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Simultaneous determination and pharmacokinetic study of six flavonoids from *Fructus Sophorae* extract in rat plasma by LC–MS/MS

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ABSTRACT

In this study, a new liquid chromatography–tandem mass spectrometry (LC–MS/MS) method has been developed and validated for the determination of six flavonoids including sophoricoside, genistin, genistein, rutin, quercetin and kaempferol in rat plasma after oral administration of *Fructus Sophorae* extract using sulfamethalazole as internal standard (IS). The plasma samples were pretreated and extracted by liquid–liquid extraction. Chromatographic separation was accomplished on a C_{18} column with a simple linear gradient elution. The detection was accomplished by multiple-reaction monitoring (MRM) scanning after electrospray ionization (ESI) source operating in the negative ionization mode. The optimized mass transition ion pairs (m/z) for quantitation were 431.1/267.9 for sophoricoside and genistin, 269.0/133.0 for genistein, 609.2/300.0 for rutin, 301.0/150.9 for quercetin, 284.9/93.0 for kaempferol and 252.0/155.9 for IS. The total run time was 8.0 min. Full validation of the assay was implemented including specificity, linearity, accuracy, precision, recovery and matrix effect. This is the first report on determination of the major flavones in rat plasma after oral administration of *Fructus Sophorae* extract. The results provided a meaningful basis for the clinical application of this herb.

1. Introduction

Traditional Chinese medicine (TCM), which uses natural therapeutic agents under the guidance of the theory of traditional Chinese medical science, is one of the world's most ancient herbal medicines and has been applied by TCM practitioners for thousands of years. *Fructus Sophorae* or HuaiJiao, the dried ripe fruits of *Styphnolobium japonicum* (L.) Schott (Leguminosae), is a herbal ingredient used in TCM for its hemostatic properties [1,2]. Modern pharmacological and clinical studies have showed that some compositions in *Fructus Sophorae* possessed hemostatic properties, anticancer, anti-tumor, anti-obesity, antifertility action, anti-oxidation effects and the treatment of hypertension and hemorrhoids [1–9].

Pharmacokinetic studies on active ingredients in natural products and traditional Chinese medicines (TCM) are important to illustrate their mechanism of action. Pharmacological studies on *Fructus Sophorae* have revealed that it contains flavonoids, alkaloids, terpenoids, amino acid, saccharide, phospholipids and so on [6,8]. Specifically, flavones, such as sophoricoside, genistin, genistein, rutin, quercetin and kaempferol are the main effective components [1,6,8]. Some assays with HPLC-UV methods have been described for the determination in plasma samples [10–12]. But these assays mainly focused on the quantification of one or two components or their metabolites. Because the therapeutic effects of TCMs are based on the complex interactions of multiple ingredients, the research of the pharmacokinetic studies of multiple flavones after administration of *Fructus Sophorae* extract is essential to understand their role in human health. However, as far as we know, no analytical methods have been reported for the simultaneous determination of these six flavonoids in biological samples.

In this study, we developed a rather sensitive and selective LC–MS/MS method to simultaneously determine sophoricoside, genistin, genistein, rutin, quercetin and kaempferol in rat plasma. The structures are showed in Fig. 1. The method was applied to pharmacokinetics after oral administration of *Fructus Sophorae* extract to rats and the obtained results would be very helpful for evaluating the clinical application of this herb.

2. Materials and methods

2.1. Chemicals and reagents

Sophoricoside (11061521), genistin (11080316), genistein (11012521) and kaempferol (11042524) were purchased from Shanghai Tauto Biotech Co., Ltd., China. Rutin (100080-200707)

