	3.1	-7.3	876.2 $\pm$ 33.0	3.9	0.5
Glycyrrhetic acid	52	49.5 $\pm$ 1.0	2.2	-5.3	47.5 $\pm$ 2.5	5.4	-9.1
	209	216.3 $\pm$ 11.1	5.8	3.5	216.2 $\pm$ 15.6	7.5	3.4
	836	834.4 $\pm$ 35.3	4.7	-0.2	830.2 $\pm$ 29.0	3.6	-0.7
6-Gingerol	56	51.6 $\pm$ 2.1	4.6	-7.5	53.7 $\pm$ 3.8	7.3	-3.6
	223	201.4 $\pm$ 1.6	0.9	-9.7	220.7 $\pm$ 18.1	8.5	-1.0
	892	833.8 $\pm$ 35.6	4.8	-6.5	890.3 $\pm$ 20.1	2.3	-0.2

Note: RSD: relative standard deviation; RE: refractive error.

Table 5 Extraction recovery and matrix effect data of the 10 compounds in rat plasma (*n* = 5)

Analytes	Spiked conc. (ng·mL ⁻¹)	Extraction Recovery (%)		Matrix Effect (%)	
		Mean	RSD	Mean	RSD
Cinnamic acid	51	97.9	15.3	96.1	8.9
	204	96.3	8.7	103.3	8.5
	812	110.9	4.8	90.3	6.0
Paeoniflorin	51	94.1	5.8	111.0	8.3
	203	99.4	2.4	96.8	2.2
	812	108.2	7.1	92.6	4.8
Albiflorin	55	95.9	9.8	102.4	10.5
	218	104.8	5.8	85.4	9.4
	872	113.9	9.4	88.7	7.5
Liquiritin	51	106.3	6.2	100.1	6.2
	203	91.0	4.0	104.5	4.0
	812	91.8	5.3	116.8	9.5
Isoliquiritin	51	87.6	11.3	106.4	3.9
	203	110.3	6.6	93.0	4.0
	812	102.0	5.4	98.1	2.5
Liquiritigenin	58	97.1	3.9	97.8	2.6
	233	101.8	2.3	97.8	2.0
	932	105.5	7.3	103.3	6.5
Isoliquiritigenin	51	92.6	5.9	108.6	4.6
	203	105.2	4.9	105.8	3.2
	812	97.2	2.7	113.7	7.4
Glycyrrhizic acid	55	100.7	5.4	114.8	9.0
	218	89.1	5.9	105.7	4.3
	872	87.5	8.8	94.7	12.7
Glycyrrhetic acid	52	88.2	8.3	94.0	7.2
	209	88.1	8.2	105.4	4.7
	836	94.5	3.9	106.2	4.4
6-Gingerol	56	85.4	7.9	111.3	6.7
	223	88.0	6.9	88.6	9.1
	892	109.5	4.3	86.5	8.0

Table 6 Stability of 10 compounds under various conditions (*n* = 5).

Analytes	Spiked conc. (ng·mL ⁻¹)	12 h at 4°C (%)		Three Freeze–Thaw Cycles (%)		15 Days at –80°C (%)	
		Mean	RSD	Mean	RSD	Mean	RSD
Cinnamic acid	52	97.5	8.4	92.1	4.0	101.1	8.4
	209	111.2	5.1	102.5	6.3	109.4	8.0
	836	111.4	6.1	99.9	1.9	99.5	4.6
Paeoniflorin	51	88.8	6.2	89.6	4.0	104.6	7.7
	203	85.6	6.3	103.3	4.4	97.5	3.1
	812	99.5	8.5	99.9	2.7	100.1	4.7
Albiflorin	55	100.7	9.9	96.2	6.5	90.6	1.9
	218	90.1	8.5	100.5	4.4	104.2	8.3
	872	102.6	6.8	100.0	1.3	101.2	4.7
Liquiritin	51	92.2	5.8	89.7	2.7	93.3	0.6
	203	103.6	5.6	103.2	5.2	106.7	5.1
	812	98.2	5.8	99.8	3.8	99.7	2.0
Isoliquiritin	51	109.1	4.6	87.2	1.6	104.8	5.2

