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Simultaneous determination of puerarin, daidzein, baicalin, wogonoside and liquiritin of GegenQinlian decoction in rat plasma by ultra-performance liquid chromatography–mass spectrometry

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ABSTRACT

A specific ultra-performance liquid chromatography–mass spectrometry (UPLC–MS) method was developed for the simultaneous determination of puerarin, daidzein, baicalin, wogonoside and liquiritin in rat plasma. Chromatographic separation was performed on a C_{18} column packed with 1.7 μm particles by a linear gradient elution. The analytes and carbamazepine (internal standard, I.S.) were monitored in a selected-ion reaction (SIR) mode with a positive electrospray ionization (ESI) interface by the following ions: m/z 417.2 for puerarin, m/z 255.2 for daidzein, m/z 271.0 for baicalin, m/z 461.0 for wogonoside, m/z 441.0 for liquiritin and m/z 237.2 for carbamazepine (I.S.), respectively. The calibration curves of these analytes were linear over the concentration ranges from 0.00254–1.02 $\mu g\,mL^{-1}$ to 0.0102–10.2 $\mu g\,mL^{-1}$. Within-batch and between-batch precisions (RSD%) were all within 15% and accuracy (RE%) ranged from −10% to 10%. The extraction recoveries were on average 79.8% for puerarin, 90.8% for daidzein, 74.4% for baicalin, 70.2% for wogonoside and 84.7% for liquiritin. The validated method was successfully applied to investigate the pharmacokinetics of five bioactive compounds of GegenQinlian decoction (GQD) in rats.

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1. Introduction

GegenQinlian decoction (GQD), one of the well-known traditional Chinese formulas, originally came from "Treatise on Febrile Diseases" compiled by Zhong-Jing Zhang. And this prescription, composed of *Radix Puerariae lobatae*, *Radix Scutellariae*, *Rhizoma Coptidis* and *Radix Glycyrrhizae*, is now commonly utilized to cure virus diarrhea, bacillary dysentery, general fever and dipsesis in clinic. But more and more attention is paid on its other effects, such as anti-hypoxia, spasmolysis, anti-arrhythmia and immunoenhancement activities, in modern pharmacological studies [1]. Isoflavonoids and flavonoids from GQD are regarded as potential contributors to those pharmacological effects. Puerarin and daidzein, two bioactive isoflavonoids in *Puerariae Radix* [2], have been reported to display antithrombotic, anti-allergic [3], anti-oxidative [4] and potential antidiabetic properties [5]. Baicalin and wogonoside, primary flavonoids of *Radix Scutellaria* [6,7], share some beneficial activities with isoflavonoids such as anti-allergic and anti-oxidative [8,9] properties. Furthermore, anti-inflammatory and hepatoprotective activities [10,11] of the flavonoids were also investigated. And the latest pharmacological studies indicated that liquiritin is an effective flavonoid of *Radix Glycyrrhizae* with neuroprotective effect [12,13].

Up to now, no analytical method has been reported for the simultaneous determination of isoflavonoids and flavonoids from GQD. Therefore, a selective and sensitive analytical method for simultaneous quantification and pharmacokinetic studies of puerarin, daidzein, baicalin, wogonoside and liquiritin in plasma is required to build up in order to improve the understanding toward the relationship of pharmacokinetics, pharmacology and bioavailability of multiple components, which have influences on the clinical effects of TCM and its rational dosage regiments. Some of the absorption, distribution, metabolism and excretion studies of these components have been carried out in animals and human [14–20]. And several analytical methods, such as fluorophotometry [21], high-performance liquid chromatography with ultraviolet detection (HPLC–UV), relative fluorescence (HPLC–RF), electrochemical detection (HPLC–ECD) [22–29], high-performance liquid chromatography coupled with mass spectrometry (LC–MS) or tandem mass spectrometry (LC–MS/MS) [30–33,15,35], have also been reported to quantify some of the five ingredients mentioned above in vivo. Besides, an ultra-performance liquid chromatography – tandem mass spectrometry (UPLC–MS/MS) method was also applied to the quantitative analysis of baicalin and chlorogenic acid in human plasma [36].

Currently, UPLC as a new liquid chromatographic technique has made a considerable contribution in analysis area. The high

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flow rate, resolution, peak capacity and sensitivity are special features of this new generation of LC columns packed with pressure stable 1.7 μm hybrid material particles and novel low dead volume, high pressure (1000 bar) LC equipment [37]. It can be conveniently coupled to mass spectrometry detectors, providing good advantages for the analysis of nonvolatile compounds [38].

Till now, no reports were found on simultaneous determination of puerarin, daidzein, baicalin, wogonoside and liquiritin in plasma after oral administration of GQD by UPLC–MS. In this paper, we presented a validated method for simultaneous determination of the five compounds, including puerarin, daidzein, baicalin, wogonoside and liquiritin (Fig. 1) in rat plasma based on UPLC–MS.