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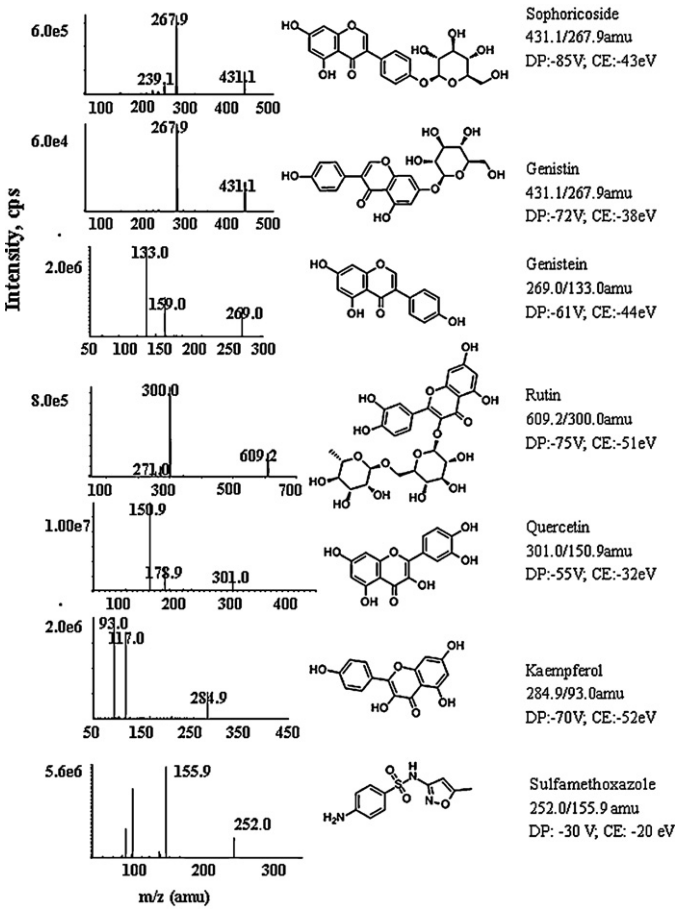

Fig. 1. The product ion scan spectra, chemical structures, monitored transitions, declustering potential (DP) and collision energy (CE) of sophoricoside, genistin, genistein, rutin, quercetin, kaempferol and sulfamethoxazole (IS).

and sulfamethoxazole were obtained from National Institute for the Control of Pharmaceutical and Biological Products. Quercetin was provided by the Department of Pharmaceutical Analysis, Hebei Medical University. The purities of the above ingredients were more than 98% according to LC analysis. Methanol and acetic acid (HPLC-grade) were purchased from DIKMA Company (USA). Analytical grade ethyl acetate, hydrochloric acid and dehydrated ethanol (Tianjin Chemical Corporation, China) were used for the preparation of plasma sample and *Fructus Sophorae* extract. Purified water was obtained from Wahaha (Hangzhou Wahaha Group Co., Ltd.). *Fructus Sophorae* was purchased from the drugstore in Hebei province in China.

2.2. Instrumentation and analytical conditions

An Agilent 1200 liquid chromatography system (Agilent Technologies, USA) equipped with a quaternary solvent delivery system, an autosampler, and a column compartment was used for all analyses. Detection was performed using a 3200 QTRAP system from Applied Biosystems/MDS Sciex (Applied Biosystems, USA), a hybrid triple quadrupole linear ion trap mass spectrometer equipped with Turbo V sources, and a turbo ion spray interface.

The chromatographic separation was performed on a Diamonsil C_{18} column (150 mm × 4.6 mm, 5 μm; DIKMA Company, USA). A linear gradient elution of eluents A (methanol) and B (0.5‰ acetic acid; v/v) was used for the separation. The following gradient condition was used: initial 0–1.5 min, linear change from A–B (35:65, v/v) to A–B (75:25, v/v); 1.5–6 min, linear change from A–B (75:25, v/v) to A–B (95:5, v/v); 6–8 min, isocratic elution A–B (95:5, v/v). The flow rate was set at 0.8 mL/min. The injection volume was 10 μL and the column temperature was maintained at 25 °C.

The ESI interface operated in the negative mode was used. The ion spray voltage was set to −4500 V, and the turbo spray temperature was kept at 650 °C. Nebulizer gas (gas 1) and heater gas (gas 2) was set at 60 and 65 arbitrary units, respectively. The curtain gas was kept at 25 arbitrary units and interface heater was on. Nitrogen was used in all cases. Multiple-reaction monitoring (MRM) was employed for determination. The precursor-to-product ion pairs, declustering potential (DP) and collision energy (CE) for each analyte is shown in Fig. 1. Other parameters were also optimized for maximum abundance of the ion of interest by the automatic tuning procedure of the instrument. All data was controlled and synchronized by Analyst software (Versions 1.4.2) from Applied Biosystems/MDS Sciex.

2.3. Preparation of Fructus Sophorae extract

Fructus Sophorae (100 g) was ground into suitable powder and extracted three times by decocting with boiling water (1:20,1:20, and then 1:10, w/v) for 50 min per time. The extraction solutions were combined for filtration and concentrated to 900 mL, and then added in 300 mL dehydrated ethanol for precipitation, stored at 4 °C for 24 h. The precipitation was filtered and the ethanol was removed under reduced pressure. Then aqueous solutions were concentrated to 90 mL to get the *Fructus Sophorae* extract with a concentration equivalent to 1.11 g/mL of the *Fructus Sophorae*.

