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Simultaneous determination of six compounds in rat plasma by ultra-performance liquid chromatography with tandem mass spectrometry: Application in the pharmacokinetic study of Qing Gan-Shu Yu-Fang

Original Article

Simultaneous Determination of Six Compounds in Rat Plasma by Ultra-Performance Liquid Chromatography with Tandem Mass Spectrometry: Application in the Pharmacokinetic Study of *Qing Gan-Shu Yu-Fang*

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Abstract

A rapid and high selective ultra-performance liquid chromatography (UPLC) with tandem mass spectrometry method for simultaneous determination of six compounds including albiflorin, paeoniflorin, picroside I, picroside II, saikosaponin A, and saikosaponin D in rat plasma was developed and validated using butyl p-hydroxybenzoate as an internal standard. One-step direct protein precipitation with acetonitrile was used to extract the compounds from the rat plasma samples. Chromatographic separation was achieved using an ACQUITY UPLC BEH C_{18} column (100 mm × 2.1 mm, 1.7 μm) at a flow rate of 0.4 mL/min, using gradient mode containing 0.1% formic acid in water and acetonitrile were used as the Mobile phase A and B. Electrospray ionization in negative ion mode and multiple reaction monitoring were used to identify and quantify active components. Calibration curves showed good linearity ($R^2 > 0.9908$) over a wide concentration range for all compounds. The intra- and interday precision (relative standard deviation) ranged 2.4%–7.0% and 2.6%–8.0%, respectively. The accuracy (relative error) was from −13.0% to 13.2% at all quality control levels. The recovery ranged from 81.1% to 92.5%. The validated method was successfully applied to pharmacokinetic study in rats after oral administration of *Qing Gan-Shu Yu-Fang*. The results show that one can draw a conclusion that these six active ingredients can be quickly absorbed and play a pharmacodynamic role rapidly *in vivo*.

Keywords: Pharmacokinetics, traditional Chinese medicine prescriptions, ultra-performance liquid chromatography with tandem mass spectrometry

Introduction

Traditional Chinese medicine prescriptions (TCMPs) have been widely applied in treating diseases for thousands of years in China. Based on complex multicomponent composition, active components of TCMP play a multilink, multitarget, and multilevel treating effect.[1,2] Compatibility of Chinese Materia Medica is a characteristic and advantage in TCMP. Like drug–drug interactions of the modern medicine, the TCMP contain two or more medicinal herbs to introduce new pharmacological effects through a synergistic action or antagonistic effect.[3] However, the synergistic action or antagonistic effect might have influence on ADME (absorption, distribution, metabolism, and elimination) of some components in the TCMP *in vivo*.[4] Therefore, a specific authoritative

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method should be established that provides recommendations for pharmacokinetic (PK) studies of TCMP.

Qing Gan-Shu Yu-Fang (QGSYF) is a clinical experience formula which has been used to the treatment of chronic viral hepatitis that was summed up by the Chinese Medicine master Professor Fujin Duan in several decades of Chinese medicine clinical treatment. The compound prescription composed of *Picrorhiza rhizome*, *Paeoniae radix alba,* and *Bupleuri radix* (1:1:1, g/g/g). The chemical constituents of QGSYF mainly include iridoid glucosides (including picroside I and picroside II), monoterpene glycosides (including albiflorin and paeoniflorin), and triterpenoid saponins (including saikosaponin A, and saikosaponin D). In consideration of constituents in individual herb from QGSYF, the main active ingredients of *Picrorhiza rhizome* are picroside I and picroside II. Recent studies have exhibited that picroside I and picroside II have protective,[5,6] anti-inflammation,[7,8] antioxidant,[9,10] potentiation of nerve growth factor action,[11] and anticancer[12] activities. Paeoniflorin and albiflorin are the primarily effective ingredients in *Paeoniae radix alba,* and paeoniflorin has been used as a target marker for the quality control (QC) of *Paeoniae radix alba* in Chinese Pharmacopoeia. Pharmacological studies show that paeoniflorin and albiflorin have liver protecting, anti-inflammatory,[13] anticoagulant,[14,15] antioxidant,[16,17] and neuron protective[18] activities. The major components of *Bupleuri radix* have been identified as a series of triterpenoid saponins, saikosaponin A, and saikosaponin D as the principal component have been observed have hepatoprotective effects,[19] activation of antitumor effector cells,[20] and anti-inflammatory activities.[21]