	203	90.9	5.4	104.0	4.5	95.8	7.9
	812	96.1	7.6	99.8	4.0	100.9	1.3
Liquiritigenin	58	95.4	9.5	89.1	2.6	95.6	7.9
	233	102.9	3.4	103.4	6.1	109.4	6.3
	932	108.3	7.3	99.8	4.5	99.3	2.1
Isoliquiritigenin	51	109.5	8.8	90.7	2.5	97.5	8.8
	203	90.2	5.2	102.9	5.6	101.5	2.3
	812	97.5	6.7	99.9	5.1	99.9	4.0
Glycyrrhizic acid	54.555	99.3	5.8	89.8	4.4	98.0	1.8
	218	101.0	1.8	103.2	2.1	104.7	4.3
	872	102.9	5.7	99.9	5.8	99.8	1.6
Glycyrrhetic acid	52	100.4	8.5	90.5	5.1	103.6	7.5
	209	99.5	6.8	105.2	6.2	108.3	3.7
	836	105.6	6.4	99.7	2.9	99.6	4.2
6-Gingerol	56	98.8	5.1	87.5	4.6	97.5	7.0
	223	99.3	4.6	103.9	4.3	100.1	4.1
	892	103.5	7.0	99.8	3.5	100.0	0.3

Note: Mean: measured concentration; RSD: relative standard deviation.

Pharmacokinetic results

The validated HPLC-MS/MS methods were applied to the pharmacokinetic study of the 10 compounds in rat plasma after oral administration and intravenous injection of GZD. The pharmacokinetic parameters were calculated by Phoenix Winnonlin 8.1 software (Certara, USA). Mean blood concentration–time curves (C-T) of the 10 compounds taken orally were displayed in Fig. 3, and the main pharmacokinetic parameters were listed in Table 7. Simultaneously, the pharmacokinetic results of the compounds administered intravenously were mainly shown in Fig. 4 and Table 8.

After oral administration of GZD, 10 compounds were detected in plasma. Except for glycyrrhetic acid (metabolite), the other compounds reached the maximum blood concentration quickly, whose $T_{\max}$ was about 0.1-0.2 min. This pharmacokinetics phenomenon coincided with the characteristic of GZD as the Jiebiao formula (TCM term), which took effect quickly. All the AUC and $C_{\max}$ values of those 10 compounds at different doses were positively correlated with the dose, and $T_{1/2}$ was independent with the dose, indicating that the compounds were in line with the first-order kinetic process in vivo.

A total of 9 compounds were detected after intravenous injection of GZD. The plasma concentration-time curve of these compounds declines rapidly at the beginning, and then decreased slowly, indicating that the plasma concentration-time curves were double exponential function curves. Initially, these compounds did not reach the dynamic balance (the effects of distribution and elimination combined together). After a period of time, only the elimination process existed in vivo. The metabolites, glycyrrhetic acid, was detected in plasma. However, it could not be fitted with winnonlin software due to its concentration was too low. The V_F values of paenoflorin, liquiritigenin, and isoliquiritigenin were higher than those of other compounds, which indicated they had broad distribution in tissues. Glycyrrhizic acid had the longest $T_{1/2}$ and MRT. The compounds eliminated slowly in vivo.

Fig. 3 Mean blood concentration–time curves of the 10 analytes in rat plasma after oral administration of GZD (H: high dose, 40.0 g crude drug·kg⁻¹, M: medium dose, 20.0 g crude drug·kg⁻¹, L: low dose, 10.0 g crude drug·kg⁻¹; each point represents mean + SD, *n* = 6).

Fig. 4 Mean blood concentration–time curves of the 9 analytes in rat plasma after intravenous injection of GZD at a dose of 2.0 g crude drug·kg⁻¹ (each point represents mean ± SD, *n* = 5).

Table 7 Pharmacokinetic parameters of 10 compounds in rat plasma after oral administration of GZD in three doses (H: high dose, 40.0 g crude drug·kg⁻¹, M: medium dose, 20.0 g crude drug·kg⁻¹, L: low dose, 10.0 g crude drug·kg⁻¹; n = 6)