The contents of six flavonoids in the *Fructus Sophorae* extract solution were quantitatively determined with an external standard method using the same chromatography conditions as described above. The contents of sophoricoside, genistin, genistein, rutin, quercetin and kaempferol in the extract were 68, 13.45, 2.16, 15.65, 0.367, and 0.61 mg/mL, respectively.

2.4. Preparation of standard solution, calibration standards and quality control (QC) samples

The stock solutions of the investigated flavones were prepared in methanol, respectively. The appropriate amounts of sophoricoside, genistin, genistein, rutin, quercetin and kaempferol were separately weighed and dissolved as the stock solutions. Then, the six stock solutions were mixed and diluted with methanol to prepare a final mixed standard solution containing 1248.00 ng/mL of sophoricoside, 12480.00 ng/mL of genistin, 1250.00 ng/mL of genistein, 1501.50 ng/mL of rutin, 3485.00 ng/mL of quercetin and 2511.00 ng/mL of kaempferol, respectively. A series of working solutions of these analytes were obtained by diluting mixed standard solution with methanol at appropriate concentrations. A quantity of sulfamethoxazole was dissolved in methanol to produce the IS solution with a concentration of 400 ng/mL.

Calibration standards were prepared by spiking the appropriate amount of the standard mixture working solutions into 50 μL drug-free rat plasma to give nominal concentration range of 1.00–499.20 ng/mL for sophoricoside, 9.98–4992.00 ng/mL for genistin, 1.00–500.00 ng/mL for genistein, 1.20–600.60 ng/mL for rutin, 2.79–1394.00 ng/mL for quercetin, 2.01–1004.40 ng/mL for kaempferol.

For validation of the method, three concentration levels of the standard solution containing sophoricoside (6.24, 124.80, and 998.40 ng/mL), genistin (62.40, 1248.00, and 9984.00 ng/mL), genistein (6.25, 125.00, and 1000.00 ng/mL), rutin (7.51, 150.15, and 1201.20 ng/mL), quercetin (17.43, 348.50, and 2788.00 ng/mL) and kaempferol (12.56, 251.10, and 2008.80 ng/mL) were used for preparing the quality control (QC) plasma samples.

2.5. Preparation of plasma samples

A simple liquid–liquid extraction (LLE) method was applied to extract the six flavonoids and IS from rat plasma. To a 50 μL of the rat plasma, 20 μL of the IS and 20 μL of methanol (volume of the corresponding working solution for calibration curve and QC samples) and 50 μL of 0.25 mol/L hydrochloric acid were added. Then the mixture was vortexed for 1 min and extracted with 1 mL of ethyl acetate by shaking on a vortex-mixer for 5 min at room temperature. The upper layer was transferred to a clean tube after centrifugation at 4000 rpm for 5 min. The upper organic phase was evaporated to dryness under a gentle stream of nitrogen. The obtained residue was reconstituted in 50 μL of 50% methanol and centrifuged at 12,000 rpm for another 5 min. Subsequently, aliquots of 10 μL were injected into the HPLC–MS system for analysis.

2.6. Method validation

The accuracy and precision of the established method were evaluated by QC samples at low, medium and high concentrations. The accuracy was determined by using the formula RE% = [(measured value − theoretical value)/theoretical value] × 100. Three validation batches, each containing six replicates of QC samples at low, medium, and high concentration levels were assayed to assess the precision and accuracy of the method on 3 consecutive validation days.

The stability of the analytes in rat plasma was assessed by analyzing QC samples at three concentration levels through three freeze–thaw cycles (−20 °C to room temperature as one cycle), on the bench at room temperature for 24 h (short-term stability), at −20 °C in the freezer for 21 days (long-term stability), and in the stock solutions at room temperature for 24 h (extracted sample/autosampler stability).

The LLOQ of the assay was defined as the lowest concentration of the standard curve that could be quantitated (LLOQ, S/N = 10). The LOD was defined as the amount that could be detected (LOD, S/N = 3).

The extraction recoveries of analytes at three QC levels were evaluated by determining the peak area ratios of the analytes in the post-extraction spiked samples to that acquired from pre-extraction spiked samples. The matrix effects were measured by comparing the peak areas of the analytes dissolved in the pre-treated blank plasma with that of pure standard solution containing equivalent amounts of the analytes.

2.7. Peak identification

Identification of each analyte is a prerequisite for successful quantification. For structural identification, the information-dependent acquisition (IDA) method was used to trigger the enhanced product ion (EPI) scans by analyzing MRM signals [13]. According to the comparison of retention time, parent and product ions with standards in MRM-IDA-EPI spectra, all the peaks of target compounds were unambiguously identified.

