

Simultaneous Determination of Kaempferide, Kaempferol and Isorhamnetin in Rat Plasma by Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry and its Application to a Pharmacokinetic Study

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A selective, rapid and sensitive method using ultra-high performance liquid chromatography was developed and used for the simultaneous determination of kaempferide, kaempferol, and isorhamnetin in rat plasma after oral administration of *Sedum sarmentosum* Bunge extract. A 6430 triple-quadrupole tandem mass spectrometer in multiple reaction monitoring mode, with a negative electrospray ionization source, was used for the detection. A Waters Symmetry C18 column with isocratic elution (methanol:5 mM ammonium acetate at 75:25, v/v) was used for separation at a flow rate of 0.3 mL min⁻¹. The method was linear for all analytes over the investigated concentration range ($r > 0.9900$). The intra- and inter-day precisions ranged between 3.88 and 7.74% and the accuracies ranged between 88.63 and 98.74%. The mean recoveries and matrix effect were higher than 85.66 and 92.36%, respectively. All the three compounds tested were stable during storage and analyses. The method was successfully applied to a pharmacokinetic study.

Keywords: *Sedum sarmentosum* Bunge, kaempferide, kaempferol, isorhamnetin, pharmacokinetics

Introduction

Traditional Chinese medicine (TCM) has been used for thousands of years in China and other Asian countries. *Sedum sarmentosum* Bunge (SSB), a perennial herb widely distributed throughout China, was mainly used for the treatment of chronic viral hepatitis and some inflammatory diseases.¹⁻⁵ It was also officially listed in the 2015 edition of the Chinese Pharmacopoeia.⁶ Previous pharmacological studies revealed that SSB can inhibit the production of inflammatory exudates and has significant anti-inflammation, anti-tumor and anti-viral activity.^{7,8} The aqueous extract exhibits anti-proliferative activity against HepG2 cells by down-regulating the expression of Bcl-2, vascular endothelial growth factor (VEGF), and phosphorylated signal transducer and activator of transcription 3 (pSTAT3) and by inducing apoptosis.^{9,10} Additionally, *in vivo* and *in vitro* renal anti-fibrotic effects

suggested that the extract may have therapeutic potential for fibrotic kidney diseases.^{11,12}

Phytochemical investigations have shown that this plant contains various components including alkaloids, sarmenosin, flavonoids and triterpenoid among others.^{13,14} Some investigations have shown that sarmenosin is the active constituent in hepatitis therapy, but its chemical properties were unstable.^{10,15} Recently, researchers isolated several bioactive flavonoids such as isorhamnetin, kaempferide, kaempferol and quercetin that exhibit pharmacological activities. Isorhamnetin is an *O*-methylated flavonol, a chemical compound derived from several plants that exhibits anti-inflammatory, anti-oxidant and anti-cancer properties.^{16,17} Kaempferide and kaempferol have similar structures with both exhibiting anti-oxidant activity.¹⁸⁻²⁰

Although some methods including high-performance liquid chromatography (HPLC) or liquid chromatography-mass spectrometry (LC-MS) have been reported for the detection of kaempferide, kaempferol or isorhamnetin in rat plasma,²¹⁻²⁴ simultaneous determination of these three constituents in plasma samples after oral administration

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of *Sedum sarmentosum* Bunge extract (SSBE) has not been reported. Thus, it was important to develop a method to investigate the pharmacokinetic parameters of SSBE. In this study, a fast, sensitive and simple ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method was validated for the determination of the three active compounds in plasma and applied to a pharmacokinetic study using rat plasma.