Analytes	Dose	AUC _{0-∞} (h·μg·L ⁻¹)	T _{1/2} (h)	T _{max} (h)	C _{max} (μg·L ⁻¹)	V _{z_F} (L·kg ⁻¹)	Cl _F (L·h ⁻¹ ·kg ⁻¹)	MRT _{0-t} (h)
Cinnamic acid	H	1059.3±221.1	0.5±0.1	0.1±0.0	4027.6±1046.7	5.2±3.5	3.3±0.6	0.5±0.1
	M	396.5±124.3	0.3±0.1	0.1±0.1	1004.0±253.0	1.9±0.8	4.8±2.0	0.4±0.1
	L	186.4±63.8	0.2±0.0	0.1±0.0	831.3±250.1	1.3±0.4	5.1±2.0	0.2±0.1
Paeoniflorin	H	353.1±123.8	2.1±0.4	0.1±0.1	221.3±64.1	952.8±338.6	319.8±93.3	2.6±0.5
	M	175.2±46.7	1.8±0.4	0.1±0.0	100.7±31.9	618.2±170.1	316.5±94.3	2.0±0.2
	L	46.8±8.7	2.8±3.4	0.5±0.7	31.0±25.1	858.5±599.4	519.8±60.2	4.2±4.8
Albiflorin	H	138.9±55.9	1.8±0.3	0.1±0.1	107.1±42.1	992.9±335.6	394.8±159.7	2.3±0.6
	M	67.1±33.1	1.5±0.4	0.1±0.0	52.6±19.3	886.5±381.0	426.6±179.3	1.9±0.5
	L	46.8±8.7	1.6±1.2	0.5±0.7	31.0±25.1	1037.8±1304.7	262.4±51.4	2.2±1.1
Liquiritin	H	47.1±11.3	0.9±0.3	0.1±0.0	37.6±7.9	2834.8±574.8	2417.7±450.2	1.3±0.3
	M	27.6±12.8	0.9±0.5	0.1±0.1	19.2±2.3	2554.0±656.6	2277.9±794.6	1.3±0.6
	L	12.3±1.7	0.5±0.1	0.5±0.4	13.7±4.1	1626.9±280.2	2261.8±335.8	0.8±0.1
Isoliquiritin	H	8.0±3.7	0.9±0.5	0.2±0.1	7.8±0.9	2984.1±1349.0	2491.4±898.8	1.3±0.6
	M	5.0±1.8	1.2±1.6	0.1±0.1	4.7±1.3	1976.4±1291.6	1779.6±940.7	1.8±2.3
	L	1.5±0.1	1.1±0.8	0.2±0.2	2.2±0.4	1084.1±70.7	2584.6±212.1	1.2±1.0
Liquiritigenin	H	102.0±39.2	3.3±1.9	0.6±0.5	42.3±17.4	192.8±57.9	36.7±8.5	4.2±2.3
	M	72.3±0.3	4.1±0.4	0.8±0.4	19.9±7.6	170.9±16.8	29.1±0.1	5.8±0.8
	L	16.1±9.7	3.9±0.1	0.8±0.3	13.4±6.8	198.2±46.1	43.0±3.9	4.3±1.7
Isoliquiritigenin	H	4.5±0.9	0.9±0.2	0.1±0.0	6.8±3.4	299.7±98.4	227.7±51.8	1.2±0.3
	M	2.9±1.1	1.1±0.8	0.1±0.1	2.7±1.6	356.2±101.2	211.1±95.3	2.0±1.6
	L	1.1±0.1	0.6±0.3	0.2±0.4	1.8±0.7	196.4±120.1	229.0±31.6	0.8±0.4
Glycyrrhizic acid	H	174.8±36.9	3.0±0.3	0.2±0.2	73.6±20.9	530.9±116.0	120.9±24.2	4.2±0.4
	M	109.9±43.0	3.2±1.0	0.5±0.8	44.7±25.6	334.2±173.9	107.2±49.8	3.2±1.5
	L	54.8±18.5	3.8±2.1	0.5±0.8	15.3±10.0	540.7±194.0	105.1±47.9	5.8±3.1
Glycyrrhetic acid	H	18958.4±6548.3	6.6±2.3	8.0±0.0	2000.2±854	10.5±3.2	1.2±0.4	12±2.6
	M	9781.7±2315.9	6.4±1.9	8.0±0.0	1021.9±425.1	10.3±4.7	1.1±0.3	11.6±0.8
	L	3275.4±993.6	6.2±2.9	7.3±3.0	283.8±115.9	14.1±6.4	1.7±0.4	12.3±4.7

6-Gingerol	H	76.0±64.3	3.2±1.9	0.2±0.2	24.2±10.1	155.6±14.8	43.7±24.2	4.5±2.7
	M	45.0±5.0	2.5±0.9	0.6±0.7	17.5±16.9	67.8±17.3	19.5±2.2	3.4±1.5
	L	28.1±0.0	2.9±1.1	0.1±0.1	9.9±1.5	151.2±146.1	31.5±22.6	3.6±0.6