2. Experimental

2.1. Chemical and reagents

The reference standards of puerarin, daidzein, baicalin, wogonoside, liquiritin, and carbamazepine (I.S.) with a purity of over 98% were purchased from National Institute for the Control of Pharmaceutical and Biological Products of China (Beijing, China). Methanol, formic acid and ammonium acetate (HPLC grade) were bought from Merck (Shanghai, PR China). De-ionized water used throughout the experiments was purified by a Milli-Q academic water purification system (Millipore, Bedford, MA, USA). And all other chemicals were of analytical grade.

Radix Puerariae lobatae, *Radix Scutellaria*, *Radix Coptidis* and *Radix Glycyrrhizae* were all provided by Kangqiao Medicinal Materials Electuary Co. Ltd (Shanghai, PR China), and the GQD decoction was prepared following the traditional craft with a final concentration of 1 g mL^{-1}. For oral administration, the concentrations of puerarin, daidzein, baicalin, wogonoside and liquiritin were determined by UPLC–MS described in this paper.

2.2. UPLC–MS conditions

Chromatography was performed on an ACQUITY^{TM} UPLC system (Waters Corp., Milford, MA, USA) with a conditioned auto-sampler at 4 °C. The separation was carried out on an ACQUITY UPLC^{TM} BEH C_{18} column (100 mm × 2.1 mm i.d., 1.7 μm; Waters Corp., Milford, MA, USA) maintained at 30 °C. Mobile phase component A was methanol and B was water (containing 0.1% formic acid and 5 mmol L^{-1} ammonium acetate), and the flow rate was set at 0.2 mL min^{-1}. The column was eluted with a linear gradient of 35–45% A over 0–1 min, 45–55% A over 1–6 min, and the composition was maintained 55% A for 0.5 min and then returned to the initial condition. The injection volume was set at 5 μL, partial loop mode was applied for sample injection.

Mass spectrometric detection was carried out on a Micromass® Quattro Ultima^{TM} PT mass spectrometer (Waters Corp, Milford, MA, USA) with an electrospray ionization (ESI) interface. The ESI source was operated in positive ionization mode. Quantifications were performed in multiple channels in selected ion monitoring (SIM) mode using target ions at $[M+H]^+$ m/z 417.2 for puerarin, 255.2 for daidzein, 461.0 for wogonoside, $[M\text{-gluc}+H]^+$ m/z 271.0 for baicalin, $[M+Na]^+$ m/z 441.0 for liquiritin and $[M+H]^+$ m/z 237.2 for carbamazepine (I.S.), respectively. The optimal MS parameters obtained were as follows: capillary 3.0 kV, source temperature 120 °C and desolvation temperature 300 °C. The flow rate of desolvation gas and cone gas were 500 L h^{-1} and 10 L h^{-1}, respectively. Argon was used as the collision gas, and the pressure of which was approximately 2.76×10^{-3} mbar. Collision energies were 45 eV for daidzein and liquiritin, 40 eV for baicalin and puerarin, 30 eV for wogonoside and carbamazepine (I.S.), respectively. All data collected in centroid mode were processed using MassLynx^{TM} NT 4.0 software with a QuanLynx^{TM} program (Waters Corp., Milford, MA, USA).

2.3. Preparation of standard and quality control samples

Puerarin, daidzein, baicalin, wogonoside and liquiritin were all dissolved together in methanol for the final concentrations of 98.4 μg mL^{-1}, 50.8 μg mL^{-1}, 96.0 μg mL^{-1}, 102.0 μg mL^{-1} and 51.2 μg mL^{-1}, respectively. A series of working solutions of these analytes were obtained by further diluting the stock solution with methanol. The I.S. solution was prepared to the concentration of 8.0 μg mL^{-1} with the mixture of methanol and water (1:1, v/v). All solutions were stored at 4 °C.

The samples for standard calibration curves were prepared by spiking the blank rat plasma (100 μL) with 10 μL of standard working solutions. The plasma concentration ranges were 0.00984–2.46 μg mL^{-1} for puerarin, 0.00254–1.02 μg mL^{-1} for daidzein, 0.00960–4.80 μg mL^{-1} for baicalin, 0.0102–10.2 μg·mL^{-1} for wogonoside and 0.0102–1.02 μg mL^{-1} for liquiritin, respectively. For the validation of the method, three concentrations of quality control (QC) samples were prepared containing puerarin (0.0246, 0.0984, 0.984 μg mL^{-1}), daidzein (0.00508, 0.0508, 5.08 μg mL^{-1}), baicalin (0.0192, 0.192, 1.92 μg mL^{-1}), wogonoside (0.0204, 0.510, 5.10 μg mL^{-1}) and liquiritin (0.0256, 0.102, 0.512 μg mL^{-1}) in the same manner.

