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ORIGINAL RESEARCH
PAPER

Simultaneous determination of three active flavonoids in rat plasma by HPLC-MS/MS: Application to a comparative pharmacokinetic investigation after oral administration of *Schizonepeta tenuifolia* aqueous extract with and without its volatile oil

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ABSTRACT

Schizonepeta tenuifolia Briq. (ST) has been used as an aromatic exterior-releasing medicine in clinical practice for thousands of years in China. Previous researches have revealed both volatile oil (STVO) and aqueous extract (STAE) from ST showed significant pharmacological activities, such as anti-virus, anti-inflammation, anti-oxidation, and immunoregulation. However, the influence between each other was still unknown. The purpose of this study was to compare the pharmacokinetic profiles of three main flavonoids (luteoloside, apigenin, and hesperidin) in STAE to illustrate the influence of STVO. A liquid chromatography-tandem mass spectrometry (HPLC-MS) method was established to quantitatively analyze the three absorbed ingredients in the plasma of healthy rats. Biological samples were analyzed on an Agilent Eclipse Plus C₁₈ column (3.0 mm × 150 mm, 3.5 μm) with gradient mobile phase (containing 0.2% formic acid and acetonitrile) at a flow rate of 0.8 mL/min. All the analytes and quercitrin (IS) were investigated with an electrospray ionization source (ESI) using multiple-reaction monitoring (MRM) in negative ionization mode. In addition, this quantitative method showed good linearities ($r \geq 0.9995$) and the lower limits of quantification were 0.590–1.19 ng/mL. The intra- and inter-day precisions ranged 3.47–10.45% and 4.29–11.28% for the three analytes. The mean extraction recoveries were in the range of 77.41–109.79% and the average matrix effects were within 83.41–112.67%. The validated method has been fully applied to compare the pharmacokinetic parameters of the three flavonoid glycosides in rat plasma after oral administration of STAE and STAE+STVO. In comparison of luteoloside, apigenin, and hesperidin in STAE group, it was found that different degree of increasing existed for the time to reach the maximum concentration (T_{max}), elimination half-life time ($T_{1/2}$), the area under the concentration curves ($AUC_{0 \rightarrow t}$ and $AUC_{0 \rightarrow \infty}$) and the maximum concentrations (C_{max}) in STAE+STVO group. As can be seen from above results, STVO could improve the absorption and bioavailability of the three analytes. These findings would provide some active and strong basis of safe clinical application for ST and further exploitation for STVO from the perspective of drug–drug interaction.

KEYWORDS

HPLC-MS/MS, *Schizonepeta tenuifolia*, pharmacokinetics, rat plasma

ABBREVIATIONS

ST, *Schizonepeta tenuifolia*, STVO, *Schizonepeta tenuifolia* volatile oil, STAE, *Schizonepeta tenuifolia* aqueous extract, HPLC-MS/MS, high performance liquid chromatography-tandem mass spectrometry

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INTRODUCTION

Schizonepeta tenuifolia (ST) is a common medicinal plant of *Lamiaceae* family widely spread in China, Korea, and Japan. This medicinal plant has been widely used for treatment of common cold, sore throat, headache, allergic dermatitis, eczema, psoriasis and otitis media for a long time. Lines of evidence in modern pharmacological studies have demonstrated its various biological properties, such as immunomodulation [1, 2], anti-inflammation [3, 4], anti-oxidation [5], and anti-diabetes [6]. Thus, this phytomedicine is still recorded in the present Chinese Pharmacopoeia [7].

For a long time, due to its fragrance, ST volatile oil (STVO) has been considered as the fundamental material basis of the phytomedicine and the bioactive volatile terpenoids have been listed as its quality markers, such as (+)-pulegone, (–)-menthone, (–)-limonene and (+)-menthofuran, which was supported by many studies [8–12]. On the other hand, as the classical dosage form of traditional Chinese medicine (TCM) for thousands of years, aqueous extract (STAE) also exhibited similar effects with ST [4, 13–15]. What's more, there were many kinds of nonvolatile components from STAE, especially the flavonoid glycosides with multiple bioactivities including hesperidin (hesperetin-7-O-rutinoside), luteoloside (luteolin 7-O-glucoside) and apigetrin (apigenin 7-O-glucoside) [16–18], possibly responsible for the efficacies of ST. For instance, hesperidin could ameliorate pancreatic beta-cell dysfunction and apoptosis in streptozotocin-induced diabetic rat model and attenuate LPS-induced acute lung injury in mice by inhibiting HMGB1 release [19, 20]. Luteoloside showed its anti-inflammatory and anti-allergic activity through reducing the number of T cells, mast cells and histiocytes in mouse skin with inflammation or atopic dermatitis [21]. Apigetrin was found to inhibit LPS-induced inflammation through the inactivation of AP-1 and MAPK signaling pathways in vivo and in vitro [22].

