LC-MS/MS determination and pharmacokinetics study of puerarin and daidzein in rat plasma after oral administration of Gegenqinlian decoction and *Radix Puerariae* extract

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ABSTRACT

Background: Gegenqinlian decoction (GQD) is a famous traditional medicine recipe. It is composed of four herbs including *Radix Puerariae* (GG), *Radix Scutellariae* (HQ), *Rhizoma Coptidis* (HL) and *Radix Glycyrrhizae* (GC), which is widely used for treating gastro-intestinal disorders in the clinical practice of Traditional Chinese medicine (TCM). The aim of this study was to compare the pharmacokinetics of puerarin and daidzein in rats following oral administration of Gegenqinlian Decoction and *Radix puerariae* extract. Thus, a sensitive and selective liquid chromatography/tandem mass spectrometry (LC-MS/MS) method was developed and validated for simultaneous determination of puerarin and daidzein in rat plasma following oral administration of Gegenqinlian Decoction and *Radix Puerariae* extract. **Materials and Methods:** Chromatographic separation was performed on a Shiseido CAPCELL PAK C_{18} analytical column (100 mm × 2.0 mm i.d., 5 μm) by linear gradient elution, with water (0.1% formic acid)-acetonitrile (0.1% formic acid) as mobile phase. Detection was carried out by multiple reaction monitoring (MRM) mode using electrospray ionization in the positive ion mode. **Results:** The calibration curves were linear over a range of 7.80-1560 ng/mL for puerarin and 6.30-1260 ng/mL for daidzein. The intra- and inter-day precision values were less than 13.6% and their average recoveries was in the range of 77.8% and 88.6% for puerarin and was between 76.3 and 86.8% for daidzein, respectively. **Conclusion:** The validated method was applied to the comparative pharmacokinetic studies of puerarin and daidzein after oral administration of Gegenqinlian Decoction and *Radix Puerariae* extract. The pharmacokinetic parameters showed that puerarin and daidzein from Gegenqinlian Decoction were absorbed more effectively with slower elimination in rat plasma than that from *Radix Puerariae* extract. These results revealed that as far as the *Radix Puerariae* extract was concerned, it is very valuable to be used as a clinical directions of Gegenqinlian Decoction.

Key words: Daidzein, gegenqinlian decoction, liquid chromatography/tandem mass spectrometry, pharmacokinetics, puerarin, *Radix Puerariae*

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INTRODUCTION

Traditional Chinese medicine (TCM) formula, which is prescribed in combinations that are aimed either to obtain synergistic effects or to reduce possible adverse reactions. Pharmacokinetic studies could help greatly in understanding and confirming the efficacy and action mechanism of drugs as well as optimizing benefit and reducing harm.[1] Therefore, it is valuable to perform pharmacokinetic studies to evaluate the rationality and compatibility of TCM formula.

Gegenqinlian Decoction (GQD) is a famous traditional medicine recipe and was first described in Shang Han Lun, a treatise on exogenous febrile diseases written by the famous Chinese physician Zhang Zhongjing (150 to 219 A.D. in the Chinese Eastern Han Dynasty). It is composed of four herbs including *Radix Puerariae lobatae, Radix Scutellariae, Rhizoma Coptidis and Radix Glycyrrhizae*. Gegenqinlian Decoction has been used to treat gastro-intestinal disorders[2] in the clinical practice of TCM with a long

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history. Recent pharmacological studies have demonstrated that Gegenqinlian Decoction has a variety of therapeutic effects. It has bacteriostatic and anti-diarrhea effect,[3] and it also has anti-inflammatory effects,[4] antipyretic[5] and anti-hyperglycemic properties.[6]

Radix Puerariae (GG) is a well-known TCM acts as the emperor herb in Gegenqinlian Decoction to treat inflammation, fever, hepatitis, allergic diseases and hypertension.[7,8] Puerarin and daidzein are the major bioactive isoflavonoids isolated from the roots of *Radix Puerariae*.[9] Puerarin is used as a phytochemical marker for the quality control of *Radix Puerariae* in the Chinese pharmacopoeia and daidzein is also a major isoflavonoid. It has been reported that recent investigations reveal that puerarin shows antioxidant and neuro-protective activities, antihyperglycemic effects.[10,11] Daidzein also shows anti-thrombotic, anti-allergic, antioxidant and anti-diabetic activities.[12,13] Their chemical structures are shown in Figure 1.