2.4. Sample preparation

After plasma samples of 100 μL were transferred into polypropylene tubes, 10 μL of carbamazepine solution (8.0 μg mL^{-1}), 200 μL of methanol containing 5% formic acid and 10 μL of 10% ascorbic acid were added and vortex-mixed vigorously for 2 min. Then the mixtures were extracted with 3 mL of ethyl acetate by vortex-mixing for 4 min. After centrifugation at 5000 rpm for 10 min at −20 °C the organic layer was pipette-transferred and evaporated to dryness using nitrogen gas at 37 °C in a water bath. To dilute the plasma samples obtained from high concentration levels, a 500 μL of mobile phase solution was added to the dry plasma extraction and a 100 μL aliquot solution was used to dissolve other samples. The reconstituted extraction was thoroughly mixed, and then centrifuged again at 12,000 rpm for 10 min. Only 5 μL aliquots was injected into the UPLC–MS system in partial loop mode.

2.5. Method validation

2.5.1. Specificity

The specificity of the method was investigated by screening analysis of six individual rat blank plasma samples. The chromatogram of each blank plasma sample was compared with the spiked rat plasma. Two other blank plasma samples containing I.S. (8.0 μg mL^{-1}) were prepared and checked for the interference. As a result, no significant endogenous interference was observed in the blank rat plasma.

2.5.2. Linearity and lower limits of quantification (LLOQ)

The linear relationship of the method was evaluated by preparing eight different concentrations of samples in plasma using the previous extraction procedure. In addition, blank plasma samples were analyzed to confirm absence of the interference of endogenous. The calibration curves were established by plotting peak area ratios of the five constituents to the I.S., versus the respective standard concentration. Eight non-zero standards in the calibration curve should meet the following criteria: not more than 20% deviation around LLOQ and not more than 15% deviation for standards above the LLOQ. The contents of the five constituents in the test samples were calculated using the regression parameters obtained

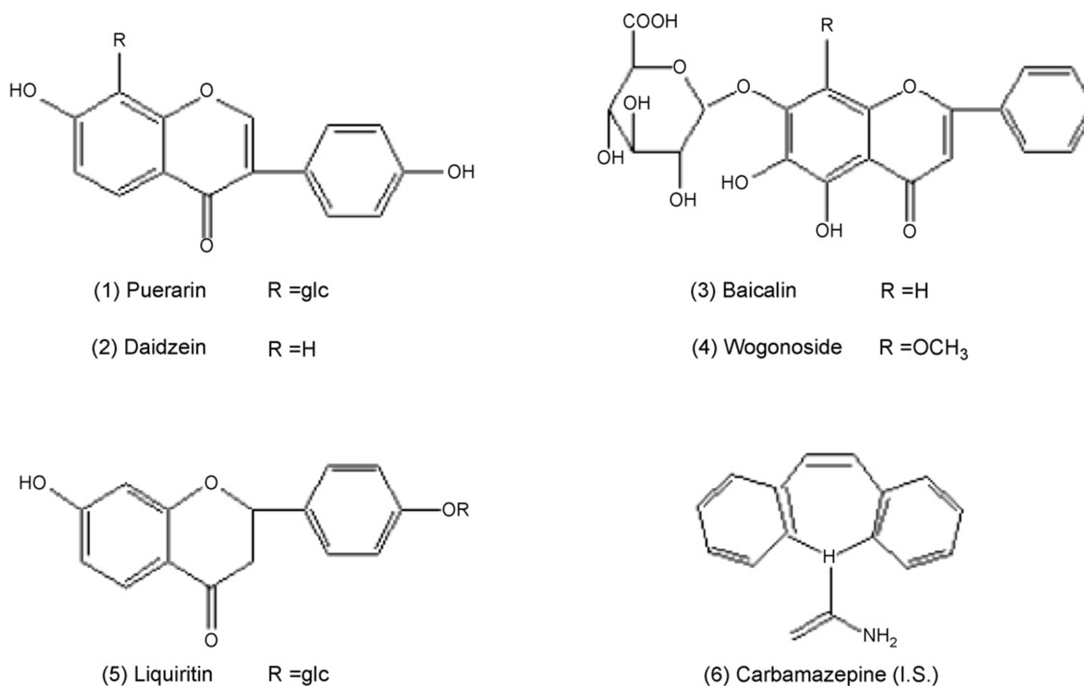

Fig. 1. Structures of the investigated compounds and internal standard carbamazepine. (1) Puerarin, (2) daidzein, (3) baicalin, (4) wogonoside, (5) liquiritin and (6) carbamazepine (I.S.).

from the standard curve. In addition, concentrations of the diluted plasma samples should be multiplied by 5 after calculation.

The lower limit of quantification (LLOQ) was defined as the lowest concentration giving a signal-to-noise ratio of at least 10-fold with acceptable accuracy within 20% deviation of the nominal concentration and precision below 20%.