To the best of our knowledge, there are many PK reports about TCMP, only determination of the active ingredients from the single herb in rat plasma.[22-24] However, there are rare literatures on pharmacokinetic studies of active ingredients in SGQYF. Obviously, the study on multiple activities of TCMP *in vivo* becomes very important in the development and clinical application of this potential new drug. In previous study, we have reported a high-performance liquid chromatography coupled with photodiode array (HPLC-PDA) method for simultaneous quantification of picroside I, II and albiflorin, paeoniflorin of QGSYF particles and a HPLC coupled with evaporative light scattering detector (ELSD) method for simultaneous quantification of saikosaponin A and saikosaponin D of QGSYF particles in *vitro*.[24] Moreover, the six ingredients are the major component of QGSYF, which to play a treatment role. Hence, it is urgent to establish a rapid and sensitive ultra-performance liquid chromatography with tandem mass spectrometry (UPLC-MS/MS) method and to perform the PK research of the six active constituents from QGSYF *in vivo*. Unlike conventional HPLC, UPLC has the advantages of a greater resolution, higher sensitivity, shorter running time, and less solvent consumption.[25] In addition, MS/MS technology could overcome the shortcoming that the absence of chromophore saikosaponin A and saikosaponin D hampers their detection usingan ultraviolet detector, so as to achieve the objective to simultaneously determining the six ingredients. The aim of the quantitative study *in vitro* is only to establish an accepted standard for primarily effective ingredients of herbs or prescriptions. However, to further improve dose regimen, understand herb–herb interaction mechanism and exert maximal therapeutic efficacy for drugs which will need to establish a series of studies *in vivo* for QGSYF. PK studies are useful to explain and foresee the *in vivo* process of active ingredients after oral administration of QGSYF and to improve dose regimen and to avoid adverse effects.

In the present article, we developed and validated a rapid, efficient, and sensitive UPLC-MS/MS method for the simultaneous determination of albiflorin, paeoniflorin, picroside I, picroside II, saikosaponin A, and saikosaponin D in rat plasma. It was expected the results of this study could not only facilitate to apprehend the action mechanism of QGSYF but also help to make rational use of it.

Experimental

Materials and reagents

The reference standards of picroside I and picroside II (purity >98%) were purchased from the Munster Biological Technology Co., Ltd. (Chengdu, China). The reference standard of albiflorin (purity >98%) was purchased from Yifang Biological Technology Co., Ltd. (Tianjin, China). The reference standard of paeoniflorin and saikosaponin A and saikosaponin D (purity >98%) was purchased from the National Institutes for Food and Drug Control (Beijing, China). The reference standard of butyl p-hydroxybenzoate (internal standard [IS]) (purity >98%) was purchased from the Guangfu Fine Chemical Research Institute (Tianjin, China). Pieces prescription was collected from the Bozhou Traditional Chinese Medicine Market of Anhui, China in October, 2014 and was identified by Professor Lianjie Su of Heilongjiang University of Traditional Chinese Medicine. Acetonitrile of HPLC grade was obtained from Tedia (Fairfield, OH, USA). Formic acid of HPLC grade was obtained from Dikma (Richmond Hill, NY, USA). Ultra-pure water was prepared from a Milli-Q water purification system (Bedford, MA, USA). All other reagents were of analytical grade.

Instrumentation and analytical conditions

Chromatographic separation was achieved using an ACQUITY UPLC system (Waters Corp., Milford, MA, USA) comprised a binary pump with integrated degasser, autosampler with thermostat, and thermostatted column compartment. Chromatographic separation was achieved on an ACQUITY UPLC BEH C_{18} column (100 mm × 2.1 mm, 1.7 μm). A gradient elution with water (containing 0.1% formic acid) (A)-acetonitrile (B) was used. The flow rate was 0.4 mL/min. The gradient program was as following: 0–4.0 min, 90%–82% A, 4.0–4.5 min, 82%–70% A, 4.5–6.0 min, 70%–67% A, 6.0–6.5 min, 67%–50% A, 6.5–9.5 min, 50%–42% A, 9.5–10.0 min, 42%–90%, and

10.0–11.5 min, 90% A. All sample extracts were maintained in the autosampler at 4°C and the injection volume was 5 μL.

The LC system was coupled with an API 4000 QTRAP mass spectrometer system (Applied Biosystems/MDS SCIEX, USA) through a triple quadrupole linear ion trap mass spectrometer in conjunction with an electrospray ionization source in the negative ion mode for detection. Trace Finder TM version 1.6.2 software (Thermo Scientific, Bremen, Germany) was used for instrument control, data acquisition, and data analysis. All compounds were quantitatively determined by the multiple reaction monitoring (MRM) mode with the transitions of *m/z* 479.3 → 121.0 for albiflorin, *m/z* 479.3 → 121.2 for paeoniflorin, *m/z* 491.0 → 147.0 for picroside I, and *m/z* 511.0 → 235.0 for picroside II, *m/z* 779.8 → 617.8 for saikosaponin A, *m/z* 779.7 → 617.5 for saikosaponin D, and *m/z* 193.0 → 91.9 for IS. The structures and MS^2 spectra of these compounds are shown in Figure 1. The mass spectrometric parameters were set as follows: ion spray voltage 4500 V; turbo spray temperature 550°C; nebulizer gas (gas 1), 55 psi; heater gas (gas 2), 55 psi; and curtain gas 20 psi; nitrogen was used in all cases. The declustering potential and collision energy are optimized for each compound and selected values are showed in Table 1.