Up until now, the separation and quantification of puerarin and daidzein in *Radix Puerariae* or related preparations have been reported by using capillary electrophoresis (CE),[14] high-performance liquid chromatography (HPLC),[15-18] near infrared spectroscopy (NIRS)[19] micellar electrokinetic chromatography,[20] flow injection chemiluminescence (FIC),[21] high-performance capillary electrophoresis (HPCE)[22] etc. Nevertheless, these methods above were not sensitive enough for pharmacokinetic studies. To improve sensitivity *in vivo* studies, Liu *et al*., developed a on-line Solid Phase Extraction (SPE) coupled with ultra high performance liquid chromatography (UHPLC) with fluorescence detection method for the determination of puerarin and daidzein in human urine and serum.[23] Wang *et al.,* evaluated puerarin and daidzein in rat plasma following administration of Gegenqinlian Decoction using ultra-performance liquid chromatography mass spectrometry (UPLC–MS).[24] However, there was no comparative studies on pharmacokinetics of puerarin and daidzein on the prescription to analyze the mechanism of the combination. In this paper, a sensitive and selective method of liquid chromatography/tandem mass spectrometry (LC-MS/MS) is presented for the simultaneous determination of puerarin and daidzein in rat plasma. This assay was then applied to an inter-comparsion pharmacokinetic study of the two constituents after oral administration of Gegenqinlian Decoction and *Radix Puerariae* extract to rats.

MATERIALS AND METHODS

Materials and reagents

The reference standards of puerarin, daidzein and internal standard of naringin with a purity of over 98.0% were all obtained from the National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). Acetonitrile, methanol and formic acid of HPLC grade were purchased from Tedia Company Inc (Beijing, China). Purified water was prepared using a Milli Q-plus system (Millipore, Billerica, USA), the other reagents were of analytical grade. *Radix Puerariae lobatae*, *Radix Scutellaria*, *Radix Coptidis* and *Radix Glycyrrhizae* were all purchased from Kangqiao Medicinal Materials Electuary Co. Ltd (Shanghai, China).

Instruments and analytical conditions

The LC-MS/MS analyses were carried out with a Shimadzu liquid chromatography system and a triple quadrupole tandem mass spectrometer API 3200. The HPLC system consisted of Shimadzu liquid chromatography system (Shimadzu Corporation, Kyoto, Japan), equipped with two LC-20AD pumps, a SIL-HTC auto-sampler and an online DGU-20A3 vacuum degasser. Chromatographic separation was carried out on a Shiseido CAPCELL PAK C_{18} column (100 mm × 2.0 mm i.d., 5 μm) coupled with a Phenomenex C_{18} (4.0 mm × 3.0 mm i.d., 5 μm) guard column at room temperature. The mobile phase consisted of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B), A linear gradient at a flow rate of 0.3 mL/min B was run at 18% over 0-1 min, 18-55% over 1-3 min, 55-85% over 3-7 min and maintained at 85% for 1 min and then returned to initial condition. The samples were kept at 4°C in the auto-sampler and a volume of 10μL was injected onto the HPLC system.

Mass spectrometric detection was performed on a triple quadrupole tandem mass spectrometer API 3200 (Applied Biosystems/MDS Sciex, Toronto, Canada) equipped with a turbo ion spray source operated in the positive ionization mode. The MS operating conditions were optimized as follows: The ion spray voltage was set at 2 KV and the