Experimental

Reagents and materials

The reference standards of isorhamnetin (purity > 98.0%), kaempferide (purity > 98.0%), and kaempferol (purity > 98.0%) were purchased from Chengdu Must Bio-Technology Co., Ltd. (Chengdu, Sichuan, China). Tinidazole (purity > 99.9%) was used as an internal standard (IS) and purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). SSB was obtained from Suzhou Tianling Chinese Herbal Medicine Co. Ltd. (Jiangsu, China) and identified by Professor Jianwei Chen in Nanjing University of Chinese Medicine. β -Glucuronidase and sulfatase were supplied by Sigma-Aldrich (St. Louis, MO, USA). Methanol and acetonitrile were of HPLC grade (Merck, USA). All other reagents were of AR (analytical reagent) grade. Ultrapure water used for the UHPLC-MS/MS was from Milli-Q system (Millipore, Billerica, MA, USA).

Preparation of SSBE

Dried slices (200 g) of SSB were immersed in 2 L of water and were extracted twice (2 h each time). After filtration through two layers of gauze, the combined filtrate was condensed, desiccated and pulverized. The powder was used as SSBE, containing kaempferide 7.9-8.9 mg g⁻¹, kaempferol 5.4-6.2 mg g⁻¹, and isorhamnetin 16.1-18.2 mg g⁻¹. Meanwhile, the SSBE for this pharmacokinetic study contained kaempferide 8.4 mg g⁻¹, kaempferol 5.8 mg g⁻¹, and isorhamnetin 17.2 mg g⁻¹.

Preparation of calibration standards and quality control (QC) samples

Stock solutions of kaempferide (1.005 mg mL⁻¹), kaempferol (1.004 mg mL⁻¹), and isorhamnetin (1.187 mg mL⁻¹) were prepared in methanol. Working solutions were prepared by diluting the stock solutions with methanol and stored at 4 °C until use. IS solution was

diluted to a final concentration of 100 ng mL⁻¹ with methanol. Working solutions were combined and used to spike blank plasma to prepare the calibration standards and QC samples. A series of calibration standards were prepared at 0.1, 0.5, 2, 10, 50, 150, 400 ng mL⁻¹ for kaempferide; 0.1, 0.4, 1.6, 6.4, 25.6, 51.2, 64 ng mL⁻¹ for kaempferol; and 0.2, 1, 5, 25, 125, 250, 625 ng mL⁻¹ for isorhamnetin. QC samples were prepared at 0.2, 200, 350 ng mL⁻¹ for kaempferide; 0.2, 25.2, 51.4 ng mL⁻¹ for kaempferol; and 0.4, 375, 500 ng mL⁻¹ for isorhamnetin. All calibration standards and QC samples were stored at -20 °C until analysis.

UHPLC-MS/MS instrumentation and chromatographic conditions

The Agilent UHPLC 1290 system (Agilent, USA) consisting of a quaternary pump, an auto-sampler, and an online degasser was used to perform the analysis. The chromatographic separation was performed on a Waters Symmetry C18 reversed phase analytical column (50 × 2.1 mm, 3.5 μ m). Methanol:water (75:25; v/v) containing 5 mM ammonium acetate solvent system was used as the mobile phase. The flow rate was 0.3 mL min⁻¹ and the sample injection volume was 2 μ L. The temperature of the column and auto-sampler was maintained at 35 and 4 °C, respectively. The total elution time was 2 min.

The UHPLC-MS/MS system consisted of G6430 tandem quadrupole mass spectrometer, with turbo ion spray ionization as the electro spray ionization (ESI) source, operated in a negative ionization mode, using multiple reaction monitoring (MRM) to monitor precursor to product ion transition. The ion MS of isorhamnetin, kaempferide, kaempferol and IS were given in Figure 1. All analytical data were processed using MassHunter software, version B.05.00 (Agilent).

Procedure for sample extraction

All samples were thawed at room temperature before use. 50 μ L of plasma samples and 10 μ L IS solution (100 ng mL⁻¹) were transferred to a 1.5 mL centrifuge tube. Next, 50 μ L β -glucuronidase (activity 1500 U mL⁻¹) hydrolase and 50 μ L sulfatase (200 U mL⁻¹) hydrolase were added to each plasma sample. After being subjected to a heat shock at 37 °C for 60 min, 800 μ L of ethyl acetate/acetone solvent (1:1) was added to the mixture, which was vortexed for 3 min and centrifuged for 5 min at 12,000 rpm. Subsequently, the supernatant was dried under 40 °C nitrogen, and re-dissolved in 100 μ L 50% methanol. Finally, 2 μ L supernatant was injected into the UHPLC-MS/MS system for analysis.