Preparation of *Qing Gan-Shu Yu-Fang* extracts and determination of active compounds in the extracts

Powdered *Picrorhiza Rhizome* (150g), *Paeoniae radix alba* (150 g), and *Bupleuri radix* (150g) were mixed together (the three plant materials were mixed in a ratio of 1:1:1) and then were extracted three times with 2700 mL of ethanol–water (70:30, v/v) refluxing for 2 h, respectively. The extraction solution was obtained to be filtered and concentrated to 1 g/mL crude drug under reduced pressure. The administered dose of six compounds in the extract were quantitatively assessed by HPLC-ELSD method and the contents were 20.34 mg/mL (picroside I), 31.14 mg/mL (picroside II), 9.92 mg/mL (albiflorin), 14.47 mg/mL (paeoniflorin), 5.90 mg/mL (saikosaponin A), and 0.99 mg/mL (saikosaponin D), respectively.

Preparation of stock solution, standard, and quality control samples

The stock solutions of albiflorin, paeoniflorin, picroside I, picroside II, IS, and saikosaponin A, and saikosaponin D were separately prepared by dissolving the precisely weighed reference compounds in methanol. All the stock solutions were stored at 4°C until use. Stock solutions of these compounds

Table 1: Mass spectrometric parameters of six compounds and IS

Analyte	Precursor ions	Product ion (*m/z*)	DP (V)	CE (V)
Albiflorin	479.3	121.0	−80.42	−29.38
Paeoniflorin	479.3	121.2	−80.42	−29.38
Picroside I	491.0	147.0	−109.90	−21.05
Picoside II	511.0	235.0	−79.60	−35.86
Saikosaponin A	779.8	617.8	−175.03	−51.37
Saikosaponin D	779.7	617.5	−175.03	−51.37
IS	193.0	91.9	−81.74	−33.48

DP: Declustering potential, CE: Collision energy, IS: Internal standard

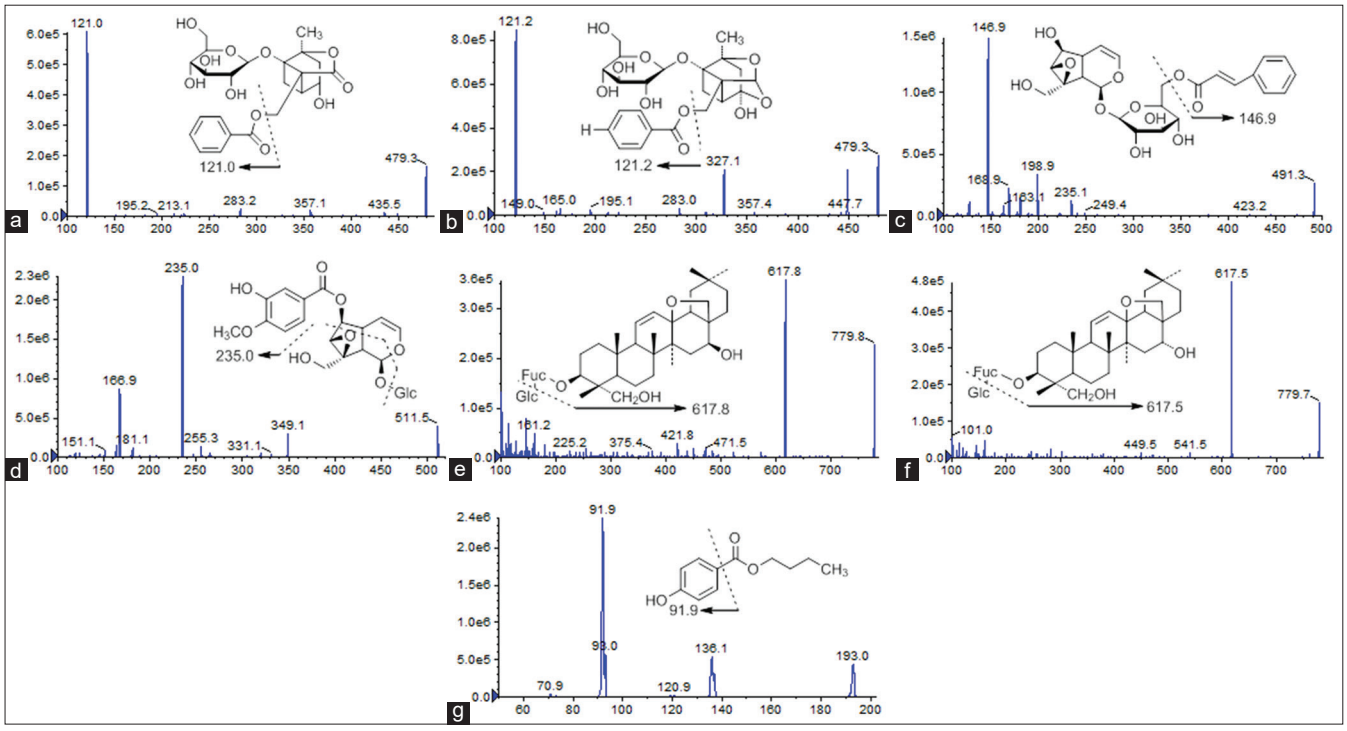

Figure 1: Product ion mass spectra of the (a) albiflorin, (b) paeoniflorin, (c) picroside I, (d) picroside II, (e) saikosaponin A, (f) saikosaponin D, and (g) internal standard