In addition, in the preparation of modern Chinese medicine, ST is often extracted to yield STVO by steam distillation method. At the same time, the decoction is also obtained and considered as STAE. Then, the concentrated STAE, STVO and some pharmaceutical excipients would be mixed and prepared to produce some Chinese patent medicines. Many similar examples containing ST could be found in the present Chinese Pharmacopoeia, such as Chuanxiong Chatiao Pills, Xiaer Jindan Pills, Xiaer Chiqiao Qingre Granules, and Niu Huang Shangqing Pills [7]. This pharmaceuticals technology not only utilizes the phytomedicine completely, but also demonstrates the two parts' effects to the full extent. Then, besides the combined effects, what influences would this combined application demonstrate on the active components in STVO and STAE as far as pharmacokinetics is concerned? To our knowledge, there have been some pharmacokinetic articles of luteoloside, apigetrin, and hesperidin in the administration form of monomeric compound, plant extract, and Chinese patent medicine [23–27]. The interaction studies of their combination with other

drugs especially volatile oils were hardly involved. Therefore, when combination, whether the pharmacokinetic characteristics of the three active flavonoids would change is an interesting issue worth investigating.

In this study, in view of the effect of STVO on bioavailability of luteoloside, apigetrin, and hesperidin in STAE, a simple, rapid and sensitive HPLC–MS method was developed for the simultaneous determination of the three bioactive flavonoids in rat plasma and applied to indicate their behavioral changes under the influence of STVO in normal rat from the perspective of pharmacokinetics. Furthermore, we hope the obtained results would be helpful for safe utilization of ST and even for understanding of volatile oil's role in combined administration of drugs.

EXPERIMENTS

Materials and reagents

ST was purchased from Chinese herbal medicine market in Anguo City, Hebei Province in July 2019, and then was authenticated by Prof. Qinan Wu, a botanist of Nanjing University of Chinese Medicine. The voucher specimens were kept in the laboratory.

The reference standards of luteoloside, apigetrin, hesperidin, and quercitrin (IS) were bought from Nanjing Jin Yibai Biological Technology Co., Ltd. Their purities were determined as above 98% by HPLC method. They were stored in accordance with their instructions.

Methanol and acetonitrile were both of LC-MS grade and obtained from Merck (Merck, Darmstadt, Germany). Formic acid of HPLC grade was purchased from Anaqua Chemicals Supply (ACS, Houston, USA). Ultrapure water was purified by a Milli-Q water purification system (Millipore, Burlington, MA, USA). All other reagents were of analytical grade.

Preparation of STVO and STAE from ST

Using a set of standard apparatus including a round-bottom flask, a volatile oil extractor, a condenser pipe and heating equipment, STVO with a yield of 1.08% was obtained by a steam distillation method recorded in the present Chinese Pharmacopoeia [7]. To remove the trace water, some anhydrous sodium sulfate was added into STVO, which was then stored in the dark glass bottle at 4 °C and filtered before use. At the same time, to remove some impurities, the decoction in the round-bottom flask was transferred into a separating funnel and added with three times the amount of 95% ethanol. After the mixture was kept at 4 °C over night, the supernatants were collected and evaporated by a vacuum-rotary unit and dried in a vacuum desiccator to prepare STAE with a yield of 10.22%.

Instrument and chromatographic conditions

Chromatographic analysis was carried out on a Shimadzu Nexera X2 series LC system (Shimadzu, Tokyo, Japan),

equipped with a LC-30AD quaternary pump, a SIL-30AC autosampler, a DGU-20A on-line degasser, and a CTO-20AC thermostat. An Agilent Eclipse Plus C₁₈ column (3.0 mm × 150 mm, 3.5 μm) was used for separation. The mobile phase consisted of with 0.2% formic acid (A) and acetonitrile (B) at a flow rate of 0.8 mL/min with the gradient elution as follows: 0.0–2.0 min, 5–15% B; 2.0–6.0 min, 15% B; 6.0–10.0 min, 15–53% B; 10.0–12.0 min, 53–95% B; 12.0–14.0 min, 95–5% B; 14.0–17.1 min, 5% B. The column temperature was maintained at 30 °C and the injection volume was 2 μL.