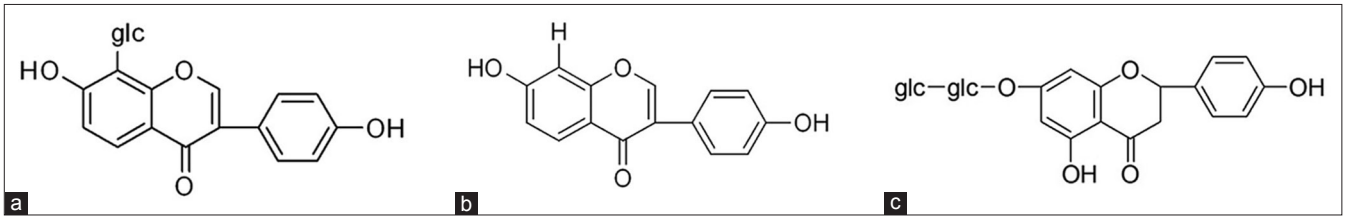

Figure 1: Chemical structures of puerarin (a), daidzein (b) and internal standard naringin (c)

source temperature was maintained at 450°C; The collision energy for puerarin, daidzein and naringin was set at 35, 35 and 23 V, respectively. Nitrogen was used as the collision gas. The flow rates of the curtain gas, nebulizer gas1 and gas2 were set at 12, 8, 12 L/min, respectively. The operation of the LC-MS/MS and data analysis were performed using the analyst 1.4 software (Applied Biosystems/MDS Sciex, Toronto, Canada).Quantification was obtained by using multiple reaction monitoring (MRM) mode of the transitions at m/z 417.1 → 296.9 for puerarin, at m/z 255.2 → 199.0 for daidzein and at m/z 581.4 → 273.0 for naringin (IS) respectively.

Preparation of Gegenqinlian decoction and *Radix Puerariae* extract

Pieces of *Radix Puerariae* 30.0 g, *Radix Scutellaria* 18.0 g, *Radix Coptidis* 18.0 g and *Radix Glycyrrhizae* 12.0 gm were mixed and decocted twice with water (800 mL and then 600 mL) for 1 h.The extracted solution was concentrated to 78 mL to obtain Gegenqinlian Decoction. *Radix Puerariae* 30.0 gm was decocted with water as the same manner above for 1h and the extracted solution was concentrated to 30 mL to obtain *Radix Puerariae* extract. To calculate the administration dose, the contents of puerarin and daidzein in Gegenqinlian Decoction and *Radix Puerariae* extract were determined by LC-MS/MS described in this paper. The contents of puerarin and daidzein were 4.86 and 0.29 mg/g in Gegenqinlian Decoction and 10.75 and 0.61 mg/gm in *Radix Puerariae* extract, respectively.

Preparation of the standard and quality control samples

Stock solutions of puerarin, daidzein and naringin (IS) were prepared by dissolving the accurately weighed reference compounds in methanol. A series of working solutions at concentrations over 7.80-1560 ng/mL for puerarin, 6.30-1260 ng/mL for daidzein and 100 ng/mL for naringin were obtained by diluting the stock solutions with the mixture of methanol and water (1:1, *v*/*v*). All solutions were stored at 4°C.

Calibration standards of puerarin (7.80, 23.4, 78.0, 312.0, 624.0, 780.0 and 1560 ng/mL) and daidzein (6.30, 18.9, 63.0, 252.0, 504.0, 630.0 and 1260 ng/mL) were prepared by spiking the blank rat plasma (100 μL) with 10 μL of standard working solutions. Quality control (QC) samples were prepared in the same way at concentrations corresponding to 23.4, 312.0 and 780.0 ng/mL for puerarin and 18.9, 252.0 and 630.0 ng/mL for daidzein.

Animals

Male Sprague-Dawley (SD) rats weighing 220-240 gm were supplied by Sino-British Sippr/BK Lab Animal Ltd. (Shanghai, China). The rats were maintained in an air-conditioned animal quarter at a temperature of 22 ± 2°C and a relative humidity of 50 ± 10%, free access to water and feeding with a laboratory rodent chow (Shanghai, China). All the experimental procedures had been approved by the University Ethics Committee for the use of experimental animals and all animal studies were carried out according to the Guide for Care and Use of Laboratory Animals.