were mixed and a series of standard working solutions were further prepared by dilution with methanol to provide seven standards of suitable desired concentration. Calibration samples were prepared by spiking 150μL rat blank biological matrix with 20 μL mixed working solutions. The calibration standards were prepared by spiking the working solution into blank rat plasma at concentrations of 4.70–940.0 ng/mL for albiflorin, 4.80–960.0 ng/mL for paeoniflorin, 5.10–1020 ng/mL for picroside I, 0.98–490.0 ng/mL for picroside II, 0.55–220.0 ng/mL for saikosaponin A, 0.60–90.00 ng/mL for saikosaponin D, and 15.00 ng/mL for IS, respectively. The QC samples were extracted at high, medium, and low concentrations for each compound (18.80, 94.00, and 940.0 ng/mL for albiflorin; 19.20, 96.00, and 960.0 ng/mL for paeoniflorin; 20.40, 102.0, and 1020 ng/mL for picroside I; 9.80, 49.00, and 490.00 ng/mL for picroside II; 11.00, 44.00, and 220.0 ng/mL for saikosaponin A; and 12.00, 24.00, and 120.00 ng/mL for saikosaponin D) in the same procedure with the standard calibration samples.

Plasma sample preparation

Plasma samples were transferred out of the refrigerator (−40°C) and thawed at room temperature. The thawed samples were extracted using a simple liquid–liquid extraction technique by direct protein precipitation with acetonitrile. To an aliquot, 20.0 μL of the IS (butyl p-hydroxybenzoate, 15.00 ng/mL) and 600 μl acetonitrile were added to 150 μl of the plasma sample were combined and vortex-mixed vigorously for 3 min. The mixture was centrifugation at 12000 rpm for 10 min at 4°, and 700 μL of supernatant was transferred to a clean test tube and was completely evaporated to dryness under a gentle stream of nitrogen gas at 40°C. Finally, the residue was reconstituted in 100 μl of methanol/water (50:50, v/v), vortex-mixed for 3 min, centrifuged at 12,000 rpm for 10 min, and a 5 μL of supernatant was injected into the UPLC-MS/MS system for measurement.

Method validation

Specificity

The specificity of the method was assessed by comparing the chromatograms at the retention times of blank plasma collected from six different rats, plasma spiked with compounds and IS and plasma obtained after gavage of extract of the drug pair to investigate the potential interferences and to ensure no interference at the peak region of endogenous matrix compounds.

Calibration curve and lower limit of quantification

The linearity of calibration curve was assessed by analyzing seven concentration levels of standard plasma samples and constructed by plotting the peak area ratios (Y) of compounds to IS against the nominal concentration samples in plasma of compounds (X, ng/mL), using a $1/x^2$ weighted linear least squares regression model. The lower limit of quantification (LLOQ) was define as the lowest amount of compounds in plasma samples that can be detected precisely with a level of acceptable accuracy (relative error [RE] ± 20%) and precision (relative standard deviation [RSD] < 20%) under a stated experimental condition. In addition, the signal-to-noise ratio(S/N)of the LLOQ sample considering should be at least five times the signal of the baseline noise.

Precision and accuracy

Three concentration levels of QC samples (n = 5) by replicate analysis in each run to assess the intraday precision and accuracy (on 1 day) and interday precision and accuracy (between three different days) of the assay. The precision and accuracy of analytical procedure were assessed as the RSD and RE of a series of measurements, respectively. The values of the precision (RSD%) and accuracy (RE%) were acceptable were all within ± 15% deviation from the nominal concentration.

Stability

Stability of the six compounds in rat plasma was evaluated by analyzing replicate QC samples (n = 5) of three levels under following storage conditions: short time stability (exposing QC samples at room temperature for 4 h which longer than the time of routine preparation samples), long time stability (keeping QC samples at −20°C for 2 weeks), postpreparation stability (placing QC samples in the autosampler at 4°C for 12 h), and freeze-thaw cycle stability (through three freezes-thaw cycles from −20°C to room temperature on consecutive days). Freshly calibration curve was prepared used to analyzing the stability of QC samples.

Extraction recovery and matrix effect

The extraction recovery of six compounds was evaluated by analyzing the ratio of the peak area for postextraction blank plasma-spiked compounds against preextraction blank plasma added compounds at three QC levels (low, medium, and high concentration levels). The matrix effects were assayed by comparing the peak responses of compounds spiked into the pretreated blank rat plasma with those of corresponding concentration pure standard solutions, the IS was determined through a similar process method.