An AB Sciex 4500 Qtrap™ mass spectrometer (AB Sciex, Foster City, CA, USA) with electron spray ionization (ESI) in the negative mode was utilized in this study. The precursor-to-product ion transitions in multiple-reaction monitoring (MRM) mode were used for mass analysis and quantification. Some MS setting conditions were defined as follows: curtain gas pressure at 20 psi, ion spray voltage at 5,000 V, turbo gas temperature at 300 °C, both nebulizer gas 1 and auxiliary gas 2 at 55 psi. Nitrogen of high purity was employed as both nebulizing gas and drying gas. All the data were obtained and analyzed by Analyst 1.6.3, a data acquisition and processing software.

Animal experiments

In the pharmacokinetic experiment, SPF male Sprague-Dawley rats (220–250 g) were supplied by Shanghai Jiesijie Experimental Animal Co., Ltd. (SCXK (Hu) 2018-0004, Shanghai, China). The rats were housed under ambient temperature of 23 ± 2 °C with a 12 h light/dark cycle and 50% relative humidity in the laboratory. They were well looked after in the plastic cages with fresh water and free pellet food and were acclimatized for a week. Prior to the experiment, all the rats were fasted for 12 h with free access to water. To minimize the pain and suffering of the rats, the experimental procedures were performed in line with the guidelines of the Animal Care and Use Committee of Nanjing University of Chinese Medicine (Nanjing, China). The protocol was also approved by the animal experimental committee of Nanjing University of Chinese Medicine (201909A008).

Preparation of standard solutions and quality control samples

The reference standards of the three target flavonoids were separately and accurately weighed and dissolved in methanol to prepare the stock standard solutions with the concentrations of 0.238 mg/mL for luteoloside, 0.193 mg/mL for apigetrin, and 0.118 mg/mL for hesperidin. Then, the calibration standard solutions were obtained by mixing and serially diluting above three stock standard solutions with methanol. Additionally, IS stock solution of quercitrin was prepared with the concentration of 200 ng/mL in methanol. All these solutions were enclosed and stored at 4 °C until analysis.

The plasma samples of calibration standards were prepared by spiking 10 μL of appropriate standard solution into

90 μL of blank plasma to obtain the final concentrations of 1.19–238 ng/mL for luteoloside, 0.965–193 ng/mL for apigetrin, 0.590–118 ng/mL for hesperidin, respectively. Quality control (QC) samples at low, middle and high concentrations (1.19, 119, and 190 ng/mL for luteoloside; 0.965, 96.5, and 154 ng/mL for apigetrin; 0.590, 59.0, and 94.4 ng/mL for hesperidin) were also prepared according to the similar procedures.

Biological sample preparation

One hundred microliter of plasma samples and 10 μL of IS solution was added to a centrifuge tube and mixed with 500 μL acetonitrile for protein precipitation. The mixture was vortexed for 3 min and then centrifuged at 13,000 rpm for 10 min at 4 °C. The supernatant was transferred to another centrifuge tube and then desiccated with an Eppendorf Concentrator Plus (Hamburg, Germany). Finally, the residue was redissolved and vortex-mixed with 120 μL of acetonitrile/water (50:50, v/v) for 5 min and centrifuged at 13,000 rpm for 10 min. The supernatant was then injected into the LC-MS/MS system for analysis.

Method validation

Based on the bioanalytical guidance of Food and Drug Administration (FDA) [28] and International Conference on Harmonization (ICH) [29], the method validation was carried out in terms of its specificity, linearity, sensitivity, accuracy, precision, matrix effect, recovery and stability.

The chromatograms of some biological samples were compared to investigate the method specificity, including blank plasma samples, plasma samples spiked with luteoloside, apigetrin, hesperidin at lower limits of quantification (LLOQ) and IS, plasma samples after oral administration of STAE+STVO and STAE.

The calibration curve of each analyte was obtained by plotting its peak area ratio (y) to IS versus its nominal concentration (x) with weighted ($1/x^2$) least-squares linear regression. The method sensitivity was evaluated by LLOQ of each analyte with its signal-to-noise ratio of 10:1 in the matrix-analyte samples, which was conformed to the lowest concentration of the standard curve with an acceptable accuracy ($RE \leq \pm 20\%$) and precision ($RSD \leq 20\%$).