2.5.3. Precision and accuracy

The within-batch and between-batch precision and accuracy were investigated by determining QC samples at three different concentrations (six replicates for each concentration level) over 5 consecutive days. The concentrations were calculated using calibration curves obtained daily. The precision of the method at each QC concentration was expressed as the relative standard deviation (RSD) and the accuracy was described as relative error (RE). The suitability of the precision and accuracy was assessed by the following criteria: the RSD should not exceed 15% and the accuracy should be within 15% of the actual values for QC samples.

2.5.4. Recovery and matrix effect

The extraction recovery were determined at three concentrations levels by comparing the analyte peak areas of six extracted QC samples with those obtained from the corresponding pure reference standards prepared at the same concentrations of five replicates.

The matrix effect was evaluated by comparing the peak area ratio of post-extraction control plasma spiked with standard solutions of analytes and I.S. solution (part 1) to that of after-extraction blank plasma obtained from five rats spiked with the neat standard solutions of analytes and I.S. solution (part 2) at three QC levels. When the peak area ratio of analytes and I.S. solution in part 1 to that of in part 2 were within 15%, the matrix effect may be considered as negligible.

2.5.5. Stability

The stability of QC samples was also investigated at three concentration levels, including (a) stability of the extracted samples at room temperature for 24 h, (b) stability after three freeze-thaw cycles with the frozen temperature of $-20\,^{\circ}C$ and thawing temperature of $25\,^{\circ}C$, (c) stability of plasma samples at $-20\,^{\circ}C$ for 10 days, and (d) stability of plasma samples at room temperature for 4 h. In addition, stock solution stability at $4\,^{\circ}C$ for 1 month was also assessed.

2.6. Application of the assay to pharmacokinetic studies

Six male Sprague-Dawley rats, weighing 180–220 g, were supplied by Laboratory Animal Center of the Shanghai University of TCM. The rats were kept in an air-conditioned animal quarter at a temperature of 22–24 °C and a relative humidity of $50 \pm 10\%$, and had access to the standard laboratory food and water. After fasted for 12 h, the rats were given GQD (in which the concentrations of puerarin, daidzein, baicalin, wogonoside and liquiritin were 15.2, 0.312, 30.7, 5.79, 0.395 $mg\,mL^{-1}$) at a dose of $10\,mL\,kg^{-1}$ by oral administration. Blood samples (0.2 mL) were collected from the suborbital veniplex before intragastric gavage and after that at 0.083, 0.25, 0.50, 0.75, 1.0, 2.0, 4.0, 8.0, 12.0, 24.0 and 32.0 h, transferred to heparinized tubes, and centrifuged at 6000 rpm for 5 min to separate out plasma. The plasma was then transferred to clean tubes and stored at $-20\,^{\circ}C$ for preservation. Animal experiments were carried out in accordance with the local institutional guidelines for animal care of Shanghai University of TCM.

3. Results and discussion

3.1. Optimization of UPLC–MS

Chromatographic separation of the five analytes of interest was realized via gradient elution using a mobile phase of methanol (A) and $5\,mmol\,L^{-1}$ ammonium acetate and 0.1% formic acid in water (B) within 7.0 min. The addition of a small amount of formic acid to the mobile phase proved to have positive effects on the sensitivity of the analytes in positive ion mode of the UPLC–MS, and ammonium acetate was to improve the chromatographic peak symmetry

of the detected compounds. The additives in mobile phase were also helped to decrease the interference by matrix effects [34]. Due to higher efficiency and linear velocities, the use of small particles of stationary phase allowed UPLC to push the limits of both peak capacity and speed of analysis without compromising resolution [39]. However, the chromatographic column packed with 1.7 μm particles was more easily blocked than the common one in HPLC system. Therefore, the sample extract procedure is very important. Gradient elution was, furthermore, used to provide a better peak shape and prolong the column life.

MS examination of the analytes and I.S. standard solutions in positive and negative ionization modes by direct full scan method revealed that the signals obtained from the electrospray ionization source in positive mode had a good resolution and high intensity to permit quantitative measurement. The electrospray ionization of puerarin and daidzein produced the abundant protonated molecular ions ($[M+H]^+$) at *m*/*z* 417.2 and 255.2 using different collision energies of 40 eV and 45 eV. As it is easily for baicalin to lose a molecular of glucuronic acid, we chose a fragment ion $[M\text{-gluc}+H]^+$ at *m*/*z* 271.0 as the detected one and $[M+H]^+$ at *m*/*z* 461.2 for wogonoside. The collision energies of 40 and 30 eV were set for obtaining the two ions. In addition, the predominantly protonated molecules $[M+Na]^+$ and $[M+H]^+$ at *m*/*z* 441.0 and 237.2 collided with the energies of 45 and 30 eV, were selected for analyze liquiritin and carbamazepine (I.S.), respectively. Each mass spectrum of detective ions was shown in Fig. 2. In our studies, cross-talk effect produced by using different channels in mass system was not observed.