Animal

Healthy male Sprague-Dawley rats (300–350 g) were supplied by the Experimental Animal Center of Heilongjiang University of Traditional Chinese Medicine. The rats were kept in an environmentally controlled room (25 ± 2°C, relative humidity 50 ± 10%), with a natural light–dark cycle for 7 days before the experiment was carried. Before the drug administration, they were fasted for 12h and free access to water. All animal experiments were achieved according to the regulations of experimental animal administration approved by the State Commission of Science and Technology of the People's Republic of China.

Pharmacokinetic and statistical analysis

Six rats were orally administered QGSYF at a single dose which equivalent to 6 g/kg body weight of crude drug was used to investigate the PKs of compounds of drug pair extract. The rat blood samples about 300 μl were gathered from the suborbital vein into heparinized tubes before dosing

and 0.08, 0.16, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24h after oral administration. And then, the samples were immediately centrifuged at 12000 rpm for 10 min, and the supernatants were transferred into polypropylene tubes and stored at −40°C until analysis. The daily calibration curves were constructed to calculate the concentration of the six compounds in rat plasma samples at different time points. All the data were expressed as mean ± SD. The PKs parameters including maximum plasma concentration (C_{max}), the time to reach C_{max} (T_{max}), elimination of half-life time ($T_{1/2}$), and area under the curve (AUC_{0-t} and $AUC_{0-\infty}$).

Results

Mass and mobile conditions optimization

In our study, to get symmetric peak shapes, appropriate retention time, and optimized responses for the compounds. Methanol, acetonitrile, pure water, and 0.1% formic water were tested as potential phases, the final gradient elution phases were chosen with acetonitrile and 0.1% formic water to achieve the optimal peak shapes, running time, and sensitivity. Butyl p-hydroxybenzoate as an IS was selected for its similarity retention time, ion mode, and stable-labeled structure with those compounds. In addition, the MRM mode was used to simultaneous determination of multiple components of QGSYF. To obtain the optimal ionization mode, the ESI full scan mode was chosen, the ultimate results demonstrated that all compounds and IS were more suitable in negative ion mode which show stronger relative intensity than positive ion mode.

Method validation

Specificity

The typical chromatograms of blank rat plasma, blank plasma spiked with mixture of six compounds and IS, and the plasma samples collected after administration of QGSYF extract are shown in Figure 2. Under the established chromatographic conditions, the endogenous and impurities interferences have not been discovered at the peak region of compounds and IS. The retention time of albiflorin, paeoniflorin, picroside I, picroside II, saikosaponin A, saikosaponin D, and IS was found to be about 3.11, 3.67, 6.13, 5.58, 8.05, 8.56, and 8.55 min, respectively.

Linearity and lower limit of quantification

The typical calibration curves of six compounds all shared good linearity with satisfactory correlation coefficients ($R^2 > 0.9908$). The results for LLOQs of six compounds are appropriate for quantitative detection studies. In addition, the LLOQs for albiflorin, paeoniflorin, picroside I, picroside II, saikosaponin A, and saikosaponin D were 4.70, 4.80, 5.10, 0.98, 0.55, and 0.60 ng/mL, respectively. Finally, the regression equations, linear range, correlation coefficients, and LLOQs were shown in Table 2.

Precision and accuracy

One could draw conclusions that all the values for intra- and interday precision and accuracy of QC samples at three concentrations was within the accepted variable limits, the established method was overall precise and accurate. The intra- and interday precision were ranged from 2.4% to 7.0% and 2.6% to 8.0%, respectively, the accuracy was within 13.0% to 13.2%. The results were displayed in Table 3.

Stability

The stability assay results were summarized in Table 4. The results indicating that the six compounds in rat plasma samples were stable under different store conditions for 4 h at room, 2 weeks at −20°C, three freeze-thaw cycles, and 12 h after pretreatment in the autosampler. The range of variation for concentrations was within 15% of the actual value.

Extraction recovery and matrix effect

As displayed in Table 5, the mean extraction recovery of compounds at three concentration levels in plasma samples was all between 81.1% and 92.5%, within acceptable range. The matrix effect of six compounds ranged from 90.1% to 101.7%, these data indicated that there was no obvious matrix effect under the current condition. Hence, further proof that the established method was stable and reliable.

Pharmacokinetic studies

The established method was successfully applied to simultaneous determination of the six compounds in rat blood plasma samples for PK study. Drug and Statistics (DAS) 2.0 software (Mathematical Pharmacology Professional Committee, Shanghai, China) was used to obtain correlation PK parameters and are present in Table 6. The mean plasma concentration-time profiles of six compounds are illustrated in Figure 3.