2.8. Pharmacokinetic study in rat plasma

Male Sprague–Dawley rats, weighing 250–300 g, were supplied by Experimental Animal Research Center, Hebei Medical University, China.

The rats were kept at a temperature of 22–24 °C and a relative humidity of 50 ± 5%, and had access to standard laboratory food and water. This project all animal experiments were carried out according to guide lines for experimental animal management committee of Hebei Medical University, China. Before being administered *Fructus Sophorae* extract, the rats were fasted for 12 h but with access to water. Twelve rats were divided into two groups at random and then were both given single doses of *Fructus Sophorae* extract (2 mL/kg). Blood samples of approximately 0.3 mL were collected from the vein of the eye ground at 0, 10, 25, 45, 60, 75, 90, 120, 180, 270 min (the first group) and 0, 1.5, 3, 4.5, 6, 8, 10, 12,18, 24, 30, 36, 48, 72 h (the second group) after a single oral administration. The blood samples were immediately transferred to heparinized tubes and centrifuged at 4000 rpm for 10 min. Then the plasma layer was transferred to clean tubes and stored at −20 °C. Blank plasma was obtained from the rat without oral administration and was used to investigate the assay development and validation.

Non-compartmental pharmacokinetic analysis of concentration–time data was performed using Excel software. The pharmacokinetic parameters, such as maximum plasma concentration (C_{max}) and time of maximum concentration (T_{max}), were obtained directly from the plasma concentration–time plots [14]. The elimination rate constants (k) were determined by linear regression analysis of the logarithmic transformation of the last four data points of the curve [14]. The elimination half-life ($T_{1/2}$) was calculated using the following equation: $T_{1/2} = 0.693/k$ [14].

3. Results and discussion

3.1. Method development

3.1.1. Mass spectrometry

First, for developing the method, the optimization of precursor ions and product ions of the analytes and IS for MRM mode analysis by syringe pump infusion was a prerequisite. The standard solutions of the analytes and IS were infused into the mass spectrometer separately to obtain detected ions and to optimize mass parameters such as DP and CE. Then it was found that negative ESI was used for good sensitivity, reproducibility and fragmentation. In the full scan mass spectra, the deprotonated molecular ions $[M-H]^-$ of sophoricoside, genistin, genistein, rutin, quercetin, kaempferol and IS (m/z, 431.1,431.1, 269.0, 609.2, 301.0, 284.9, and 252.0) were stable and exhibited higher abundance. Thus $[M-H]^-$ were chosen as the precursor ions for MS/MS fragmentation analysis. The optimized mass transition ion pairs (m/z) for quantitation were 431.1/267.9 for sophoricoside and genistin, 269.0/133.0 for genistein, 609.2/300.0 for rutin, 301.0/150.9 for quercetin, 284.9/93.0 for kaempferol and 252.0/155.9 for IS. Fig. 1 shows the product ion scan spectra of the analytes and IS.

3.1.2. Chromatography

On the analytes, sophoricoside and genistin are structural isomers, then genistein is the hydrolysis product of them [11]. Thus the selection of the LC conditions was key in order to obtain chromatograms with better resolution of adjacent peaks, especially when similar components were analyzed. To achieve higher peak responses and shorter analysis times for the target compounds in chromatograms, the effect of different mobile phase compositions was compared and found there was no obviously distinguish between methanol–water and acetonitrile–water. Because of the high-toxicity and high-price of acetonitrile, methanol–water was chosen. Besides several mobile phase additives such as ammonium acetate (0.5, 1, and 2 mmol/L), formic acid (0.1‰, 0.5‰, and 1‰) and acetic acid (0.1‰, 0.5‰, and 1‰) were used to achieve the high sensitivity. It was found that the peak shapes and responses of analytes were becoming better with eluent A (methanol) and B (0.5‰, v/v acetic acid). Satisfactory separation was achieved in 8 min by gradient elution using the HPLC conditions described above. Representative extract ion MRM chromatograms of blank plasma, blank plasma spiked with the six analytes and IS, and sample plasma after a single oral administration of *Fructus Sophorae* extract are shown