3.2. Optimization of the extraction procedure

Protein precipitation and liquid–liquid extraction method were first carried out during sample preparation. It was much simpler and less time-consuming for protein precipitation, but matrix effects could not be reduced because of the presence of co-eluting compounds and the sensitivities of puerarin, baicalin, wogonoside and liquiritin were not satisfactory (LLOQs > 0.10 μg mL^{-1}) due to a high demand for precipitant. Liquid–liquid extraction concentrated the plasma samples, thus offered higher sensitivity than protein precipitation, but analytes (especially to liquiritin) were interfered by endogenous substances. Moreover, this method often caused column clogging and needed frequent cleaning of MS source. Fortunately, when these two methods were combined together, results showed that plasma samples were separated thoroughly from interferences with sufficient sensitivities.

To improve the recoveries of the five analytes, several organic solvents were compared for the two-step extraction method. For precipitation, methanol, acetonitrile and their mixture in different ratios were investigated. Methanol was eventually proved to be the best precipitant among the mentioned solutions in terms of the higher extraction recovery for all analytes. For liquid–liquid extraction, ethyl acetate, ethyl ether, and their mixture in different ratios were compared. Results showed that ethyl acetate was the best extraction solvent with absolute recoveries of the five analytes and I.S. at least above 60% and no interferences were found at the retention times of analytes and I.S. Thus, ethyl acetate appeared to be optimal. In addition, the recoveries of applying 3 mL of ethyl acetate for extraction almost doubled that of using 1 mL of extraction solvent and were similar to the use of 4 mL. Considering the recovery efficiency and consuming time of blow-drying samples, 3 mL of ethyl acetate was employed. Based on these results, we established the sample extraction procedure as described in Section 2.4.

3.3. Method validation

3.3.1. Specificity

The specificity of the method was tested by analysis of blank plasma samples from six different rats. There was no significant chromatographic interference around the retention times of the analytes and I.S. in drug-free specimens (Fig. 3). The retention times

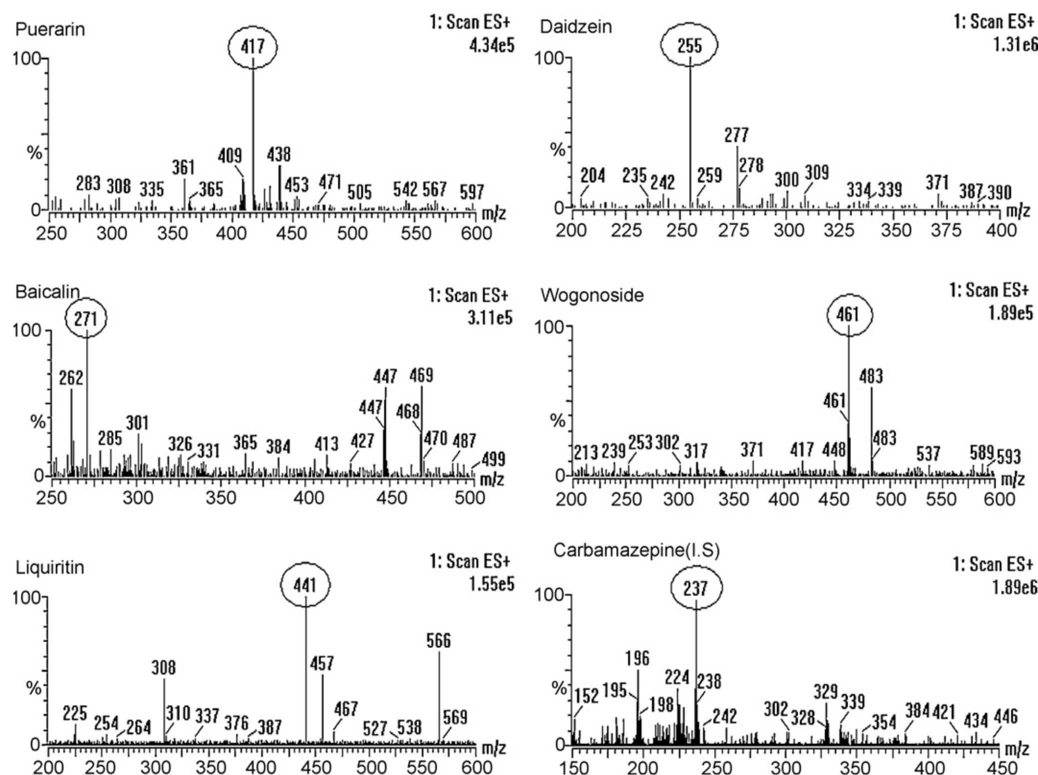

Fig. 2. Positive ion electrospray mass scan spectra of puerarin, daidzein, baicalin, wogonoside, liquiritin and carbamazepine (I.S.).

