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RESEARCH ARTICLE

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A UPLC-MS/MS method for simultaneous determination of five flavonoids from *Stellera chamaejasme* L. in rat plasma and its application to a pharmacokinetic study

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Abstract

Stellera chamaejasme L. has been used as a traditional Chinese medicine for the treatment of scabies, tinea, stubborn skin ulcers, chronic tracheitis, cancer and tuberculosis. A sensitive and selective ultra-high liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) method was developed and validated for the simultaneous determination of five flavonoids (stelleranol, chamaechromone, neochamaejasmin A, chamaejasmine and isochamaejasmin) of *S. chamaejasme* L. in rat plasma. Chromatographic separation was accomplished on an Agilent Poroshell 120 EC-C_{18} column (2.1 × 100 mm, 2.7 μm) with gradient elution at a flow rate of 0.4 mL/min and the total analysis time was 7 min. The analytes were detected using multiple reaction monitoring in positive ionization mode. The samples were prepared by liquid–liquid extraction with ethyl acetate. The UPLC-MS/MS method was validated for specificity, linearity, sensitivity, accuracy and precision, recovery, matrix effect and stability. The validated method exhibited good linearity ($r \geq 0.9956$), and the lower limits of quantification ranged from 0.51 to 0.64 ng/mL for five flavonoids. The intra- and inter-day precision were both <10.2%, and the accuracy ranged from −11.79 to 9.21%. This method was successfully applied to a pharmacokinetic study of five flavonoids in rats after oral administration of ethyl acetate extract of *S. chamaejasme* L.

KEYWORDS

flavonoids, pharmacokinetics, rat plasma, *Stellera chamaejasme* L, UPLC-MS/MS

1 | INTRODUCTION

Stellera chamaejasme L. (Thymelaeaceae), a perennial weed, is distributed widely across the grasslands of Russia, Mongolia and China (Shirai et al., 2015; Yan et al., 2014; Yan, Zeng, Jin, & Qin, 2015). Its roots are commonly known as 'Ruixianglang du' in Chinese, and have been used in China as a traditional Chinese medicine for the treatment of scabies, tinea, stubborn skin ulcers, chronic tracheitis, cancer and tuberculosis (Xu, Qin, Li, & Xu, 2001; Zhang et al., 2013; Pan et al., 2015). Previous phytochemical studies of *S. chamaejasme* L. have shown that it contains various kinds of chemical components, including diterpene, lignans, coumarins, phenylpropanoid, flavonoids and volatile oil (Li, Shen, Bao, Chen, & Li, 2014; Yan et al., 2014; Zhang et al., 2015). Modern studies have suggested that flavonoids from *S. chamaejasme* L. are the main components that mostly contribute to the pharmacological efficacy (Liu et al., 2012; Liu & Zhu, 2012; Liu, Han, et al., 2014; Liu, Yang, et al., 2014), such as anti-cancer activity (Liu et al., 2012; Li, Zhang, Pang, ZhengChen, & Gan, 2014), anti-fatty liver (Wang, Li, Han, Wang, & Li, 2015) and anti-HIV (Asada et al., 2013).

In our earlier research, some flavonoids have been isolated and identified from ethyl acetate extract of *S. chamaejasme* L. (ESL), and five flavonoids – stelleranol, chamaechromone, neochamaejasmin A, chamaejasmine and isochamaejasmin – have been detected in the rat plasma and tissues after oral administration of ESL. All of them are active compounds of the herbal preparation with pharmacological

Abbreviations: ESL, ethyl acetate extract of *S. chamaejasme* L.; LLE, liquid–liquid extraction.

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effects. Stelleranol was found to have potential antiviral activity against respiratory syncytial virus (Huang et al., 2010). Yang and Chen (2008) demonstrated that chamaechromone has and anti-viral effect on hepatitis B virus. Neochamaejasmin A, meanwhile, was reported to induce apoptosis in human prostate cancer cells. According to previous research, chamaejasmine has a profound anti-proliferative effect on human osteosarcoma, breast cancer, lung adenocarcinoma A549 and HEp-2 larynx carcinoma cells (Yu et al., 2011; Zhang, Yu, Dong, Cai, & Bai, 2013; Yang, Wang, & Ren, 2015). In addition, Zhang et al. discovered that isochamaejasmin could induce apoptosis in leukemia cells through inhibiting the Bcl-2 family proteins (Zhang, Shan, Li, Li, & Zhang, 2015).