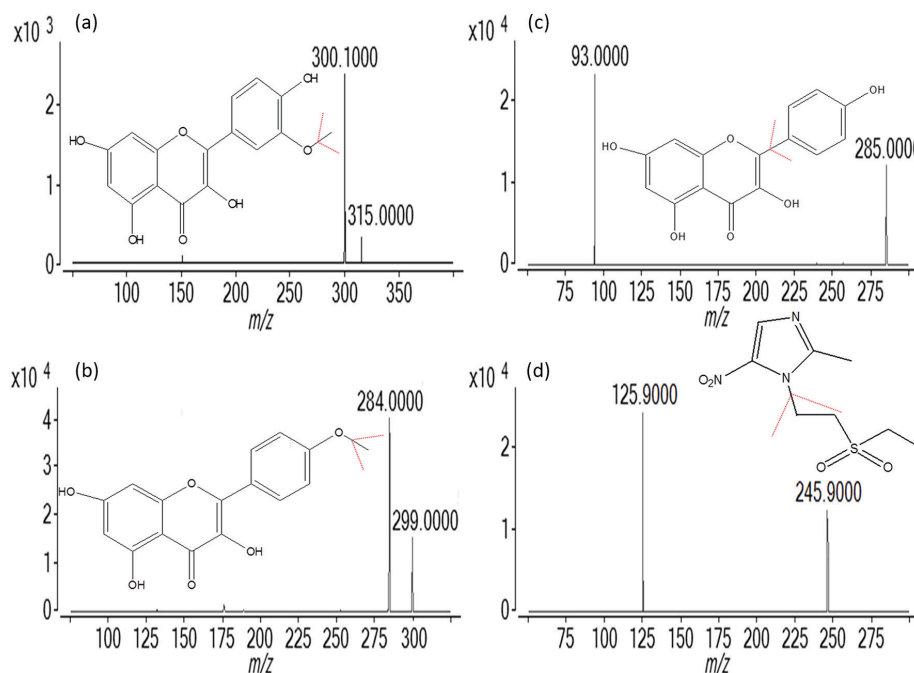


Figure 1. The fragmentation pathways for isorhamnetin (a), kaempferide (b), kaempferol (c) and IS (d).

Method validation

The method was verified adequately for specificity, selectivity, carryover, linearity (*r*), extraction recovery, matrix effect (ME), precision, accuracy and stability.

Specificity and selectivity

Specificity was determined by comparing the retention times of six blank plasma samples and blank plasma spiked with kaempferide, kaempferol, isorhamnetin, and IS from the respective chromatograms. Additionally, 2 μ L working solution was diluted with mobile phase and injected to check for interference.

Carryover

Carryover was evaluated by three injections of an upper limits of quantification (ULOQ) sample of the calibration curve, immediately followed by three injections of a blank plasma sample. Carryover was considered acceptable if the mean peak area counts of analytes and ISs were not more than 20% for analytes, and 5% for IS, compared to the area counts in the lower limits of quantification (LLOQ) sample.

Linearity and LLOQ

In order to assess the linearity for each analyte, seven non-zero concentrations of calibration samples were determined to establish the calibration curves. Blank plasma samples were analyzed to discard the presence of interferences. The linearity was confirmed through the comparison of the ratio

of the peak area of the analytes to that of the IS solution with the analyte concentrations through least squares linear regression analysis, which is described in the form of $Y = aX + b$ (weighting factor = $1/x^2$).