Biosampling

Twelve rats were divided into two groups randomly ($n = 6$) and after oral administration of Gegenqinlian Decoction of 15.0 g/kg of rat weight (containing 72.90 mg/g for puerarin and 4.35 mg/g for daidzein) and *Radix Puerariae* extract at a dose of 7.0 g/kg (containing 75.25 mg/g for puerarin and 4.27 mg/g for daidzein) to rats. Blood samples (300 μL) were collected from the suborbital vein prior to dosage (0 min) and at 0.083, 0.25, 0.50, 0.75, 1.0, 2.0, 4.0, 6.0, 8.0, 12.0, 24.0 and 32.0 h, thereafter. The blood samples were immediately transferred to heparinized tubes and centrifuged at 6000 rpm for 5 min. The plasma samples obtained were stored at −20°C until analysis.

Rat plasma 100 μL was mixed with 10 μL internal standard solution (naringin, 1.03 μg/mL) 350 μL hydrochloric acid (0.5 mol/L) and 1 mL ethyl acetate in an eppendorf tube, after vortex-mixing 3 min and centrifugation for 12,000 rpm for 5 min, the supernatant was separated out and blown to dryness with nitrogen at 37°C. Then the residue was reconstituted in 100 μL mobile phase and a 10 μL aliquot of the final testing samples was injected onto the LC-MS/MS system for analysis.

Method validation

Following the bio-analytical method validation guidance (FDA, 2001), the analytical method was validated to demonstrate the specificity, linearity, precision and accuracy, recovery, matrix effects and stability of samples. Specificity was assessed by a comparison of the chromatograms from blank plasma with those obtained from the corresponding plasma spiked with puerarin, daidzein and IS and plasma samples after the oral administration of Gegenqinlian Decoction and *Radix Puerariae* extract. Linearity was obtained by plotting the measured peak area ratios of analytes to IS. Calibration curves representing peak area ratios versus analyte concentrations were described in the form of $y = a + bx$ (weighing factor $1/x$).The lower limit of quantification (LLOQ), defined as the lowest concentration at which both precision and accuracy were less than or equal to 20%, was evaluated by analyzing samples in six replicates.The intra-day and inter-day precision and accuracy were evaluated from the results of QC samples. Five replicates of QC samples at three

concentration levels (low, medium and high concentrations) were determined on the same day and on three consecutive validation days, respectively. Precision was expressed as the relative standard deviation (RSD in %) and accuracy was expressed by (mean measured concentration/spiked concentration) × 100%. The extraction recoveries of puerarin and daidzein at three QC levels were measured in a set of six replicates by comparing the peak areas of the extracted (pre-spiked) QC samples with those of the post-spiked standard plasma samples at an equivalent concentration at the same concentrations. The recovery of the IS was measured in the same way at the concentration of 100 ng/mL. The matrix effects were performed by comparing the peak areas obtained from samples where the extracted matrix was spiked with standard solutions to those obtained from the pure reference standards solutions at the same concentration. The stability of puerarin and daidzein in rat plasma was evaluated by analyzing samples at three QC concentrations under different storage conditions: In the auto-sampler at 4°C for 24 h, frozen at −70°C for 4 weeks and three freeze-thaw cycles from −70°C to 20°C, Stability was assessed by comparing the mean concentration of the three stored QC samples with that of the freshly prepared ones.

Pharmacokinetic study

Twelve male Sprague-Dawley (SD) rats were randomly divided into two groups. The animals were acclimatized to the facilities for 5 days and then fasted, free access to water for 12h prior to experiment. Six in each group: Animals in the first group were administered an oral dose of 15.0 gm/kg Gegenqinlian Decoction (72.90 mg/kg puerarin and 4.35 mg/kg daidzein) and animals in the second group were administered an oral dose of 7.0 g/kg *Radix Puerariae* extract (75.25 mg/kg puerarin and 4.27 mg/kg daidzein). Blood samples (300 μL) were obtained from the oculi chorioideae vein before dosing and subsequently at 0.083, 0.25, 0.50, 0.75, 1.0, 2.0, 4.0, 6.0, 8.0, 12.0, 24.0 and 32.0 h following administration, transferred to a heparinized eppendorf tube and centrifuged at 6000 rpm for 10 min. The plasma obtained was frozen at −70°C until analysis. The pharmacokinetic parameters were calculated using Data Acquisition Station (DAS) 2.0 software (Mathematical Pharmacology Professional Committee of China, Shanghai, China).The results were expressed as mean ± SD. The differences in pharmacokinetic parameters among groups were performed by SPSS 19.0 (Statistical Package for the Social Science) using independent samples *t*-tests. $P < 0.05$ were considered statistically significant for all the tests. Pharmacokinetic parameters including half-life ($t_{1/2}$), time of maximum plasma concentration (T_{max}), area under the curve (AUC_{0-t}), peak concentration (C_{max}) and the elimination rate (CL/F).