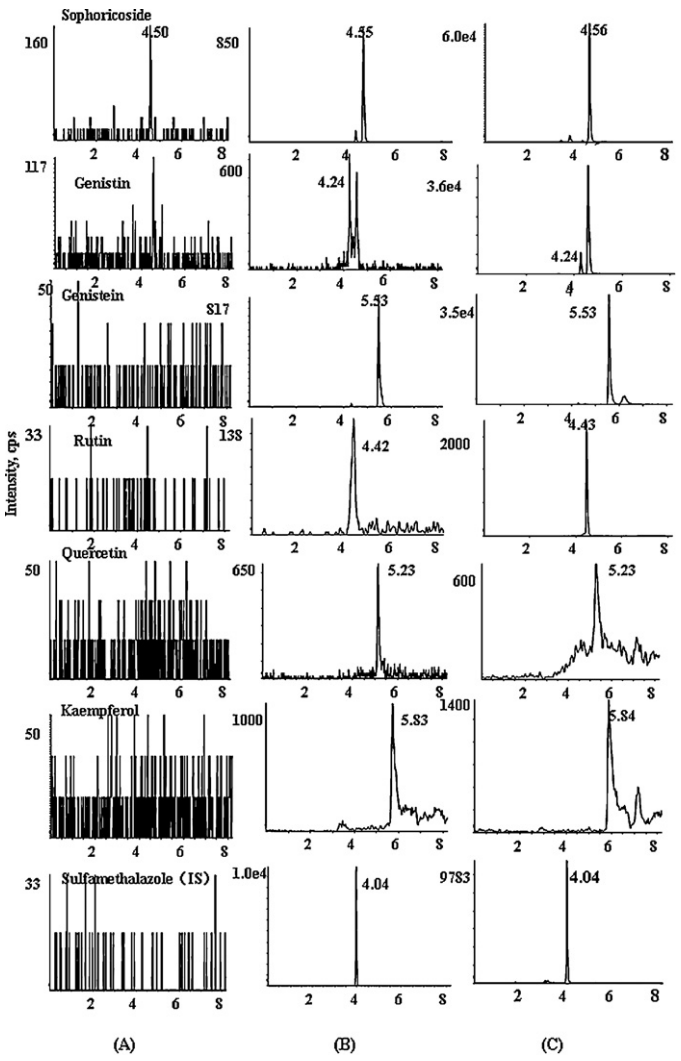

Fig. 2. Representative extract ion MRM chromatograms of sophoricoside, genistin, genistein, rutin, quercetin, kaempferol and sulfamethoxazole (IS): (A) blank plasma; (B) blank plasma spiked with the six analytes at LLOQ and IS; (C) 1.5 h sample plasma after a single oral administration of *Fructus Sophorae* extract.

in Fig. 2. Blank plasma yielded relative clean chromatograms without interfering peaks. The retention times of the sophoricoside, genistin, genistein, rutin, quercetin, kaempferol and IS were 4.55, 4.24, 5.53, 4.42, 5.23, 5.83, and 4.04 min, respectively.

3.1.3. Selection of IS

Suitable IS is the key of biological sample analysis. During the quantitative processing, it was used to correct the unknown losses. The internal standard was used to compensate for variability during the extraction procedure and the ionization process. Flavonoids are the main chemical constituents in *Fructus Sophorae* [1,6,8], which implied that it would need great effort to find a flavonoid as an ideal IS. Finally, comprehensive consideration of the interference in biological specimen, stability under this analysis procedure, and consistency with the analytes at retention time and extraction efficiency, a chemical synthetic drug named sulfamethalazole was chosen as an IS.

3.1.4. Optimization of extraction conditions

Liquid–liquid extraction (LLE) and a protein precipitation method were compared during sample preparation. In spite of the latter was much simpler and less time consuming, this method was still discarded because of its high noise level and interference by endogenous substances. Finally, LLE was applicable to use as it offers a pure sample and low matrix effect. In this study, three factors were considered: proper extraction agent (acetic ether, chloroform and diethyl ether), vortex mixing time (2, 3, and 5 min), and the temperature during centrifugation (4 °C and room temperature). Finally, ethyl acetate was selected as extraction solvent with the highest extraction efficiency and lowest noise level. However, the stronger polarity makes flavonoid glycoside difficult to be directly extracted with ethyl acetate from plasma. Thus we added appropriate concentration of hydrochloric acid (0.25 mol/L) to acid plasma to improve the extract efficiency [15]. 5 min was selected as vortex mixing time and the blood samples were centrifuged at room temperature.

3.2. Method validation

The calibration curves of six flavonoids exhibited good linearity with correlation coefficients (r) within the range from 0.9956 to 0.9988. The LLOQs were sufficient for quantitative detection of analytes in the pharmacokinetic studies. The regression equations, linear ranges, LLOQs, and LODs were shown in Table 1.

Intra-day and inter-day precision and accuracy were determined by measuring QC samples at three concentration levels. The relative errors (REs) were obtained ranging from −10.80% to 3.50% in intra-day accuracy and from −8.63% to 3.91% in inter-day accuracy with RSD less than 14.0%. The results were listed in Table 2 and indicated that the method was acceptable.