Six QC samples at each level (low, medium, high) were used to in the method precision and accuracy test. The intra-day precision test was performed by repeatedly analyzing each QC sample in a day while the inter-day precision test was carried out by analyzing each QC sample on three consecutive days. The analytical precision was expressed by relative standard deviation (RSD) and relative error (RE), respectively. RE was calculated with this formula: $RE (\%) = (\text{measuring value} - \text{nominal value}) \times 100\% / \text{nominal value}$. The pass criterion for RSD was required to be less than 15%, and that for RE was required to be within $\pm 15\%$.

The extraction recovery and the matrix effect were evaluated with six replicates by the formulas as follows: $(A_1/A_2) \times 100\%$ and $(A_2/A_3) \times 100\%$, respectively. A_1 : the peak areas obtained from the post-treated plasma samples spiked

with the target components at the three QC levels; A_2 : the peak areas obtained from the pre-treated plasma samples spiked with the target components at the three QC levels; A_3 : the peak areas obtained from the standard solutions at the equivalent concentrations. The extraction recovery and matrix effect of IS were also measured by the same approach at the identical concentration.

The stability of each analyte in rat plasma was evaluated by analyzing six replicates of the QC samples at low, middle, and high concentrations in the following practical experimental conditions: storage at 4 °C for 12 h, three freeze-thaw cycles (freezing at –80 °C for 24 h and thawing at room temperature) and long-term storage at –80 °C for 2 weeks. The acceptable RE was within $\pm 15.0\%$ of the nominal concentration.

Pharmacokinetic study

For pharmacokinetic study, all the rats were divided into two groups ($n = 6$ per group) randomly. The given dosages of two test extracts were equivalent to about 5g ST/kg. STAE group: the rats were administrated orally with STAE (500 mg/kg), dissolved in 0.5% carboxy methyl cellulose sodium (CMC–Na) solution. STAE+STVO group: the rats were administrated orally with STAE (500 mg/kg) and STVO (50 mg/kg), dissolved in 0.5% CMC–Na solution. Blood samples were collected from the venous plexus of the eye socket and transferred in a heparinized centrifuge tube (1.5 mL) after oral administration (0, 5, 10, 20, 30, 45, 60, 120, 240, 480, and 720 min). Each sample was immediately centrifuged at 13,000 rpm for 10 min at 4 °C to acquire the plasma. At last, the plasma was immediately stored at –80 °C until analysis.

Data analysis

The plasma concentrations of the three flavonoids during the experiment were calculated according to the daily calibration curve. With Drug And Statistics Version 3.2.8 (DAS 3.2.8) software (Mathematical Pharmacology Professional Committee

of China, Shanghai, China), a non-compartmental method was employed to analyze some representative pharmacokinetic parameters, such as the time to reach maximum concentration (T_{max}), elimination half-life time ($T_{1/2}$), the area under the concentration curves ($AUC_{0 \rightarrow t}$ and $AUC_{0 \rightarrow \infty}$) and the maximum concentration (C_{max}). Data were demonstrated as the mean $\pm$ standard deviation (SD) with triplicate. The differences of pharmacokinetic parameters from two groups were tested by Student's t -test. Statistical analysis was operated by Graphpad Prism 7.0 software.

RESULTS AND DISCUSSION

Method development

As far as peak shape and analysis time were concerned, it was revealed upon comparison that an Agilent Eclipse Plus C_{18} column (3.0 mm $\times$ 150 mm, 3.5 μ m) showed better performance than a Phenomenon Luna C_{18} column (4.6 mm $\times$ 250 mm, 5 μ m), a Hanbon Megres C_{18} column (4.6 mm $\times$ 250 mm, 5 μ m). The mobile phase system consisting of 0.2% formic acid and acetonitrile was employed for gradient elution with the flow rate of 0.8 mL/min.

Due to similar chemical structure, appropriate retention time and nonexistence in ST and rats, quercitrin was selected as the internal standard for quantification of luteoloside, apigenin, and hesperidin (Fig. 1). For the responses of these four flavonoid glycosides, the negative mode was much better in comparison with the positive mode during MS detection. So, their deprotonated ions $[M-H]^-$ were considered as the precursor ions (Q1) and the products of glycosidic bond cleavages were selected as the product ions (Q3). Compound dependent MS parameters including collision energy (CE), collision cell exit potential (CXP) and declustering potential (DP) values were optimized and designed by Applied Biosystems/MDS Sciex Analyst software 1.5.2. These parameters were demonstrated in Table 1.