However, there is almost no report on pharmacokinetic studies of simultaneous determination of those five flavonoids in rat plasma after administration of ESL. In this study, a rather selective and sensitive UPLC-MS/MS method was developed and validated for simultaneously determining stelleranol, chamaechromone, neochamaejasmin A, chamaejasmine and isochamaejasmin in rat plasma. The results of this study would be helpful for improving clinical application and further pharmacological studies of *S. chamaejasme* L.

2 | EXPERIMENTAL

2.1 | Chemicals and reagents

Stelleranol, chamaechromone, neochamaejasmin A, chamaejasmine, isochamaejasmin and quercetin were purchased from Nature Standard (Shanghai, China) (purity >98.0%). Quercetin was used an an internal standard (IS). The chemical structures of these compounds are shown in Figure 1. Acetonitrile, ethanol, ethyl acetate, petroleum ether and formic acid were of HPLC grade, and supplied by Merck (Germany). Ultra-pure water was prepared using a PW water system (Heal Force, Hong Kong).

2.2 | Instruments and UPLC-MS/MS conditions

The UPLC system was an Agilent Technology 1290 series equipped with an online degasser, a G7120A binary pump, a G7167B autosampler and a G7116B thermostatic column. Chromatographic separation was achieved on an Agilent Poroshell 120 EC-C_{18} column (2.1 × 100 mm, 2.7 μm) at 25°C. The mobile phase consisted of methanol–water containing 0.1% formic acid with a gradient elution starting at 45% methanol and progressing linearly to 75% methanol over 6 min then returning to 45% methanol. The flow rate was 0.4 mL/min and the total analysis time was 6 min. A 5 μL aliquot of sample solution was injected, with a needle wash process that was used to wash the outer wall of sample needle after each injection.

The mass spectrometric detection was performed on a 6470 triple quad mass spectrometer (Agilent, USA) in positive ionization mode. An Agilent Mass Hunter workstation (Agilent, USA) was used to control the equipment and for data acquisition and analysis. The quantification was obtained using multiple reaction monitoring of the precursor–product ion transition at *m/z* 559.2 → 153.0 for stelleranol, 543.3 →