RESULTS AND DISCUSSION

Optimization of the mass spectrometric and chromatographic conditions

The determination of puerarin and daidzein was done with MRM detection since higher ion intensities were observed using positive relative mode Electronic Spray Ion (ESI). According to the full-scan ESI (+) mass spectra, the protonated molecule $[M + H]^+$ was observed for all of the analytes. By manual optimization using infusion with a syringe pump, the most suitable ion transitions and collision energy with MRM for all target analytes were selected. As shown in Figure 2, those precursor and product ions were m/z 417.1/296.9 for puerarin, m/z 255.2/199.0 for daidzein and m/z 581.4/273.0 for naringin (IS).

To achieve symmetric peak shape as well as a short run time for the simultaneous analysis of the three compounds, the chromatographic conditions were optimized through trials and errors. In positive ionization mode, the use of an acidic solvent in mobile phase further enhanced the response of $[M + H]^+$ ions It was found that addition of formic acid in the mobile phase provided a higher response and better peak shape. Finally, water (0.1% formic acid)-acetonitrile (0.1% formic acid) as mobile phase by linear gradient elution was employed and low background noise and proper retention time were provided.

Method validation

Specificity

Typical chromatograms obtained from a blank, a spiked plasma sample with the analytes and IS and a plasma sample after an oral dose are presented in Figure 2. The retention time of puerarin, daidzein and naringin was found to be approximately 2.30, 6.78 and 5.25 min, respectively, There was no significant chromatographic interference around the retention times of the analytes and IS. in drug-free specimens [Figure 2], indicating good resolution of the two isoflavonoids and the IS.

Linearity of calibration curves and LLOQ

The calibration curve of puerarin was y = 0.00365x + 0.00364 (r = 0.9984) and y = 0.00410x + 0.00191 (r = 0.9994) for daidzein, the concentration range of 7.80-1560 ng/mL for puerarin and 6.30-1260 ng/mL for daidzein. All calibration curves showed good linearity. LLOQ was set at 7.80 ng/mL for puerarin and 6.30 ng/mL for daidzein in rat plasma. The LLOQs are appropriate for quantitative detection of analytes in the pharmacokinetic studies.

Precision and accuracy

The intra-day and inter-day precision and accuracy data are shown in Table 1. The intra- and inter-day precision were less than 10.8 and 13.6% for puerarin and 8.6 and 12.9%

for daidzein. The accuracy ranged from 89.32 to 95.17% for puerarin and from 94.97 to 107.4% for Daidzein. The results revealed good precision and accuracy of this present method.

Recovery and matrix effect

The extraction recoveries of two analytes under the liquid-liquid extraction conditions with ethyl acetate from rat plasma were shown in Table 2. The recovery of the QC samples of three concentration levels was in the range of 77.8% and 88.6% for puerarin and was between 76.3% and 86.8% for daidzein. The recovery of the IS was 91.3 ± 5.2% at the concentration used (100 ng/mL). The observed matrix effects ranged from 88.0 to 92.4% for puerarin and from 82.2 to 96.3% for daidzein. It is suggested that the method to be considered reliable and free from the matrix effect.

Stability experiments

The stability of the analytes during the sample processing procedures and storing was evaluated by analysis three levels of QC samples. These results were summarized in Table 3.The results showed that the two analytes remained stable after 24 h in the auto-sampler at 4°C, 4 weeks storage at −70°C and three freeze-thaw cycles.