Fig. 1. Chemical structures of luteoloside (1), apigenin (2), hesperidin (3) and quercitrin (4, IS)

Table 1. MS parameters of luteoloside, apigetrin, hesperidin, and quercitrin (IS)

Compound	t_R (min)	Q1 (m/z)	Q3 (m/z)	DP (V)	CXP (eV)	CE (eV)
Luteoloside	7.86	447.1	285.0	210	25	36
Quercitrin (IS)	8.54	447.2	301.0	180	15	30
Apigetrin	10.19	431.1	269.0	185	19	34
Hesperidin	12.22	609.2	301.0	170	21	32

It is well known that protein precipitation, liquid-liquid extraction and solid phase extraction are the common methods for plasma sample preparation. In our trial test, low extraction recoveries of the analytes were found in liquid-liquid extraction and could not meet the specifications of pharmaceutical analysis. Too many cartridges would be used and significant time would be required when solid phase extraction was employed. Thus, protein precipitation with acetonitrile was selected to prepare plasma samples owing to its easy operation, high efficiency and low cost.

Method validation

The representative chromatograms acquired from blank plasma, blank plasma added with the three target flavonoids at LLOQ and IS, and plasma sample at 1 h after oral administration of STAE and STAE+STVO are shown in Fig. 2. Due to the efficient sample preparation and high selectivity of MRM, there was no obvious endogenous interference for the separation and analysis of the four flavonoids in the plasma.

The linear regression equations, correlation coefficients (r) and LLOQs of luteoloside, apigetrin, and hesperidin were summarized in Table 2. The correlation coefficients (≥ 0.9995) indicated their good linearities in 1.19–238 ng/mL for luteoloside, 0.965–193 ng/mL for apigetrin, and 0.590–118 ng/mL for hesperidin. The LLOQs (0.590–1.19 ng/mL) were also appropriate for quantitative detection.

As shown in Table 3, the results of the intra- and inter-day precision and accuracy of the analytes in QC samples were exhibited. For the three QC levels of the analytes, in the intra-day test, RSD values varied from 3.47 to 10.45%, while RE values ranged between –3.82 and 5.43%. Similarly, in the inter-day test, RSD values varied from 4.29 to 11.28%, while RE values ranged between –7.09 and 9.24%. All these precision and accuracy results were within the acceptable ranges for analysis in biological media, indicating the reliability and reproducibility of the method.

As for the three QC levels, the average extraction recoveries of luteoloside, apigetrin, and hesperidin ranged

Table 2. The regression equations, linear ranges, correlation coefficients and LLOQs of luteoloside, apigetrin, and hesperidin

Analyte	Regression equation	Linear range (ng/mL)	R	LLOQ (ng/mL)
Luteoloside	$y = 0.0750x + 0.1836$	1.19–238	0.9998	1.19
Apigetrin	$y = 0.0598x - 0.1277$	0.965–193	0.9995	0.965
Hesperidin	$y = 0.0711x + 0.2943$	0.590–118	0.9996	0.590

Fig. 2. Representative MRM chromatograms of luteoloside (1), apigetrin (2), hesperidin (3) and quercitrin (4, IS) in rat plasma (A. blank plasma; B. blank plasma added with the three analytes at LLOQ and IS; C. plasma sample at 1 h after oral administration of STAE; D. plasma sample at 1 h after oral administration of STAE and STVO)

Table 3. Intra-day and inter-day precision and accuracy of luteoloside, apigetrin, and hesperidin

Analyte	Concentration (ng/mL)	Intra-day		Inter-day	
		Precision (RSD, %)	Accuracy (RE, %)	Precision (RSD, %)	Accuracy (RE, %)
Luteoloside	1.19	10.45	−1.47	8.86	−0.37
	119	5.69	1.43	5.71	3.63
	190	3.47	−1.29	4.29	−7.09
Apigetrin	0.965	9.52	3.85	11.28	7.14
	96.5	8.95	−3.82	9.69	2.54
	154	5.53	0.91	4.53	1.90
Hesperidin	0.590	9.28	5.43	7.46	9.24
	59.0	6.03	1.36	7.62	4.81
	94.4	5.77	−1.34	5.08	−1.00

from 77.41 to 109.79% while their average matrix effects were between 83.41 and 112.67% (Table 4). Besides, all of the RSD values were below 15.00%. Thus, the results within the acceptable ranges demonstrated that the analytes could be extracted from the plasma efficiently and matrix effects were seldom found for this method.