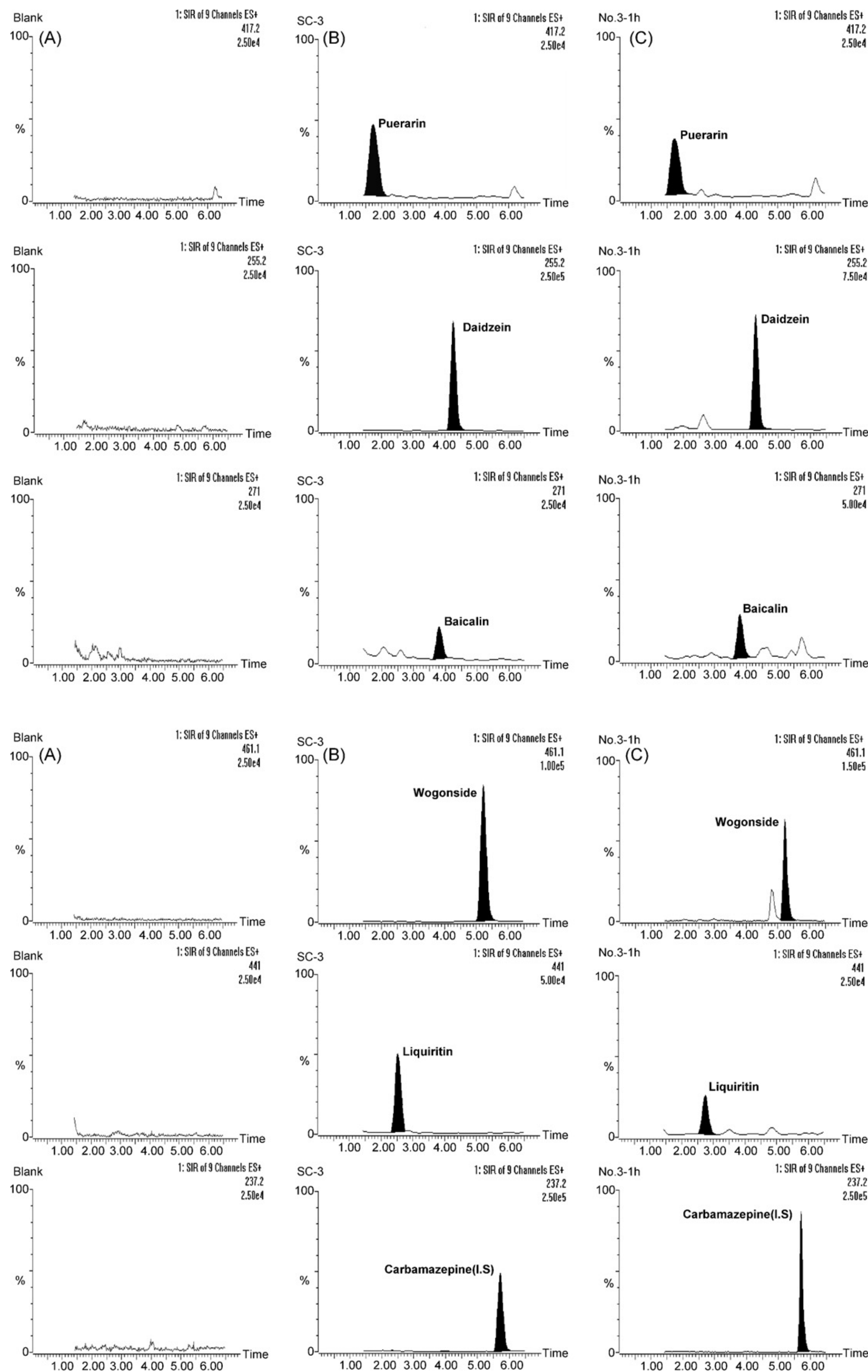

Fig. 3. Chromatograms of the five compounds in plasma (A) blank plasma, (B) blank plasma spiked with the five components and I.S. and (C) plasma sample obtained 1 h after intragastric administration of GQD (10 mL kg^{-1}).

Table 1
Regression data and LLOQs of the multi-components determined.

Components	Linear range ($\mu g\,mL^{-1}$)	Linear regression equation	Correlation coefficient (r)	LLOQ ($ng\,mL^{-1}$)
Puerarin	0.00984–2.46	$y = 0.1422x - 0.0005$	0.998	9.84
Daidzein	0.00254–1.02	$y = 1.4293x + 0.0133$	0.999	2.54
Baicalin	0.00960–4.80	$y = 0.0224x + 0.0001$	0.998	9.60
Wogonoside	0.0102–10.2	$y = 0.3263x + 0.003$	0.999	10.2
Liquiritin	0.0102–1.02	$y = 0.1554x - 0.0012$	0.995	10.2

Table 2
Precision, accuracy, and extraction recoveries of puerarin, daidzein, baicalin, wogonoside and liquiritin for UPLC–MS method.