The stability of the analytes in rat plasma was investigated for three freeze–thaw cycles, for 24 h at room temperature, for 21 days at −20 °C in the freezer, and for 24 h in the stock solutions at room temperature. Stability data in Table 3 showed good stability for all the analytes over four storage conditions and determination with a concentration variation of less than 5.0% of the initial values.

The mean extraction recoveries of the investigated flavones in plasma at three different concentration levels were found to be 66.2–85.1% with RSD less than 6.7%, and no significant matrix effect for sophoricoside, genistin, genistein, rutin, quercetin, kaempferol was observed. The data are summarized in Table 4. The recoveries of IS with 400.00 ng/mL were 84.5% and the matrix effect of IS were 98.4%.

3.3. Pharmacokinetics study of free flavones in rats

The developed and validated method was applied to the pharmacokinetic evaluation of *Fructus Sophorae* in rats following oral administration. Plasma samples were obtained after intragastric gavage. The mean plasma concentration–time profiles of the investigated components were shown in Fig. 3. The pharmacokinetic parameters are presented in Table 5.

As seen from Fig. 3 and Table 5, the analytes were divided into two clusters: flavonoid glycoside (sophoricoside, genistin and rutin) and flavonoid aglycones (genistein, quercetin and kaempferol). Flavonoid glycoside could achieve the maximum plasma concentration at 1 h while flavonoid aglycones could achieve the maximum plasma concentration between 10 and 12 h after oral administration. The results show that the flavonoid glycoside in *Fructus Sophorae* was absorbed firstly, then the aglycones were absorbed. The values of the elimination rate constants k ranged from 0.0117 to 0.0201 for flavonoid glycoside and from 0.0011 to 0.0016 for flavonoid aglycones, which indicated that the flavonoid aglycones had slower elimination rates. A double-peak phenomenon of genistein is presented in Fig. 3. The first peak appeared at about 1.5 h, and the second peak appeared at about 10 h which is higher than the first peak. This phenomenon may be relevant to entero-hepatic recirculation. Bacterial metabolism in the intestine may play a significant role. Metabolic process such as

Table 1
The regression equations, linear ranges, LLOQs, and LODs of the six flavonoids.

Compounds	Regression equation	r	Linear range (ng/mL)	LLOQ (ng/mL)	LOD (ng/mL)
Sophoricoside	$y = 0.0190x + 0.7677$	0.9956	1.00–499.20	1.00	0.39
Genistin	$y = 0.0007x + 0.0943$	0.9973	9.98–4992.00	9.98	3.96
Genistein	$y = 0.0205x + 0.2628$	0.9987	1.00–500.00	1.00	0.31
Rutin	$y = 0.0008x + 0.0370$	0.9973	1.20–600.60	1.20	0.54
Quercetin	$y = 0.0139x + 0.0808$	0.9981	2.79–1394.00	2.79	0.96
Kaempferol	$y = 0.0019x + 0.1697$	0.9988	2.01–1004.40	2.01	0.53

Table 2
The intra-day and inter-day accuracies and precisions for the determination of six free analytes ($n = 3$ days, 6 replicates per day).

Compounds and spiked concentration (ng/mL)	Intra-day ($n = 6$)			Inter-day ($n = 6$)		
	Measured concentration (ng/mL)	Accuracy (%)	Precision (%)	Measured concentration (ng/mL)	Accuracy (%)	Precision (%)
Sophoricoside						
2.50	2.41 ± 0.08	−3.70	4.40	2.43 ± 0.10	−2.93	1.90
49.92	49.08 ± 2.44	−1.70	7.20	49.49 ± 3.42	−0.87	4.00
399.36	394.55 ± 5.28	−1.20	2.20	397.16 ± 8.55	−0.55	1.70
Genistin						
24.96	24.53 ± 2.93	−1.70	11.70	25.03 ± 2.81	0.29	6.00
499.20	496.35 ± 12.25	−0.60	2.50	495.95 ± 11.71	−0.65	0.40
3993.60	3963.33 ± 85.4	−0.80	2.40	3948.56 ± 90.37	−1.13	1.20
Genistein						
2.50	2.43 ± 0.35	−2.90	14.00	2.41 ± 0.32	−3.76	3.00
50.00	50.93 ± 2.73	1.90	5.20	51.96 ± 2.70	3.91	5.10
400.00	393.67 ± 8.31	−1.60	2.30	393.33 ± 8.92	−1.67	1.60
Rutin						
3.00	3.07 ± 0.15	2.20	4.60	3.05 ± 0.13	1.76	1.70
60.06	62.13 ± 6.66	3.50	10.10	62.31 ± 5.94	3.75	1.00
480.48	463.92 ± 14.85	−3.40	3.30	466.10 ± 14.97	−2.99	2.40
Quercetin						
6.97	6.73 ± 0.37	−3.40	4.30	6.90 ± 0.31	−1.01	6.00
139.40	132.33 ± 12.32	−5.10	9.70	133.28 ± 12.75	−4.39	8.30
1115.20	1154.00 ± 55.78	3.50	4.80	1153.11 ± 51.59	3.40	0.90
Kaempferol						
5.02	5.12 ± 0.11	1.90	1.70	5.05 ± 0.10	0.68	3.50
100.44	89.58 ± 4.03	−10.80	7.80	91.77 ± 7.23	−8.63	8.70
803.52	809.67 ± 15.73	0.80	1.70	812.61 ± 13.16	1.13	1.00