The stability of the three analytes in rat plasma during the sample storing and processing procedures was fully evaluated. The results (Table 5) showed that luteoloside, apigetrin, and hesperidin were stable in rat plasma after three freeze-thaw cycles, for 12 h at 4 °C and for 2 weeks at −80 °C after plasma sample preparation. The RE values were calculated in the range from −7.13 to 11.54% and the RSD values were not more than 9.38%, which demonstrated that the sample solutions were stable under above various conditions.

Pharmacokinetic study

The developed assay method was sensitive and accurate enough to be applied in the pharmacokinetic study of luteoloside, apigetrin, and hesperidin in rat plasma following oral administration of STAE and STAE+STVO. The plasma concentration-time curves of the three analytes are present in Fig. 3. The pharmacokinetic parameters are summarized in Table 6, including T_{max} , $T_{1/2}$, C_{max} , $AUC_{0 \rightarrow t}$, and $AUC_{0 \rightarrow \infty}$.

In Fig. 3, it can be seen intuitively that the concentration-time curve of each flavonoid glycoside in rat plasma after oral administration of STAE+STVO is similar as that of STAE. Actually, the pharmacokinetic profiles of each analyte in the two groups differentiated to some extent. The results demonstrated that the mean $T_{1/2}$ values of luteoloside,

Table 4. Recoveries and matrix effects of luteoloside, apigetrin, and hesperidin

Analyte	Concentration (ng/mL)	Recovery (%)	RSD (%)	Matrix effect (%)	RSD (%)
Luteoloside	1.19	80.34 ± 3.23	4.02	88.40 ± 2.63	2.72
	119	89.48 ± 1.89	2.11	84.48 ± 2.45	7.48
	190	91.72 ± 5.44	5.93	101.56 ± 4.04	3.01
Apigetrin	0.965	77.41 ± 6.28	8.11	97.01 ± 3.85	9.53
	96.5	82.67 ± 4.43	5.36	112.67 ± 9.53	6.31
	154	94.60 ± 6.93	7.33	96.90 ± 4.73	4.70
Hesperidin	0.590	88.34 ± 7.95	9.00	83.41 ± 9.15	8.86
	59.0	109.79 ± 8.64	7.87	97.93 ± 4.46	3.51
	94.4	97.83 ± 6.49	6.63	107.38 ± 7.29	4.82

Table 5. Stability of luteoloside, apigetrin, and hesperidin

Analyte	Concentration (ng/mL)	4 °C for 12 h		3 freeze-thaw cycles		−80 °C for 2 weeks	
		RSD (%)	RE (%)	RSD (%)	RE (%)	RSD (%)	RE (%)
Luteoloside	1.19	8.56	7.35	9.38	5.15	7.61	1.47
	119	6.56	6.69	6.23	0.70	4.27	0.54
	190	4.83	−5.62	4.81	−7.13	4.60	−5.20
Apigetrin	0.965	4.93	11.54	4.62	−4.95	6.15	7.14
	96.5	4.65	6.87	3.70	1.60	6.10	1.37
	154	4.43	5.96	3.92	−1.34	5.06	−2.22
Hesperidin	0.590	8.29	4.89	7.18	−1.63	7.47	−5.43
	59.0	7.24	9.48	6.33	7.44	4.40	4.30
	94.4	4.20	8.72	4.96	−1.60	4.68	−1.00

Fig. 3. Time-dependent curves of luteoloside (1), apigetrin (2), and hesperidin (3) in STAE group and STAE+STVO group

Table 6. Pharmacokinetic parameters of luteoloside, apigetrin, and hesperidin after oral administration of STAE and STAE+STVO