The T_{max} of picroside I and picroside II were 1.0 ± 0.5 h and 1.2 ± 0.7 h, respectively. Therefore, it is difficult to accumulate *in vivo* for the two compounds with a short residence time and can be eliminated quickly. This is likely due to picroside I and

Table 2: Calibration curves, linear range, lower limit of quantification for the six compounds

Analyte	Regression equation	Linear range (ng/ml)	R^2	LLOQ (ng/ml)
Albiflorin	$y=5.28\times10^{-4}X-1.16\times10^{-2}$	4.70-940.0	0.9908	4.70
Paeoniflorin	$y=9.11\times10^{-4}X+4.76\times10^{-3}$	4.80-960.0	0.9999	4.80
Picroside I	$y=3.74\times10^{-4}X-3.00\times10^{-3}$	5.10-1020	0.9974	5.10
Picoside II	$y=5.89\times10^{-3}X-4.19\times10^{-2}$	0.98-490.0	0.9979	0.98
Saikosaponin A	$y=2.05\times10^{-3}X-1.36\times10^{-2}$	0.55-220.0	0.9968	0.55
Saikosaponin D	$y=2.23\times10^{-3}X-8.91\times10^{-3}$	0.60-90.00	0.9972	0.60

LLOQ: Lower limit of quantification

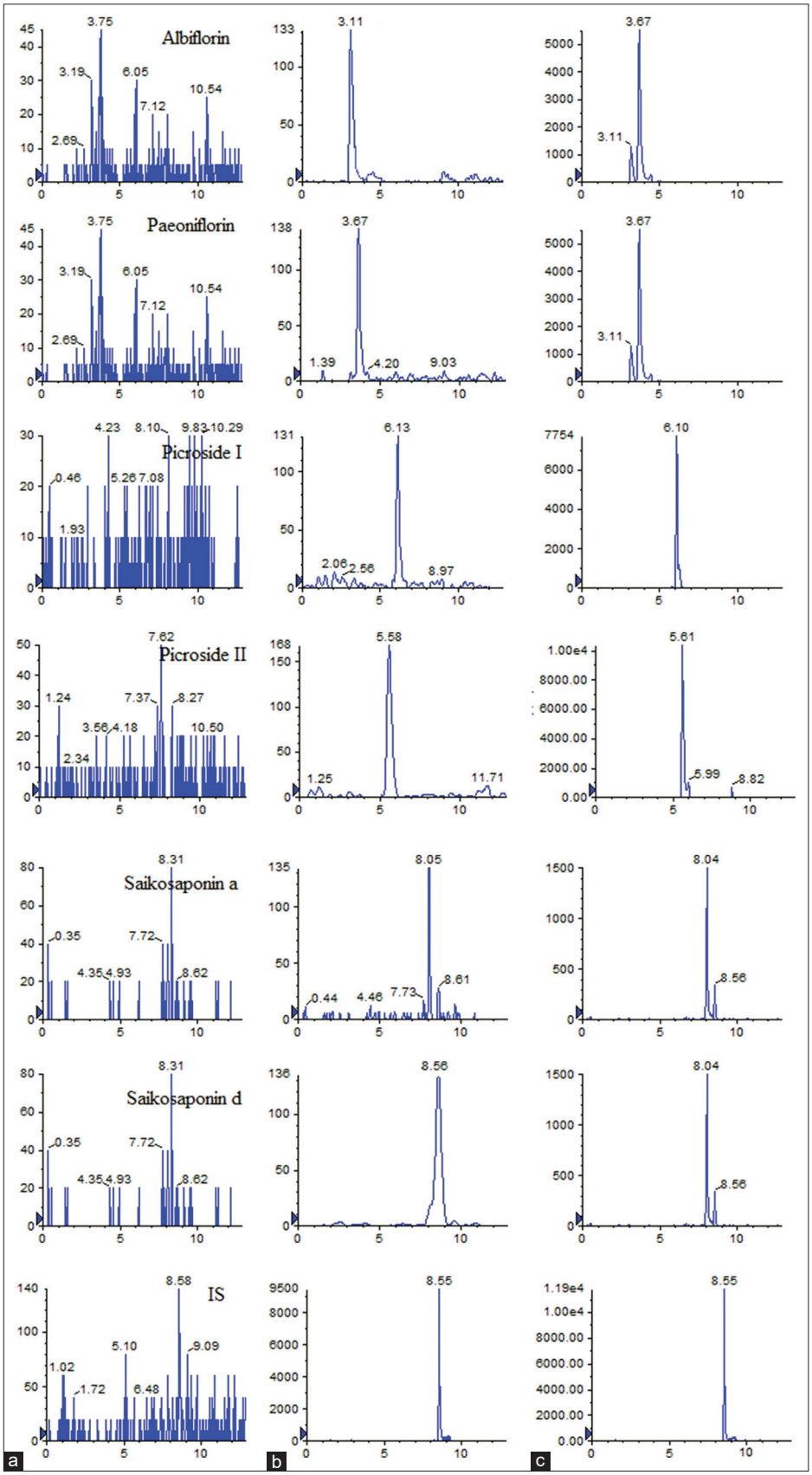

Figure 2: Representative multiple reaction monitoring chromatograms of albiflorin, paeoniflorin, picroside I, picroside II, saikosaponin A, saikosaponin D, and internal standard in rat plasma. (a) Blank plasma, (b) blank plasma spiked with six compounds at lower limit of quantification and internal standard, (c) plasma from a rat 1 h after oral administration of *Qing Gan-Shu Yu-Fang*