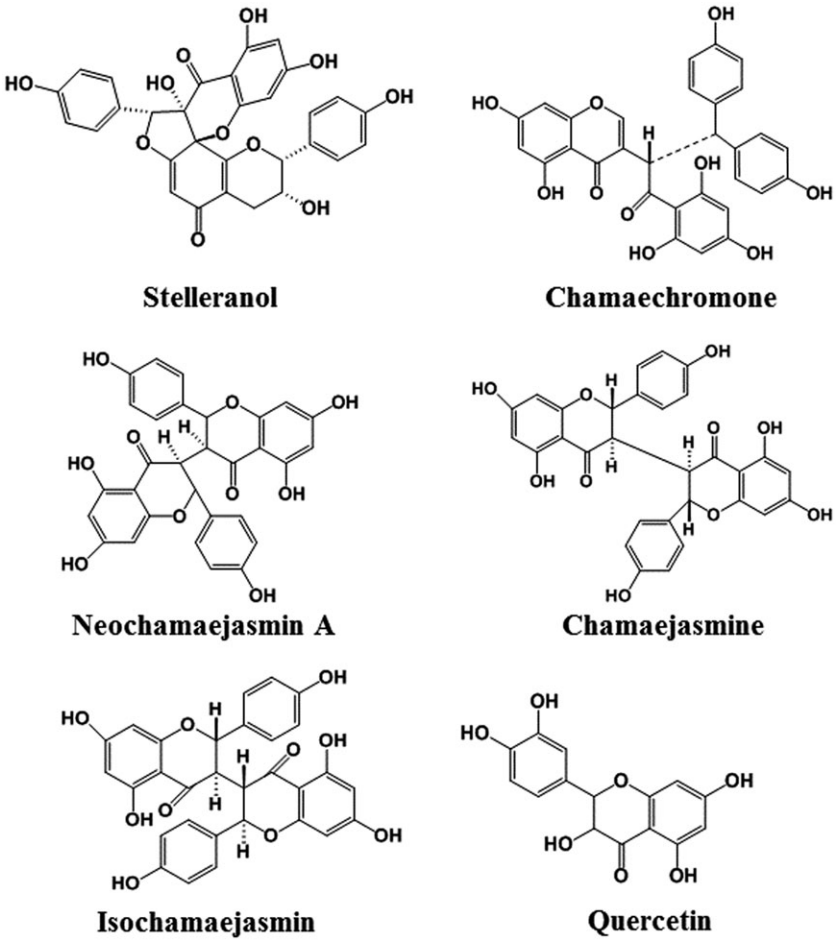

FIGURE 1 The chemical structures of five flavonoids and the internal standard

198.9 for chamaechromone, 543.2 → 153.0 for neochamaejasmin A, 543.1 → 152.9 forchamaejasmin, 543.2 → 153.0 for isochamaejasmin and *m/z* 303.1 → 153.1 for quercetin (IS). Other parameters of the mass spectrometer were set as follows: a drying gas flow of 5 L/min; a drying gas temperature of 200°C; a nebulizer pressure of 45 psig; and a capillary voltage of 4000 V.

2.3 | Preparation of ESL

For preparation of the extract, the dried powder of *S. chamaejasme* L. (1 kg) was extracted under reflux with 4000 mL ethanol–water (80:20, v/v) twice, 2 h each time, and then filtered. Ethanol was recovered under reduced pressure, and then extracted with petroleum ether and ethyl acetate successively several times. Ethyl acetate extract was obtained by vacuum freeze drying. The extract was reconstituted in 15% ethanol–water to get a concentration equivalent to 0.25 g/mL of ESL.

2.4 | Preparation of calibration standards and quality control samples

The stock solutions of stelleranol, chamaechromone, neochamaejasmin A, chamaejasmine, isochamaejasmin and the IS (quercetin) were individually prepared in methanol. The stock solutions of the standards were further diluted in methanol to produce combined standard working solutions at a series of concentrations. The IS solution (94.2 ng/mL) was obtained by diluting the stock solution in methanol. The samples for standard calibration curves were prepared by spiking the appropriate amount of the standard solutions in 100 µL blank plasma to yield calibration concentrations of 1280, 640.0, 320.0, 128.0, 64.00, 32.00, 12.80, 6.400, 1.280 and 0.6400 ng/mL for stelleranol; 1060, 530.0, 265.0, 106.0, 53.00, 26.50, 10.60, 5.300, 1.060 and 0.5300 ng/mL for chamaechromone; 1055, 527.5, 263.8, 105.5, 52.75, 26.38, 10.55, 5.275, 1.055 and 0.5275 ng/mL for neochamaejasmin A; 1160, 580.0, 290.0, 116.0, 58.00, 29.00, 11.60, 5.800, 1.160 and 0.5800 for chamaejasmine; 1020, 510.0, 255.0, 102.0, 51.00, 25.50, 10.20, 5.100, 1.020 and 0.5100 ng/mL for isochamaejasmin, respectively. Quality control (QC) samples include low QC (LQC), middle QC (MQC) and high QC (HQC). The QC samples were prepared at 1.280, 128.0 and 1280 ng/mL for stelleranol; 1.060, 106.0 and 1060 ng/mL for chamaechromone; 1.055, 105.5 and 1055 ng/mL for neochamaejasmin A; 1.160, 116.0 and 1160 ng/mL for chamaejasmine; and 1.020, 102.0 and 1020 for isochamaejasmin. Calibration work solutions and QC samples were stored at 4°C until UPLC-MS/MS analysis.