Table 3
Stability of the six flavonoids in rat plasma ($n = 3$).

Compounds and spiked concentration (ng/mL)	Freeze–thaw stability (3 freeze–thaw cycles)		Long-term stability (−20 °C for 21 d)		Short-term stability (room temperature for 24 h)		Extracted sample stability (room temperature for 24 h)	
	Measured concentration (ng/mL)	Deviation (%)	Measured concentration (ng/mL)	Deviation (%)	Measured concentration (ng/mL)	Deviation (%)	Measured concentration (ng/mL)	Deviation (%)
Sophoricoside								
2.50	2.40 ± 0.02	−4.0	2.43 ± 0.20	−2.8	2.42 ± 0.12	−3.2	2.49 ± 0.13	−0.4
49.92	48.95 ± 3.16	−1.9	47.70 ± 2.98	−4.4	47.53 ± 1.16	−4.8	48.97 ± 0.91	−1.9
399.36	396.60 ± 6.59	−0.7	391.37 ± 3.73	−2.0	394.20 ± 7.15	−1.3	406.10 ± 16.37	1.7
Genistin								
24.96	24.90 ± 1.82	−0.2	23.73 ± 4.42	−4.9	23.90 ± 3.18	−4.2	25.53 ± 2.02	2.3
499.20	502.33 ± 14.69	0.6	492.77 ± 13.78	−1.3	494.23 ± 12.27	−1.0	497.30 ± 16.20	−0.4
3993.60	3937.33 ± 83.51	−1.4	3937.67 ± 104.2	−1.4	3998.67 ± 100.01	0.1	3936.33 ± 128.89	−1.4
Genistein								
2.50	2.39 ± 0.34	−4.4	2.38 ± 0.25	−4.8	2.39 ± 0.29	−4.4	2.51 ± 0.38	0.4
50.00	51.90 ± 3.42	3.8	51.70 ± 3.15	3.4	52.10 ± 1.47	4.2	50.17 ± 2.65	0.3
400.00	387.67 ± 2.08	−3.1	391.67 ± 2.65	−2.1	388.33 ± 4.04	−2.9	403.00 ± 7.81	0.8
Rutin								
3.00	3.09 ± 0.14	3.0	3.08 ± 0.15	2.7	3.03 ± 0.16	1.0	3.03 ± 0.13	1.0
60.06	62.57 ± 6.57	4.2	62.17 ± 6.33	3.5	62.83 ± 4.92	4.6	61.83 ± 7.83	2.9
480.48	461.77 ± 10.90	−3.9	463.92 ± 13.85	−3.4	474.33 ± 13.05	−1.3	462.17 ± 4.25	−3.8
Quercetin								
6.97	6.84 ± 0.31	−1.9	6.84 ± 0.07	−1.9	7.03 ± 0.41	0.9	6.95 ± 0.07	−0.3
139.40	134.33 ± 15.04	−3.6	132.33 ± 15.32	−5.0	138.67 ± 18.77	−0.5	134.33 ± 5.51	−3.6
1115.20	1135.00 ± 56.31	1.8	1142.33 ± 66.40	2.4	1150.00 ± 76.22	3.1	1101.67 ± 48.60	−1.2
Kaempferol								
5.02	5.10 ± 0.02	1.6	5.06 ± 0.15	0.8	5.06 ± 0.06	0.8	4.93 ± 0.03	−1.8
100.44	95.57 ± 11.60	−4.8	95.50 ± 5.57	−4.9	97.00 ± 7.94	−3.4	98.58 ± 5.03	−1.9
803.52	800.67 ± 14.73	−0.4	789.67 ± 19.73	−1.7	799.67 ± 15.73	−0.5	805.67 ± 21.73	0.3

Table 4
Mean extraction recoveries and matrix effects of the six flavonoids in rat plasma ($n = 5$).