The LLOQ defined as the amount detected with a signal-to-noise ratio of 10, was determined in five replicates with a precision of less than 20% using the relative standard deviation (RSD) and an accuracy between 80 and 120% of the spiked concentration.

ME and extraction recovery

The ME was measured by comparing the peak area of an analyte added into post-extracted blank plasma samples with the average peak area obtained from corresponding standard solution of the analyte freshly prepared in the reconstitution solution. Meanwhile, the extraction recovery for kaempferide, kaempferol, and isorhamnetin was determined by comparing the peak area of the analyte in extracted samples with the average peak area of the samples spiked with the analyte after extraction. The experiments were performed at three QC levels in six different batches.

Precision and accuracy

The intra-batch precision and accuracy for kaempferide, kaempferol, and isorhamnetin were evaluated using five replicates at three QC levels in the same analytical run. Inter-batch precision and accuracy were evaluated through three different analytical runs by repeated analysis. The concentrations were determined from the

standard calibration curve. The accuracy was expressed as percentage relative error (RE, %), and the precision was defined as relative standard deviation (RSD, %). Accuracy is required to be within $\pm 15\%$ and precision should not exceed 15% for QC samples.

Stability

Kaempferide, kaempferol, and isorhamnetin stability in rat plasma was determined by assessing three replicates in different storage conditions as follows: (i) stability during sample preparation was assessed by keeping the samples at room temperature for 6 h; (ii) freeze-thaw stability for the plasma samples was analyzed over three freeze ($-20\text{ }^{\circ}\text{C}$) and thaw (room temperature) cycles; (iii) stability of the treated plasma samples in the auto-sampler was evaluated by preparing and storing QC samples at the auto-sampler temperature ($4\text{ }^{\circ}\text{C}$) for 24 h, and then injected for analysis; (iv) the long-term stability was determined by assaying the plasma at $-20\text{ }^{\circ}\text{C}$ for at least one month.

Pharmacokinetic and data analysis

Eight male Sprague-Dawley (SD) rats were used in the pre-clinical pharmacokinetic study of SSBE. The experiments were carried out under the supervision of the Animal Ethics Committee of Nanjing University of TCM, 14008. All animals were pathogen-free and kept in an environmentally controlled room ($24 \pm 1\text{ }^{\circ}\text{C}$ and 12/12 h light/dark cycle) for at least one week, with free access to standard chow and water. SD rats were given a single oral dose of 0.2 g kg^{-1} of SSBE. While water intake was permitted, the rats were subjected to a 12 h fast prior to the experiment. Plasma samples were collected at 0, 0.083, 0.167, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 8, 12, 24 h after oral administration of SSBE. Blood samples ($150\text{ }\mu\text{L}$) were collected into heparinized tubes through the fossa orbitalis vein and immediately centrifuged at 4000 rpm for 10 min. The separated plasma samples were collected into labeled plastic vials and stored at $-20\text{ }^{\circ}\text{C}$ until analysis.

To calculate the pharmacokinetic parameters including compartment and statistic parameters, the data were analyzed by Drug and Statistic (DAS) 3.0 pharmacokinetic software (Chinese Pharmacological Association, Anhui, China). Data were determined as the mean \pm standard deviation (SD) with parallel determination of three copies.

Results and Discussion

Optimization of UHPLC-MS/MS conditions

MS conditions were optimized to obtain more stable

and stronger signal intensities for kaempferide, kaempferol, isorhamnetin, and IS. We chose the positive and negative scan modes to evaluate ion signal, which showed that the negative ion mode was superior to the positive ion mode and the product ions at $m/z\ 299.0 \rightarrow 284.0$ for kaempferide, $m/z\ 285.0 \rightarrow 93.0$ for kaempferol, $m/z\ 315.0 \rightarrow 300.1$ for isorhamnetin, and $m/z\ 245.9 \rightarrow 125.9$ for IS.