Table 8 Pharmacokinetic parameters of 10 compounds in rat plasma after intravenous injection of GZD at a dose of 2.0g crude drug·kg⁻¹ (n = 6)

Analytes	C ₀ (μg·L ⁻¹)	AUC _{0-∞} (h·μg·L ⁻¹)	T _{1/2} (h)	V _{ss} (L·kg ⁻¹)	Cl _F (L·h ⁻¹ ·kg ⁻¹)	MRT _{0-t} (h)
Cinnamic acid	106.4±40.3	821.6±310.7	0.2±0.0	0.2±0.0	1.8±0.9	0.3±0.0
Paeoniflorin	2671.4±354	11038.6±2175.8	0.5±0.2	0.6±0.2	2.0±0.3	0.6±0.0
Albiflorin	987.3±226.3	4386.1±2358.1	0.2±0.0	0.7±0.4	2.6±0.7	0.2±0.1
Liquiritin	2072.0±588.0	285.2±59.0	0.2±0.0	2.2±0.5	21.2±4.4	0.3±0.0
Isoliquiritin	68.1±18.8	570.8±200.6	0.1±0.1	1.1±0.5	12.8±4.2	0.1±0.0
Liquiritigenin	11.8±1.1	57.3±7.0	0.1±0.0	0.7±0.1	4.1±0.4	0.2±0.0
Glycyrrhizic acid	5802.2±458.7	10327.2±791.6	2.3±0.6	0.3±0.0	0.2±0.0	2.5±0.3
6-Gingerol	12.9±2.6	84.4±25.4	0.1±0.0	0.6±0.2	6.7±1.3	0.1±0.0

Discussion

The validated HPLC-MS/MS method was successfully applied to determination of 10 compounds, including 9 prototype compounds and 1 metabolite in rat plasma after oral administration and intravenous injection. Besides, we also studied the bioavailability of all measured substances in plasma based on the pharmacokinetics results to explore the absorption and utilization of GZD in vivo.

Ramulus Cinnamomi was the sovereign drug in GZD. The AUC of cinnamic acid(i.g.) were bigger than other prototypic compounds, while the content of cinnamic acid in decoction was much lower. It meant that cinnamic acid was more easily absorbed into blood.

Paeoniae Radix Alba was the assistant drug in GZD. Several papers had reported that there was a mutual transformation between paeoniflorin and albiflorin[26]. The ratios of AUC and $C_{\max}$ of paeoniflorin and albiflorin were basically the same as the ratios of content in decoction (2:1) after oral or intravenous administration. This indicated that the two compounds might not be transferred into each other in vivo.

Radix Glycyrrhizae was the envoy drug in GZD. In this study, 6 compounds were detected in rat plasma after oral administration. The content of liquiritin was higher in GZD than that of other compounds, but its $C_{\max}$ and AUC values were low, indicating that it was not easily absorbed into the body. The $C_{\max}$ and AUC values of liquiritigenin were about twice that of liquiritin in vivo, but the content of liquiritigenin was much lower in GZD (1:100). The result suggested that liquiritin could be converted into liquiritigenin in vivo. Moreover, the C-T diagram of liquiritigenin showed a double peak, which might due to the transformation of liquiritin in the intestinal tract. The AUC ratio of liquiritin to liquiritigenin in the plasma during intravenous administration was similar to the ratio of their content in GZD, indicating that liquiritin could not be transformed into liquiritigenin by liver. Combining the results of oral administration, it could be speculated that the transformation process mainly occurred in intestines. Similar to liquiritin, glycyrrhizic acid had high content in GZD, but the values of $C_{\max}$ and AUC were low after oral administration, indicating that it was not easily absorbed. The $C_{\max}$ and AUC of glycyrrhetinic acid were about 60-100 times than that of glycyrrhizic acid, which shown that glycyrrhetinic acid were more easily absorbed into the body than glycyrrhizic acid. The $T_{\max}$ of glycyrrhetinic acid was about 8h, indicating that the speed of conversion was slow. After intravenous injection, the AUC ratio of glycyrrhetinic acid to glycyrrhizic acid was significantly lower than the ratio of the two compounds after oral administration, suggesting that the liver could not convert glycyrrhizic acid into glycyrrhetinic acid effectively. We speculated that after GZD injected into blood, only a small part of glycyrrhizic acid was excreted into intestine by the bile of the liver, and metabolized into glycyrrhetinic acid by intestinal flora, then re-absorbed into the blood.