Component	Mean extraction recovery (%)			Matrix effect (%)		
	Low	Medium	High	Low	Medium	High
Sophoricoside	82.5 (4.2)	82.4 (3.9)	77.3 (1.6)	90.6 (5.3)	101.8 (3.4)	96.4 (2.2)
Genistin	85.1 (4.9)	73.7 (3.6)	72.3 (3.1)	90.5 (5.2)	94.6 (4.2)	102.6 (3.1)
Genistein	80.7 (6.7)	77.2 (4.6)	76.1 (2.9)	98.6 (5.5)	85.0 (4.3)	94.7 (2.8)
Rutin	68.1 (5.2)	66.2 (4.8)	71.2 (2.9)	102.6 (7.3)	87.8 (4.3)	85.8 (2.6)
Quercetin	60.8 (4.3)	75.2 (3.2)	74.6 (2.5)	90.8 (5.6)	97.2 (4.3)	104.9 (3.1)
Kaempferol	71.7 (5.2)	80.8 (4.9)	77.2 (2.9)	88.2 (4.7)	105.9 (7.4)	103.2 (2.2)

Note: Percentage RSDs are in parentheses.

Table 5
Pharmacokinetics parameters of six flavones after an oral administration of *Fructus Sophorae* extract ($n = 6$).

Pharmacokinetic parameter	Compounds					
	Sophoricoside	Genistin	Genistein	Rutin	Quercetin	Kaempferol
C_{max} (ng/mL)	496.35	103.20	483.20	397.66	342.10	407.97
T_{max} (min)	60·	60	600	60	600	720
$T_{1/2}$ (min)	59.16	37.89	641.39	34.44	448.17	476.79
k (1/min)	0.0117	0.0183	0.0011	0.0201	0.0016	0.0015
AUC_{0-t} (ng min/mL)	34073.28	6966.23	559652.47	30835.63	426380.10	829757.21
$AUC_{0-\infty}$ (ng min/mL)	35311.12	7527.79	566945.64	34647.96	427715.64	834435.45

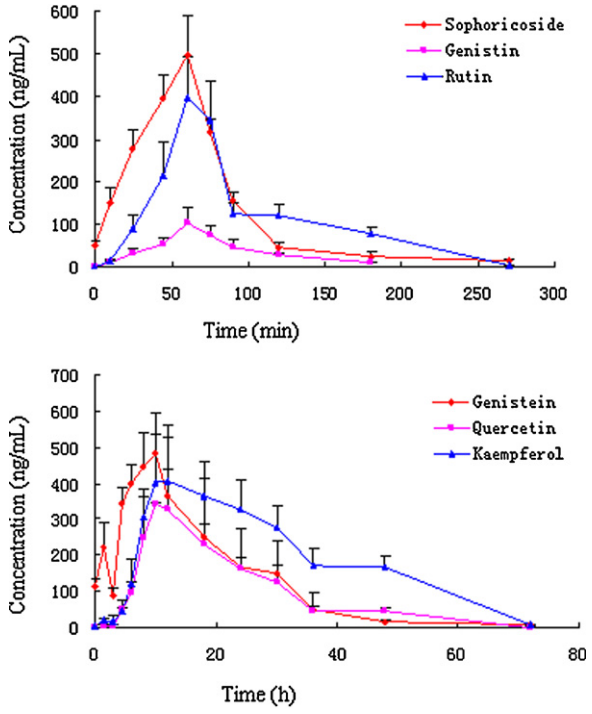

Fig. 3. Mean plasma concentration–time curves of free six flavonoids after a single oral administration of *Fructus Sophorae* extract.

de-glucose of sophoricoside and genistin might partly explain this phenomenon. These hypothesize need further investigation.

4. Conclusion

In this paper, a rapid, selective and specific LC–MS/MS method was developed for simultaneous analysis of six flavonoids in rat plasma within 8.0 min of a simple chromatographic run for the first time. The analytical procedure was successfully applied to pharmacokinetic study of the analytes after oral administration of *Fructus Sophorae* extract. Additionally, the results from this study demonstrated that the pharmacokinetic behavior of the six flavones in *Fructus Sophorae* was quite different from their pure forms or in other extracts [16–20]. The information described above might be helpful for further studies on the pharmacokinetics of *Fructus Sophorae* and may be beneficial for the application of clinical therapy.

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