The source parameters of the mass spectrometer were set as follows: capillary voltage 4.0 kV, gas temperature $400\text{ }^{\circ}\text{C}$ and gas flow 10 L min^{-1} . The dependent parameters of the compounds, such as fragmentor and collision energy, were respectively optimized as follows: 120 and 15 V for kaempferide, 140 and 10 V for kaempferol, 140 and 15 V for isorhamnetin, 110 and 20 V for IS. Dwell time was set as 200 ms for all analytes and IS.

Chromatography conditions were investigated to obtain a higher response, sensitivity, and a short run-time. Several mobile phase conditions such as acetonitrile-water and methanol-water, and different buffer solutions such as ammonium formate (2 and 5 mM) and formic acid (0.1 and 0.2%) were evaluated. Isocratic elution using a mobile phase consisting of methanol/5 mM ammonium formate (75:25, v/v), in 2 min using a Symmetry C18 column ($1.8\text{ }\mu\text{m}$, $2.1 \times 50\text{ mm}$) at a flow rate of 0.3 mL min^{-1} gave the best separation.

Sample preparation

To obtain minimum interference and high recoveries for kaempferide, kaempferol, isorhamnetin, and IS, protein precipitation using methanol and acetonitrile, ethyl acetate, acetone, chloroform or methylene chloride was investigated by liquid-liquid extraction (LLE). LLE using ethyl acetate/acetone (1:1) exhibited a four-fold higher extraction rate than that of other solvents, allowing the effective extraction of the analytes from the plasma. Due to the high recovery rates and low interference, LLE extraction using ethyl acetate/acetone (1:1) provides a simple and efficient method for sample preparation.

Method validation

Specificity and selectivity

Figures 2a-2c show the blank rat plasma chromatogram; blank plasma spiked with the three standard compounds and IS; and rat plasma samples 30 min after oral administration of SSBE, respectively.

The retention times were about 1.19, 1.21, 1.62 and 1.15 min for kaempferide, kaempferol, isorhamnetin, and IS, respectively. No endogenous peak was detected in the plasma samples within the retention times of the three

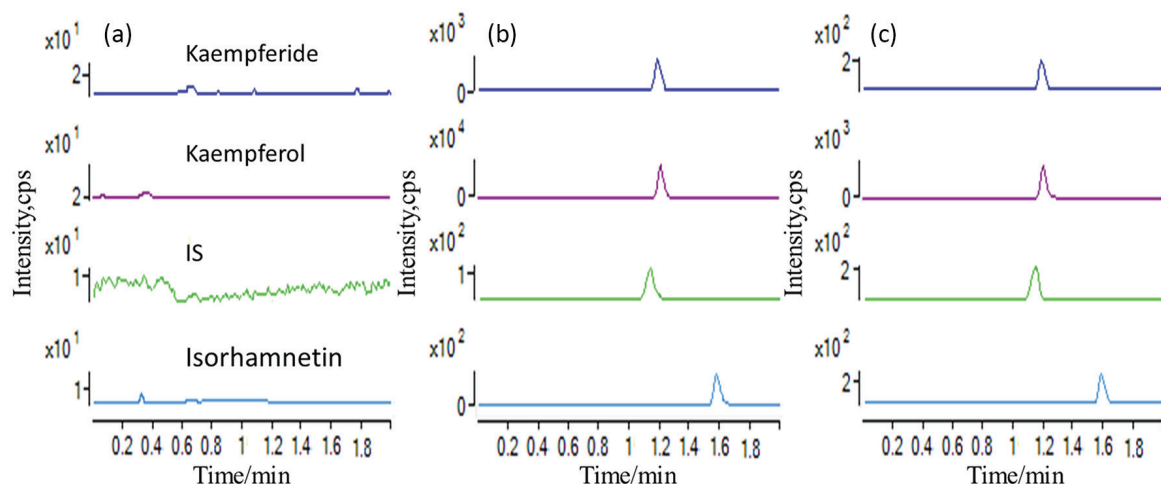


Figure 2. Compound chromatograms. (a) blank rat plasma sample; (b) blank plasma spiked with three standard compounds and IS; (c) rat plasma sample after an oral administration of SSBE at 30 min intervals.

standard compounds and IS. Therefore, it can be concluded that the method can be used to determine the levels of the three components in rat plasma.