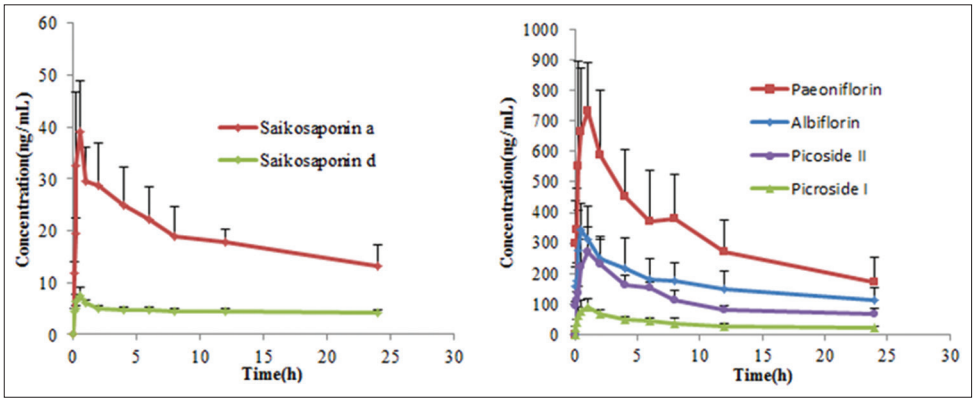

Figure 3: Mean plasma concentration time profiles of six compounds in rat plasma after oral administration of *Qing Gan-Shu Yu-Fang* (n = 6)

Table 3: Summary of accuracy and precision of the six compounds in rat plasma (n=5)

Analytes	Concentration (ng/mL)		RSD (%)		Accuracy (RE, %)
	Spiked	Measured	Intra-day	Inter-day	
Albiflorin	18.80	20.43±0.86	4.2	6.4	8.7
	94.00	102.3±2.45	2.4	5.5	8.8
	940.0	973.9±39.7	4.1	4.2	3.6
Paeoniflorin	19.20	19.54±0.88	4.5	6.1	1.7
	96.00	108.0±4.08	3.8	4.9	12.5
	960.0	942.8±30.3	3.2	4.0	−1.8
Picroside I	20.40	20.64±0.99	4.8	7.9	1.2
	102.0	103.2±5.05	4.9	6.9	1.1
	1020	1046.4±37.3	3.6	2.6	2.6
Picoside II	9.80	8.61±0.50	5.8	7.4	−12.1
	49.00	55.49±2.0	3.5	4.1	13.2
	490.0	532.8±21.0	3.9	4.2	8.7
Saikosaponin A	11.00	9.66±0.47	4.8	7.6	−12.3
	44.00	46.84±1.16	2.5	5.0	6.5
	220.0	224.4±7.00	3.1	5.8	2.0
Saikosaponin D	12.00	10.44±0.73	7.0	8.0	−13.0
	24.00	22.85±0.64	2.8	7.4	−4.8
	120.0	130.59±5.92	4.5	5.0	8.8

RSD: Relative standard deviation, RE: Relative error

picroside II are iridoid glycosides, previous reports about PKs suggest that iridoid glycosides show a less bioavailability due to their extensive metabolism through various pathways.[26,27]

Paeoniflorin and albiflorin are two major monoterpene glycosides of *Paeoniae radix alba*, which are also the most important active ingredients, play treating effect of this herb. Inthe previous report, these two compounds show similar T_{max} and similar characteristics and tendencies in plasma drug–time curve profiles after oral administration of signal herbal. This could be because of the similar structure of these two compounds.[23] However, two compounds are different T_{max} (0.9 ± 0.6h and 0.7 ± 0.3h) in plasma drug–time curve profiles after oral administration of QGSYF, even longer than in a single herbal.

Saikosaponin A and saikosaponin D are two major triterpenoid saponins of *Bupleuri radix*. Compared with another four triterpenoid saponins, saikosaponins A and Dhave lower content in this compound prescription. Recent reports suggest that it is difficult to enter the body through the intestinal membranes for saponins.[28] Moreover, the structure of the two saikosaponins was unstable and easily convertible in the gastrointestinal tract. Therefore, the two compounds showed a lower concentration in rat plasma samples. However, the current study indicates that saikosaponin A and saikosaponin D were quickly absorbed with a T_{max} about 30 min. Furthermore, saikosaponin A and saikosaponin D were usually used as marker for the QC of *Bupleuri radix*.[29] However, the $T_{1/2}$ values of saikosaponin D (14.5 ± 7.0h) are a little longer than previous studies including oral administration of monomer, bupleurum extract, and prescription.[24,30] A probable explanation was that the other unknown components of the other two herbals may inhibit the elimination of saikosaponin D in rats. This hypothesis will be one of the directions of our future research.

Discussion

The experimental results showed that the variation in bioavailability of paeoniflorin and albiflorin from different preparations attributed to the presence of some other compounds in QGSYF which are likely to inhibit the metabolism of paeoniflorin and albiflorin. The unknown compounds most likely from the other two herbals. These also results suggested that herb–herb interactions occurred *in vivo* after oral administration of QGSYF in rats. Thus, further mechanistic study has to be conducted in future to understand the actual reason for low bioavailability of paeoniflorin and albiflorin in QGSYF.