Rhizoma Zingiberis Recens were the supplementary drug in GZD. The V_z of 6-gingerol was relatively high, indicating that the compound was widely distributed in the body or accumulated in some tissues. Different from cinnamic acid, the $T_{1/2}$ of 6-gingerol (about 3h) was greater than the $T_{1/2}$ of cinnamic acid (about 0.5h), and the Cl_F and MRT values were higher than those of cinnamic acid, indicating that the elimination of 6-gingerol in the body was slow.

Based on the determination of the main compounds of GZD, we studied the pharmacokinetics of the main effective compounds of GZD through blood. These compounds are likely to be the basis of the pharmacodynamics of Guizhi Decoction. These studies will provide information to help clarify the biologically active ingredients and the mechanism of action of Chinese medicine prescriptions.

On the basis of compound chemical components research of GZD, we studied the major effective compounds observed in blood through the pharmacokinetics research, which were the mostly possible material basis of GZD[27]. Moreover, the research help to clarify the biologically active ingredients and mechanism of action in TCM prescriptions.

Conclusions

In this study, the HPLC-MS/MS analysis method was established for the quantification and determination of 10 compounds in GZD and 10 compounds (including 9 prototype compounds and 1 metabolites) in rat plasma. The results showed that the compounds in GZD were absorbed into the body with a first-order kinetics. At the dose of the study, the exposure of these compounds was

proportional to the dose. The absorption was rapid, as the $t_{\max}$ were about 0.1~0.2h. The results suggested that the intestinal digestion played an important role in the pharmacological effects of GZD. The active compounds of GZD might be the metabolisms, rather than the prototypes. This process might affect the pharmacodynamics effects of GZD. We expected this results would help us to reveal the pharmacodynamic material basis of GZD and provide a reference for the rational use of GZD in the clinic.

List of abbreviations

GZD: Guizhi Decoction; TCM: traditional Chinese medicine; HPLC-MS/MS: high-performance liquid chromatography-tandem mass spectrometry; LLOQ: lower limit of quantification; LLOD: lower limit of detection; PK: pharmacokinetics; MRM: multiple reaction monitoring; IS: internal standard; RSD: relative standard deviation; RE: refractive error.

Ethics approval and consent to participate

The animal study was approved by Animal Care and Use Committee of the Institute of Basic Theory for Chinese Medicine, China Academy of Chinese Medical Sciences (Date: 25 March 2019; No.: SYXK (Jing) 2019-008).

Consent to publish

All authors consent to publication of this study in Chinese Medicine.

Availability of data and materials

The datasets used in this article are available from the corresponding author upon request.

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

H.G., Q. G., D.B. and X.W. designed the research and performed the HPLC experiment; L.Z., H.G. and Q. G. performed the animal experiment; H.G., Q. G., and M.B. analyzed the data and wrote the paper. All authors read and approved the final manuscript.

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Figures

Figure 1

Chemical structures of analytes and internal standard (IS)

Figure 2

Multiple reaction monitoring mode (MRM) chromatograms of cinnamic acid (1), paeoniflorin (2), albiflorin (3), liquiritin (4), isoliquiritin (5), liquiritigenin (6), isoliquiritigenin (7), glycyrrhizic acid (8), glycyrrhetic acid (9), 6-gingerol (10), icariin (11; IS1), and aesculetin (12; IS2). blank rat serum (A), blank rat serum spiked with the analytes and ISs (B), rat serum samples at 5 min, 1 h, or 8 h after oral administration of GZD (C), and GZD after 1000 times dilution with methanol (D).

Figure 3

Mean blood concentration–time curves of the 10 analytes in rat plasma after oral administration of GZD (H: high dose, 40.0 g crude drug·kg⁻¹, M: medium dose, 20.0 g crude drug·kg⁻¹, L: low dose, 10.0 g crude drug·kg⁻¹; each point represents mean + SD, n = 6).

Figure 4

Mean blood concentration–time curves of the 9 analytes in rat plasma after intravenous injection of GZD at a dose of 2.0 g crude drug·kg⁻¹ (each point represents mean ± SD, n = 5).