Components	Concentration added ($\mu g\,mL^{-1}$)	Within-batch concentration measured ($\mu g\,mL^{-1}$)	Precision (%, RSD)	Accuracy (%, RE)	Between-batch concentration measured ($\mu g\,mL^{-1}$)	Precision (%, RSD)	Accuracy (%, RE)	Extraction recovery (%)
Puerarin	0.0246	0.0238 ± 0.0026	11	−3.2	0.0231 ± 0.0020	8.9	−4.9	79.0 ± 4.0
	0.0984	0.0959 ± 0.0081	8.4	−2.5	0.0964 ± 0.0098	9.1	−2.0	80.2 ± 6.3
	0.984	1.01 ± 0.045	4.5	3.1	0.957 ± 0.061	6.1	−1.2	80.3 ± 3.4
Daidzein	0.00508	0.00513 ± 0.00022	4.2	1.0	0.00514 ± 0.00035	5.9	1.1	90.9 ± 6.5
	0.0508	0.0518 ± 0.0046	8.8	2.1	0.0471 ± 0.0041	9.1	−4.6	92.1 ± 4.1
	0.508	0.504 ± 0.036	7.1	−0.73	0.495 ± 0.044	8.0	−1.9	89.5 ± 5.1
Baicalin	0.0192	0.0191 ± 0.0017	8.7	−0.67	0.0193 ± 0.0015	7.3	0.28	69.4 ± 8.2
	0.192	0.191 ± 0.0061	3.2	−0.73	0.183 ± 0.0.018	8.1	−3.3	78.6 ± 3.2
	1.92	1.85 ± 0.13	7.1	−3.9	1.93 ± 0.12	6.3	−0.52	75.3 ± 6.2
Wogonoside	0.0204	0.0188 ± 0.0011	5.9	−7.6	0.0199 ± 0.00080	4.9	−3.3	66.9 ± 4.6
	0.510	0.496 ± 0.016	3.2	−2.7	0.556 ± 0.047	8.8	5.5	70.7 ± 2.3
	5.10	5.12 ± 0.23	4.4	0.37	5.14 ± 0.24	4.3	0.62	72.9 ± 1.7
Liquiritin	0.0256	0.0266 ± 0.0021	8.0	4.1	0.0235 ± 0.0011	7.7	−4.6	79.4 ± 6.5
	0.102	0.106 ± 0.0070	6.7	3.4	0.104 ± 0.0055	9.2	0.99	85.4 ± 5.6
	0.512	0.517 ± 0.030	5.8	1.1	0.504 ± 0.017	4.0	−0.83	89.2 ± 3.8

of puerarin, daidzein, baicalin, wogonoside, liquiritin and I.S. were 1.7, 4.3, 3.9, 5.2, 2.8 and 5.8 min, respectively.

3.3.2. Linearity and LLOQs

All standard curves exhibited good linearity with coefficients of correlation (r) within the range 0.995–0.999. The LLOQs (chromatographic spectra were shown in Fig. 3) are appropriate for quantitative detection of analytes in the pharmacokinetic studies. Linear ranges, regression equations, LLOQs, and correlation coefficients obtained from typical calibration curves were shown in Table 1.

3.3.3. Precision and accuracy

The within-batch and between-batch precisions were ranged from 3.2% to 11.4% and 4.0% to 9.2%, respectively, and the accuracy, ranged from −7.6% to 5.5%, was calculated from the nominal concentration (C_{nom}) and the mean value of the observed concentration (C_{obs}) as follows: $RE\,(\%) = [(C_{obs} - C_{nom})/(C_{nom})] \times 100$. Data of assay precision and accuracy are listed in Table 2.

3.3.4. Extraction recovery and matrix effect

The extraction recoveries determined for puerarin, daidzein, baicalin, wogonoside and liquiritin were shown in Table 3. At three concentration levels of the five analytes, the absolute extraction recoveries were all in the range from 66.9% to 92.1%. The extraction recovery of the I.S. was more than 85%. These results demonstrated that the values were all in the acceptable ranges.

The RSDs of the ratio of analytes/I.S. for standards spiked post-extraction into extracts from five different lots of plasma were in the range of 7.6–12.3%. The specific values of analytes/I.S. of post-extraction control plasma spiked with standard solutions to that of after-extraction blank plasma with the neat standard solutions were not <75% and >120%. These results confirmed that the evaluated method was free from any matrix effect.