Pharmacokinetic applications

The aim of this study was to compare the pharmacokinetics of puerarin and daidzein in rats following oral administration of Gegenqinlian Decoction (GQD) and *Radix puerariae* extract. The clinical dosage of GQD is 39 g crude drug per day for human, A dose of 3.5 gm GQD (crude drug) per day for rats was corresponding to the recommended dose of 39 g GQD (crude drug) per day in clinic based on body surface area (BSA). A series of doses of 7.5, 15.0 and 30.0 g/kg/day were designed by pilot experiments to rats. As a result, with the dose of 7.5 g/kg/day after administration, the content of daidzein was too low to measure. Meanwile, the reasonable dose 15.0g/kg/day other than 30.0 g/kg/day was finally chosen in this study not only to let the rat dose more equivalent to the clinic human dose but to get the lowest dose to gain the perspective influence to compare differences on pharmacokinetic behavior.

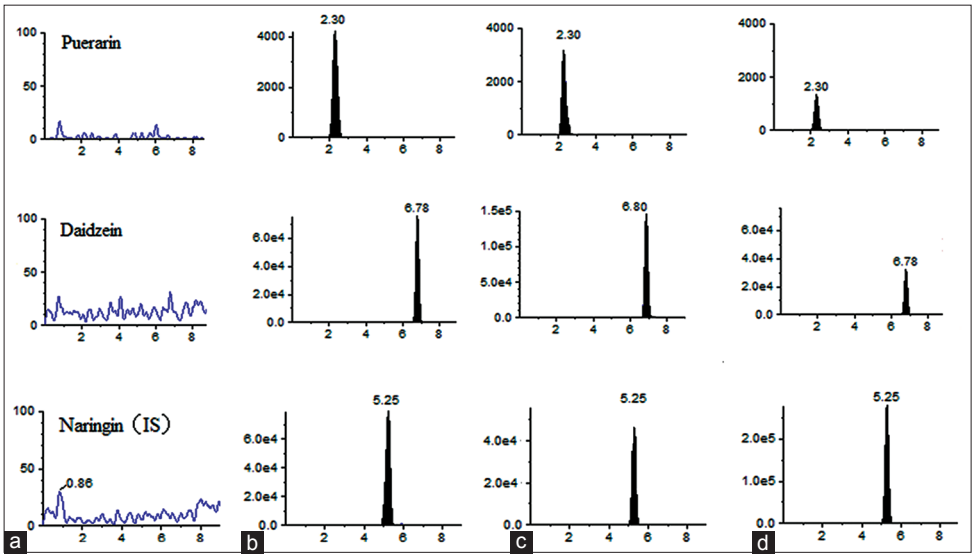

Figure 2: MRM chromatograms of the two analytes and IS in rat blank plasma (a), spiked with standard solution in blank plasma (b), plasma sample obtained 1 h after oral administration of GQD (c), plasma sample obtained 1 h after oral administration of GG extract (d)

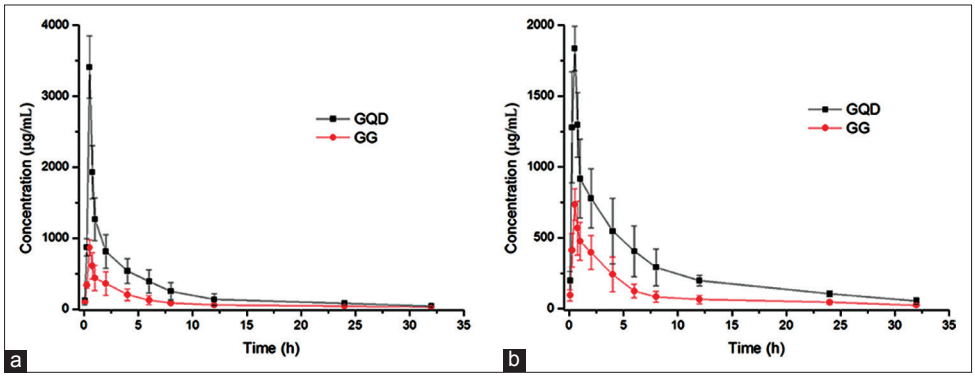

Figure 3: Plasma concentration-time profiles of puerarin (a) and daidzein (b) after the oral administration of GQD (15 gm/Kg of rat weight) and GG extract (7 gm/Kg) (*n*=6)