In short, one can draw a conclusion that these six active ingredients can be quickly absorbed and play a pharmacodynamic role rapidly *in vivo*.

Conclusion

For the first time, we have achieved simultaneously quantitative six active ingredients from QGSYF in rat plasma. Moreover, a flesh, rapid, sensitive, simple UPLC-MS/MS method was performed which used for determination of six kinds of

Table 4: Matrix effects and extraction recovery for the compounds in rat plasma (n=3)

Analytes	Concentration (ng/mL)	Stability (percentage, RSD)			
		Short-term	Postpreparative	Freeze-thaw	Long-term
Albiflorin	18.80	4.8	6.2	9.1	10.8
	94.00	6.6	9.8	6.7	9.5
	940.0	3.2	4.0	5.2	7.2
Paeoniflorin	19.20	4.3	6.7	8.5	10.4
	96.00	4.0	3.7	7.2	9.9
	960.0	4.6	3.1	8.1	11.6
Picroside I	20.40	5.1	5.8	7.1	10.9
	102.0	2.9	6.8	5.0	8.1
	1020	2.9	4.7	5.3	7.7
Picoside II	9.80	4.4	4.4	4.2	7.6
	49.00	3.6	5.0	5.9	6.6
	490.0	2.7	2.8	4.3	5.0
Saikosaponin A	11.00	4.5	3.3	6.3	11.1
	44.00	3.2	5.7	5.1	6.3
	220.0	3.1	3.4	3.5	4.2
Saikosaponin D	12.00	3.3	5.8	6.0	7.1
	24.00	5.6	5.5	6.4	6.8
	120.0	4.6	3.7	4.9	5.6

Table 5: Stability data for compounds in rat plasma under different storage conditions (n=5)

Analytes	Concentration (ng/ml)	Recovery (%, mean±SD)	RSD (%)	Matrix effect (%, mean±SD)	RSD (%)
Albiflorin	18.80	81.1±6.8	8.3	92.0±7.0	7.6
	94.00	84.6±3.7	4.4	101.7±5.8	5.7
	940.0	87.8±0.7	0.8	96.2±3.2	3.3
Paeoniflorin	19.20	84.0±8.3	9.8	90.1±1.5	1.7
	96.00	86.6±2.1	2.4	92.3±7.1	7.7
	960.0	88.6±1.9	2.1	94.8±2.8	3.0
Picroside I	20.40	81.8±7.2	8.8	91.9±8.0	8.7
	102.0	86.8±2.8	3.2	92.6±3.4	3.6
	1020	87.9±1.7	2.0	98.6±7.1	7.2
Picoside II	9.80	83.5±9.3	11.2	95.7±8.8	9.2
	49.00	89.3±6.3.	7.0	90.8±3.0	3.4
	490.0	92.5±5.3	5.8	97.0±3.4	3.5
Saikosaponin A	11.00	81.3±4.2	5.2	93.3±4.2	4.5
	44.00	83.4±3.9	4.6	94.8±5.7	6.0
	220.0	85.5±2.6	3.1	97.6±3.9	4.0
Saikosaponin D	12.00	80.8±8.0	10.0	90.5±7.9	8.8
	24.00	91.5±5.6	6.1	92.1±2.6	2.8
	120.0	90.1±2.4	2.7	93.9±2.3	2.5

RSD: Relative standard deviation, SD: Standard deviation

compound in rat plasma after oral give QGSYF. Drawing out the concentration–time curves and calculating the corresponding PK parameters of six compound, can foster better understanding of the efficacy of QGSYF, and can facilitate further to understand the herb–herb interaction and provide the theoretical basis and guidance for clinical application, the development of new drugs, and dosage form of optimization in the future.

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Table 6: Pharmacokinetic parameters after oral administration of *Qing Gan-Shu Yu-Fang* ($n=6$)

Analyte	C_{max} (ng/ml)	T_{max} (h)	T1/2 (h)	AUC_{0-t} (ng/ml)	$AUC_{0-\infty}$ (ng/ml)
Albiflorin	318.6±33.7	0.7±0.3	10.0±0.9	3393.3±451.2	5279.6±1454.6
Paeoniflorin	525.8±66.3	0.9±0.6	11.1±4.2	6216.2±955.1	7997.7±1591.7
Picroside I	91.3±14.1	1.0±0.5	14.5±0.1	878.6±76.8	1325.1±231.9
Picoside II	232.5±40.6	1.2±0.7	11.0±2.8	2442.2±206.7	2934.7±286.1
Saikosaponin A	44.2±7.7	0.5±0.3	11.5±3.7	474.7±42.7	781.9±53.5
Saikosaponin D	9.7±1.5	0.5±0.3	14.5±7.0	115.3±28.8	340.0±195.5

AUC: Area under the curve

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Conflicts of interest

There are no conflicts of interest.

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