Carryover

No peak was observed at the retention times of analytes or IS in the chromatogram of a blank sample analyzed after the injection of ULOQ sample, indicating the absence of carryover.

Linearity and LLOQ

Calibration curves for kaempferide, kaempferol, and isorhamnetin in rat plasma showed good linear relationship at 0.1-400 ng mL⁻¹, 0.1-64 ng mL⁻¹, and 0.2-625 ng mL⁻¹, respectively. The representative calibration curves are shown below: $Y = 0.8222X + 0.0009$ ($r = 0.9985$, kaempferide), $Y = 1.0213X + 0.0022$ ($r = 0.9944$, kaempferol), and $Y = 0.4105X - 0.0011$ ($r = 0.9985$, isorhamnetin).

Therefore, the LLOQ for determination of kaempferide, kaempferol, and isorhamnetin was 0.1, 0.1 and 0.2 ng mL⁻¹, respectively.

ME and recovery

The recovery and ME were estimated by analyzing the three levels of QC samples. As seen in the data summarized in Table 1, the QC sample recoveries were within 85.66-92.21% and ME was between 92.36-101.32%. These results indicate that the extraction method for the analytes is robust and reliable.

Accuracy and precision

The precision and accuracy for kaempferide, kaempferol, and isorhamnetin in rat plasma are summarized in Table 2. The accuracy data were within 88.63-98.74%, and the

Table 1. Recovery and ME (matrix effect) for kaempferide, kaempferol, isorhamnetin

Analyte	Concentration / (ng mL ⁻¹)	Recoveries (n = 5) / %	ME / %
Isorhamnetin (n = 3)	0.4000	90.11 ± 4.41	92.36 ± 5.36
	375.0	91.06 ± 2.55	96.51 ± 3.68
	500.0	90.68 ± 4.21	96.89 ± 4.33
Kaempferide (n = 3)	0.2000	85.66 ± 3.12	99.22 ± 6.21
	200.0	88.95 ± 2.22	97.67 ± 2.02
	350.0	88.19 ± 3.19	97.57 ± 3.69
Kaempferol (n = 3)	0.2000	89.26 ± 5.02	101.32 ± 4.15
	25.20	91.65 ± 2.53	100.36 ± 2.69
	51.40	92.21 ± 3.66	99.21 ± 3.68

intra- and inter-day precision RSD% between 3.88-7.23 and 4.44-7.74, respectively. The results demonstrated that the accuracy and precision values were within the acceptable range.

Stability

Stability results (n = 3) are summarized in Table 3, which show that all analytes were stable in rat plasma for 6 h at room temperature, three freeze-thaw cycles carried out at room temperature for 4 h, and after being stored at -20 °C for at least one month. There was no significant degradation when the extracted plasma samples were stored at 4 °C for 24 h in an auto sampler. The deviation was within 9.75%, which indicated that the method was reliable for routine analysis.

Application to the pharmacokinetic study

The above method was successfully used to study the pharmacokinetics of SSBE in rats with a single-dose, oral administration of 0.2 g kg⁻¹ in eight rats.

Table 2. Intra- and inter-day accuracy and precision for kaempferide, kaempferol, and isorhamnetin in rat plasma

Analyte	Concentration / (ng mL ⁻¹)	Intra-day (overall mean, n = 5)			Inter-day (overall mean, n = 15)		
		Concentration found / (ng mL ⁻¹)	Accuracy / %	RSD / %	Concentration found / (ng mL ⁻¹)	Accuracy / %	RSD / %
Isorhamnetin	0.4000	0.3754	93.85	6.12	0.3545	88.63	6.65
	375.0	368.5	98.27	4.58	361.1	96.29	6.01
	500.0	488.7	97.74	4.99	475.2	95.04	5.47
Kaempferide	0.2000	0.1886	94.30	7.23	0.1809	90.45	7.74
	200.0	195.7	97.85	4.57	192.1	96.05	4.51
	350.0	345.6	98.74	4.02	341.2	97.49	4.89
Kaempferol	0.2000	0.1907	95.35	3.88	0.1798	89.90	4.44
	25.20	18.52	96.46	4.21	18.48	96.25	5.04
	51.40	37.22	96.93	5.56	36.58	95.26	5.23

RSD: relative standard deviation.