Table 1: Precision, accuracy of analytes in rat plasma QC samples

Analytes	Spiked concentration (ng/mL)	Intra-day (*n*=5)			Inter-day (*n*=15)		
		Concentration measured (ng/mL)	Precision (RSD,%)	Accuracy (%)	Concentration measured (ng/mL)	Precision (RSD,%)	Accuracy (%)
Puerarin	23.4	21.8±2.4	10.8	93.16	20.9±2.8	13.6	89.32
	312.0	292.0±15.2	5.2	93.58	282.8±20.5	7.2	90.65
	780.0	742.3±77.0	10.4	95.17	739.3±69.8	9.4	94.79
Daidzein	18.9	18.7±1.0	5.5	98.94	17.9±2.3	12.9	94.97
	252.0	250.8±14.2	5.7	99.52	249.6±22.0	8.8	99.05
	630.0	613.3±52.8	8.6	97.35	676.7±51.6	7.6	107.4

QC: Quality control

Table 2: Extraction recovery and matrix effect of rat plasma

Analytes	Spiked concentration (ng/mL)	Recovery (%, mean±SD, *n*=6)	Matrix effect (%, mean±SD, *n*=6)
Puerarin	23.4	85.8±7.4	92.4±9.8
	312.0	77.8±3.1	90.3±5.5
	780.0	88.6±4.7	88.0±4.2
Daidzein	18.9	86.8±3.0	89.8±5.2
	252.0	81.6±2.9	82.2±6.9
	630.0	76.3±4.7	96.3±6.0

The validated method was successfully applied to the pharmacokinetic study of puerarin and daidzein in rat plasma after oral administration of Gegenqinlian Decoction and *Radix Puerariae* extract. The mean concentration time curves in the two treatments are shown in Figure 3 and the pharmacokinetic parameters are summarized in Table 4. Both the concentration time curves of puerarin and daidzein were adequately described by a two-compartment open model which were consistent with the literature.[24] That these two analytes had the similar pharmacokinetic behavior could be tentatively explained based on their similar structures. These two analytes exhibited consistent tendencies in plasma concentration–time profiles and similar T_{max} values after oral administration of either GQD or GG extract.

In this paper, we found that the T_{max} and $t_{1/2\alpha}$ values of neither puerarin nor daidzein had significant differences when orally administering Gegenqinlian Decoction and *Radix Puerariae* extract [Table 4]. However, as shown in Table 4, some other pharmacokinetic parameters (AUC_{0-t}, C_{max} and CL/F) were obviously different at almost the same dosages of puerarin or daidzein in the two preparations. The AUC_{0-t} of puerarin and daidzein after oral administration of Gegenqinlian Decoction (GQD) group was about 1.5, 1.2 fold higher than that of puerarin of oral administration of *Radix Puerariae* extract (GG) group. After oral administration of GQD, puerarin and daidzein were absorbed with C_{max} being 4.46 ± 0.48, 1.84 ± 0.16 mg/mL respectively and after oral administration of GG group, the absorption of puerarin and daidzein was sharply decreased with the C_{max} being 0.84 ± 0.21, 0.73 ± 0.11 mg/mL for both. Interestingly, however, Compared with the elimination rate (CL/F) of puerarin (19.76 ± 2.75 L/h/kg) and daidzein (7.34 ± 1.07 L/h/kg) after oral administration of GQD group, a remarkably increased CL/F of puerarin and daidzein (26.54 ± 4.06 L/h/kg for puerarin and 8.21 ± 0.55 L/h/kg for daidzein) was observed after oral administration of GG group. Therefore, it can be speculated that the slower elimination rate was a greater contribution to the improvement of the bioavailability of these compounds in GQD. In addition, a remarkable increase in the $t_{1/2\beta}$ values of puerarin was observed in the GQD group compared with the GG group, there was no obviously difference in the $t_{1/2\beta}$ values of daidzein. According to the pharmacokinetic results mentioned above, it can be inferred that the bioavailability of GQD group is better than the GG group, indicating that the coexisting constituents of other ingredients might accelerate the absorption rate of puerarin and daidzein from the gastrointestinal tract. The difference in the two treatment groups was significant ($P < 0.05$ or $P < 0.01$) by unpaired Student's *t*-test, suggesting that drug interactions occurred in this compound prescription formula, which possibly increased the absorption of puerarin and daidzein and accelerated the elimination of puerarin and daidzein in rats, it needs further clarification. These results were in agreement with those observed in our previous studies[25,26] on the effect of the intestinal absorption of puerarin and daidzein in GQD by using averted the rat gut sac model and Caco-2 cell monolayer model which verified that co-administration of other drugs in GQD could significantly elevate the intestinal absorption of puerarin in comparison with that of *Radix puerariae* alone. The pharmacokinetic parameters of puerarin and daidzein determined in the present study may imply that compound TCM prescriptions may be effective than a single Chinese medicine. Possible compound-compound interactions in GQD should be studied to further elucidate the pharmacokinetic differences between GQD and GG extracts for clinical applications.