3.3.5. Stability

The stability tests of puerarin, daidzein, baicalin, wogonoside and liquiritin were designed to cover anticipated conditions that plasma samples might experience in this study. The QC samples prepared in rat plasma, after undergoing three freeze-thaw cycles, showed no significant degradation (RE: −9.8% to 5.3%, RSD < 10.5%). In the extracts, the five analytes were stable for up to 24 h without any significant degradation (RE: −9.1% to 3.6%, RSD < 9.1%). After storage at −20 °C, the concentrations of the five analytes in plasma showed that no obvious substance loss during 10 days (RE: −8.2% to 2.3%, RSD < 9.8%). In addition, they were stable before extracted and stored at room temperature for 4 h (RE: −7.5% to 2.9%, RSD < 8.6%). Stock solutions of these analytes in methanol were stable at 4 °C for up to 30 days.

Table 3
Phamacokinetic parameters of puerarin, daidzein, baicalin, wogonoside and liquiritin after i.g. of GQD ($10\,mL\,kg^{-1}$) to rats ($X \pm SD$, $n = 6$).

Parameter	Puerarin	Daidzein	Baicalin	Wogonoside	Liquiritin
$t_{1/2}{}^{\alpha}$ (h)	4.06 ± 2.10	0.32 ± 0.19	–	–	–
$t_{1/2}{}^{\beta}$ (h)	11.37 ± 6.66	4.34 ± 1.22	–	–	–
$t_{1/2}{}^{z}$ (h)	–	–	9.15 ± 5.80	8.58 ± 4.34	–
AUC_{0-t} ($h\,mg\,L^{-1}$)	6.01 ± 2.56	2.43 ± 1.21	67.81 ± 22.91	25.07 ± 7.94	–
$t_{max}{}^{1}$ (h)	0.33 ± 0.13	0.33 ± 0.13	0.29 ± 0.10	0.54 ± 0.10	0.25 ± 0.00
$t_{max}{}^{2}$ (h)	–	–	8.67 ± 1.63	9.33 ± 2.07	–
$C_{max}{}^{1}$ ($mg\,L^{-1}$)	3.58 ± 1.98	1.85 ± 1.64	9.32 ± 2.41	3.62 ± 1.34	0.11 ± 0.03
$C_{max}{}^{2}$ ($mg\,L^{-1}$)	–	–	5.38 ± 1.50	1.65 ± 0.73	–

$t_{1/2}$ apparent elimination half-life; AUC_{0-t} areas under the plasma concentration-time curves; t_{max} time to reach peak concentration; C_{max} maximum plasma concentration.

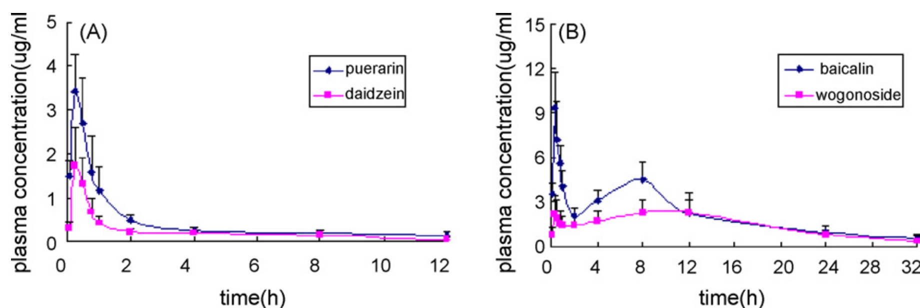

Fig. 4. Plasma concentration-time profiles of (A) puerarin, daidzein and (B) baicalin, wogonoside, after intragastric administration of GQD (10 mL kg^{-1}).

3.4. Data analysis of pharmacokinetic studies in rats

The developed assay was sensitive to measure all the five compounds except liquiritin (for its plasma concentrations in most of the time points were lower than the LLOQ) in rat plasma samples obtained following intragastric administration of GQD (10 mL kg^{-1}). The plasma concentration-time profiles of the other four components in rat plasma were shown in Fig. 4. According to DAS Ver 2.0 (Drug and Statistics for Windows) program, the concentration-time courses of puerarin and daidzein were adequately described by a two-compartment open model which were in parallel with the literature [40]. Daidzein and puerarin provide us with similar pharmacokinetic characteristics, though they had unequal contents in GQD and different concentrations in rat plasma. It has been reported that the first absorption site of baicalin was in upper intestinal and the second site was in colon in the form of aglygon [41,42]. Thus, the bimodal phenomenon of baicalin appeared in our study, as well as its analogue wogonoside, was reasonable. However, the non-compartment model of baicalin we obtained varied from one-compartment model of baicalin in dogs [43], this may be in part due to the species differences in experimental animals. The estimated pharmacokinetic parameters were listed in Table 3.

4. Conclusion

The method reported here is the first UPLC–MS quantitative assay for simultaneous determination of puerarin, daidzein, baicalin, wogonoside and liquiritin in rat plasma samples following oral administration of GQD. It is rapid, sensitive, and provides a useful alternative for analysis of the pharmacokinetics of multi-bioactive components in plasma samples.

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