Table 3. Analyte stability in rat plasma under different storage conditions (mean ± standard deviation, n = 3)

Storage condition	Kaempferide			Kaempferol			Isorhamnetin		
	Added / (ng mL ⁻¹)	Measured / (ng mL ⁻¹)	Deviation / %	Added / (ng mL ⁻¹)	Measured / (ng mL ⁻¹)	Deviation / %	Added / (ng mL ⁻¹)	Measured / (ng mL ⁻¹)	Deviation / %
Short-term stability	0.2000	0.19 ± 0.00	-6.45	0.2	0.19 ± 0.00	-4.65	0.4	0.38 ± 0.00	-4.90
	200.0	209.1 ± 2.58	4.5	25.2	18.52 ± 0.44	-3.54	375	387.5 ± 12.57	3.33
	350	342.7 ± 7.58	-2.1	51.4	37.22 ± 0.91	-3.07	500	479.8 ± 14.85	-4.04
Three freeze-thaw cycles	0.2000	0.22 ± 0.00	9.75	0.2	0.20 ± 0.00	0.90	0.4	0.37 ± 0.00	-6.38
	200.0	211.6 ± 2.84	5.8	25.2	18.08 ± 0.33	-5.83	375	364.3 ± 11.74	-2.85
	350	340.2 ± 6.88	-2.8	51.4	36.48 ± 0.85	-5.00	500	482.5 ± 10.58	-3.50
At 4 °C in the auto-sampler for 24 h	0.2000	0.19 ± 0.00	-7.6	0.2	0.19 ± 0.00	-5.35	0.4	0.36 ± 0.00	-8.55
	200.0	211.2 ± 1.59	5.6	25.2	18.18 ± 0.26	-5.31	375	384.5 ± 10.02	2.53
	350	365.9 ± 4.25	4.55	51.4	38.12 ± 0.47	-0.73	500	504.6 ± 13.13	0.92
Long-term stability	0.2000	0.19 ± 0.00	-9.3	0.2	0.19 ± 0.00	-5.60	0.4	0.42 ± 0.00	5.35
	200.0	188.7 ± 3.55	-5.65	25.2	20.25 ± 0.36	5.47	375	372.5 ± 11.57	-0.67
	350	334.6 ± 6.87	-4.4	51.4	36.97 ± 0.76	-3.72	500	486.7 ± 14.01	-2.66

Pharmacokinetic parameters such as AUC₀₋₂₄ and AUC_{0-∞} (area under the concentration time curve truncated), t_{1/2} (half-life of drug elimination during the terminal phase), C_{max} (maximum concentration observed) and T_{max} (time to reach C_{max}), for kaempferide, kaempferol and isorhamnetin were determined (Table 4). The mean plasma concentration-time curves after oral administration of SSBE are shown in Figure 3. The data conformed to a two-compartment, first-order pharmacokinetic model.

The difference in the level of the three components in the SSBE could be responsible for the variable results. The T_{max} was between 0.50 ± 0.00 h and 1.50 ± 0.58 h, which indicates the difference in metabolism of the three compounds *in vivo*. The AUC₀₋₂₄ was between 195.88 ± 9.55 and 683.64 ± 98.54 ng h mL⁻¹. These results provide a basis

for further studies on SSB to determine the effectiveness of this TCM in clinical treatment.