Table 3: Stability of analytes in rat plasma

Analytes	Spiked concentration (ng/mL)	Samples in 4°C for 24 h		Samples in −70°C for 4 weeks		Three circles' Freeze-thaw	
		Measured concentration (ng/mL)	Accuracy (%)	Measured concentration (ng/mL)	Accuracy (%)	Measured concentration (ng/mL)	Accuracy (%)
Puerarin	23.4	22.8±2.3	97.22	23.0±2.3	98.29	21.6±1.6	92.52
	312.0	291.2±21.3	93.33	282.8±20.5	90.65	299.7±24.5	95.73
	780.0	754.3±59.8	96.71	754.5±59.6	96.73	747.7±55.4	96.05
Daidzein	18.9	19.23±1.1	101.8	17.9±2.3	94.97	18.1±1.4	95.73
	252.0	247.5±19.5	98.39	249.7±22.0	99.07	246.5±25.5	97.82
	630.0	687.7±45.0	109.2	676.7±51.6	107.4	670.0±35.6	106.35

Table 4: The pharmacokinetic data for puerarin and daidzein after oral administration of GQD (15 g/Kg of rat weight) and GG extract (7 g/Kg of rat weight) (mean±SD, *n*=6)

Parameter	Puerarin		Daidzein	
	GQD	GG	GQD	GG
$t_{1/2\alpha}$(h)	4.18±0.92	3.82±0.59	0.43±0.18	0.40±0.23
$t_{1/2\beta}$(h)	12.44±1.35**	7.95±2.65	4.66±1.72	3.83±1.23
AUC_{0-t} (mg h/L)	6.83±0.88**	4.45±0.76	2.81±0.34*	2.24±0.21
C_{max} (mg/L)	4.46±0.48**	0.84±0.21	1.84±0.16**	0.73±0.11
T_{max} (h)	0.55±0.26	0.48±0.20	0.56±0.14	0.52±0.03
CL (L/h/kg)	19.76±2.75*	26.54±4.06	7.34±1.07*	8.21±0.55

GQD: Gegenqinlian decoction; GG: Radix puerariae; *P<0.05, ** P<0.01 compared with GG group

CONCLUSION

A LC-MS/MS method was developed and validated for simultaneous determination of puerarin and daidzein in rat plasma after oral administration of Gegenqinlian Decoction (GQD) and *Radix Puerariae* (GG) extract. The method was successfully applied to comparative pharmacokinetic study of the two analytes in rat plasma after oral administration of GQD and GG extract. The results of this study showed that the pharmacokinetic parameters of the two analytes of AUC_{0-t}, C_{max} and CL/F were quite different between GQD and GG extract, the pharmacokinetic parameters showed that puerarin and daidzein from GQD were absorbed more effectively with slower elimination in rat plasma than that from GG extract. Furthermore, these results revealed that as far as the GG extract was concerned, it is very valuable to be used as a clinical directions of GQD. The pharmacokinetic results may be useful for further study of the mechanism of GQD formula.

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