Analyte	Group	$T_{\max}$ (h)	$C_{\max}$ (ng/mL)	$T_{1/2}$ (h)	$AUC_{0 \rightarrow t}$ (h·µg/L)	$AUC_{0 \rightarrow \infty}$ (h·µg/L)
Luteoloside	STAE	0.667 ± 0.258	16.552 ± 2.421	4.000 ± 1.237	40.476 ± 2.905	45.064 ± 5.241
	STAE+STVO	0.958 ± 0.102	$22.913 \pm 6.192^*$	4.359 ± 1.988	$57.934 \pm 5.881^{**}$	$64.988 \pm 8.724^{**}$
Apigetrin	STAE	0.444 ± 0.086	12.283 ± 2.233	8.646 ± 1.901	25.772 ± 1.369	39.394 ± 4.607
	STAE+STVO	$0.750 \pm 0.000^{**}$	13.506 ± 2.558	9.767 ± 3.045	$31.479 \pm 1.365^{**}$	53.743 ± 10.829
Hesperidin	STAE	0.514 ± 0.134	23.882 ± 4.431	5.182 ± 1.231	31.139 ± 1.740	42.137 ± 9.395
	STAE+STVO	$0.875 \pm 0.137^{**}$	31.215 ± 5.354	5.797 ± 0.935	$55.439 \pm 3.013^{**}$	$60.206 \pm 3.672^*$

** $P < 0.01$, * $P < 0.05$ versus STAE group.

apigetrin, and hesperidin in STAE+STVO group were found 1.12, 1.09, and 1.13 fold, respectively, compared to those in STAE group. However, there were no notable differences observed, indicating some weak influence on elimination procedure of the three analytes. In addition, for the three flavonoids, after co-administration of STAE and STVO, the values of $AUC_{0 \rightarrow t}$ and $AUC_{0 \rightarrow \infty}$ were investigated significantly larger than administration of STAE alone ($P < 0.01$, $P < 0.05$), except $AUC_{0 \rightarrow \infty}$ value of apigetrin. The data reminded us promoting effect of STVO on absorption of the three analytes. For hesperidin and apigetrin, $T_{\max}$ values in STAE+STVO group were markedly longer than those in STAE group ($P < 0.01$) while $C_{\max}$ values were increased without notable differences. On the contrary, $C_{\max}$ value of luteoloside was elevated notably ($P < 0.05$) while no significant difference was found for its $T_{\max}$ value as far as the same comparison was concerned. It was concluded that STVO might ameliorate absorption and improve bioavailability of the three analytes.

In the past decades, it has become a consensus that the plant-originated volatile oils are investigated as the favorable transdermal absorption enhancing agents, such as the

volatile oils from *Rosmarinus officinalis* [30], *Magnolia fargesii* [31], *Eugenia caryophyllata* [32], and *Alpinia Officinarum* [33]. Moreover, it has been documented that the volatile oil from *Acori Tatarinowii* might have promoting effect on pharmacokinetic fates of three coumarins including byakangelicin, xanthotoxol and oxypeucedanin hydrate from *Angelicae Dahuricae* when co-administration in rat [34]. In another study, the volatile oil from Xiangfu Siwu Decoction (a famous formula of traditional Chinese medicine) has been found to improve the absorption and bioavailability of ten non-volatile components (caffeic acid, ferulic acid, gallic acid, vanillic acid, albiflorin, paeoniflorin, berberine, protopine, tetrahydrocolumbamine, and tetrahydrocolumbamine) in the same formula [35]. Taken together, these findings indicated that the volatile oils could enhance the absorption and bioavailability of some drugs not only through transdermal absorption, but also through intestinal absorption, which was consistent with the results of our present study.

Our investigation verified the rationality of oral co-administration of STAE and STVO in terms of pharmacokinetics, which would be helpful for the pharmaceutical

technology study of the Chinese herbal preparation containing ST and for the novel application exploration of STVO. However, there still existed some interesting issues. Except for the pharmacokinetic parameters, would the metabolites of the three analytes in STAE be affected by STVO? Which volatile component(s) showed absorption-promoting effect? What was the absorption-promoting mechanism of STVO? Was P-glycoprotein involved? Which effects would STAE show on the pharmacokinetic profiles of the components in STVO after oral co-administration? These would be listed as the research priorities in the future.

CONCLUSION

In the present study, a sensitive and rapid HPLC-MS/MS analytical method was firstly established for simultaneous quantitative analysis of luteoloside, apigenin, and hesperidin in rat plasma. This method provided eligible recovery and stability with fine precision and accuracy and then was successfully applied to the pharmacokinetic study of three bioactive flavonoid glycosides after oral administration of STAE and STAE+STVO. The results obtained from this study demonstrated that STVO could promote the absorption and bioavailability of the three flavonoid glycosides in vivo. Therefore, we hope that these findings would help to broaden the applied range of STVO and to provide some scientific basis and guidance for the safe clinical use of ST.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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