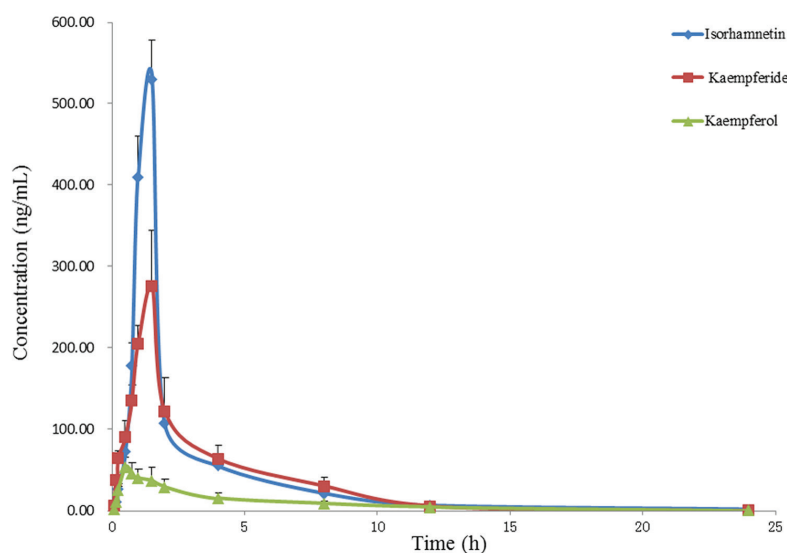
Conclusions

The primary aim of this study was to present a novel and validated UHPLC-MS/MS method, which could be used for the simultaneous analysis of kaempferide, kaempferol, and isorhamnetin in rat plasma after oral administration of SSBE. The advantages of the method presented in this paper are simple sample preparation and simultaneous determination of three analytes within a short run time (2 min), providing a high throughput approach for sample analysis. The small starting volume of plasma (50 µL) is especially useful because of the low blood volume obtained

Table 4. Pharmacokinetic parameters of the analytes after an oral administration of SSBE

Parameter	Kaempferide	Kaempferol	Isorhamnetin
C_{max} / (ng mL ⁻¹)	276.22 ± 58.51	54.03 ± 8.24	530.24 ± 86.98
T_{max} / h	1.50 ± 0.58	0.50 ± 0.00	1.50 ± 0.35
$t_{1/2\beta}$ / h	3.56 ± 1.06	2.92 ± 0.25	2.76 ± 0.19
$AUC_{(0-24)}$ / (ng h mL ⁻¹)	683.64 ± 98.54	195.88 ± 9.55	524.08 ± 23.42
$AUC_{(0-\infty)}$ / (ng h mL ⁻¹)	684.94 ± 100.35	196.57 ± 25.26	525.69 ± 20.57
Vd / L	0.51 ± 0.02	15.75 ± 4.24	9.82 ± 2.55
$MRT_{(0-t)}$ / h	3.63 ± 0.69	4.59 ± 0.77	3.13 ± 0.63
$MRT_{(0-\infty)}$ / h	3.66 ± 0.58	4.69 ± 0.81	3.19 ± 0.58
CL / (L h ⁻¹)	0.04 ± 0.01	0.95 ± 0.02	19.02 ± 1.76

C_{max} : maximum concentration observed; T_{max} : time to reach C_{max} ; $t_{1/2\beta}$: half-life of drug elimination during the terminal phase; AUC: area under the concentration time curve truncated; Vd: volume of distribution; MRT: mean residence time; CL: serum clearance.

**Figure 3.** Plasma concentration vs. time profile of the analytes with an oral administration of SSBE in rats (n = 8).

from a single rat for routine pharmacokinetic studies. Additionally, this UHPLC-MS/MS with a simple liquid extraction can ultimately be used in other pre-clinical and clinical samples (plasma, urine, tissue) for future pharmacokinetic studies.

The results of our study will be useful for future studies on the mechanism of SSB, and could provide valuable information for the clinical application of TCM.

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