2.5 | Preparation of plasma samples

The plasma samples were frozen and stored at −40°C in the refrigerator and were thawed at room temperature by vortex before use. The liquid–liquid extraction (LLE) method was applied to extract the analytes and IS from rat plasma. After 100 µL of plasma sample was transferred into a 1.5 mL centrifuge tube, 20 µL of IS working solution (94.2 ng/mL) was added. The mixture was vortex-mixed for 2 min and extracted with 900 µL of ethyl acetate by vortex-mixing for 5 min. Then the sample was centrifuged at 13,800 ***g*** for 10 min. The supernatant was transferred into another test tube and evaporated to dryness with nitrogen gas at room temperature. The residue was reconstituted in 50 µL of the initial mobile phase by vortex-mixing for 2 min and centrifuged at 13,800 ***g*** for 10 min. Finally, 5 µL supernatant was injected for UPLC-MS/MS analysis.

2.6 | Method validation

The validation method for stelleranol, chamaechromone, neochamaejasmin A, chamaejasmine and isochamaejasminin rat plasma was done following the guidelines of the US Food and Drug Administration (2013). The validation parameters included specificity, linearity, sensitivity, accuracy and precision, recovery, matrix effect and stability.

2.6.1 | Specificity

Specificity is the ability of an analytical method to differentiate and quantify the analytes in the presence of other components in the sample. In this paper, the specificity was ascertained by comparatively analyzing blank plasma samples from six individual rats, corresponding blank plasma spiked with the five analytes and IS and the plasma samples from the rats after oral administration of ESL.

2.6.2 | Linearity, lower limits of quantification and lower limit of detection

Calibration curves were prepared by assaying standard plasma samples at 10 concentration levels. The linearity of each calibration curve was determined by plotting the peak area ratio (y) of analytes to IS vs the nominal concentration (x) of analytes with a weighted ($1/x^2$) least square linear regression. Six replicate samples were used for evaluation. The LLOD and LLOQ were determined as the concentration of the analytes with a signal-to-noise ratio at 3 and 5, respectively.

2.6.3 | Accuracy and precision

Three validation batches, each containing six replicates of LLOQ and QC samples at low, medium and high concentration levels, were assayed to assess the precision and accuracy of the method on three different days. The intra- and inter-day precisions were defined as the relative standard deviation with criteria of <15% for QC samples and 20% for LLOQ; the accuracy was assessed by comparing the measured concentration with its nominal value with a criterion of within ±15% for QC samples and 20% for LLOQ.

2.6.4 | Recovery and matrix effect

The recovery of analytes and IS was determined by comparing the responses of the analytes from QC samples with the responses of analytes spiked in post-extracted blank rat plasma at equivalent concentrations. The matrix effects were evaluated quantitatively for six different lots of black rat plasma and evaluated at three QC concentrations in triplicate to know the individual distinctions of matrix effect. The matrix effects were calculated by determining the peak area ratios of the analytes in post-extracted spiked samples with those of pure standard solutions containing analytes at the equivalent concentration.

2.6.5 | Stability

The stability of the five flavonoids in samples including room-temperature stability (storage for 6 h at ambient temperature), long-term stability (storage for 30 days at −40°C), freeze–thaw stability (three freeze at −40°C and thaw cycles) and post-preparation stability (storage for 12 h in the autosampler at 4°C) was tested at LQC, MQC and HQC levels with six replicates at each level. All stability-testing QC samples were determined using the calibration curve of freshly prepared standard samples.

2.7 | Pharmacokinetic study

Six healthy 6–8-week-old Sprague–Dawley rats were purchased from the Laboratory Animal Center of Second Military Medical University (Shanghai, China). The animals were kept under fasting conditions for 12 h and fed with water prior to oral administration of the ESL at a dose of 5 g/kg. Blood samples (~400 μL) were collected from the fosse orbital vein into heparinized polythene tubes at 0 (before dosing), 0.17, 0.33, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 and 24 h after dosing, and then centrifuged immediately at 4600 ***g*** for 10 min. The obtained plasma was transferred into clean tubes and stored at −40°C until analysis. In order to guarantee enough blood volume in rats, the rats were given free access to water after 2 h of administration. The animal handling procedures were approved by the Institutional ethical committee and conformed to the principles of the International Guide for the Care and Use of Laboratory Animals. Non-compartmental-pharmacokinetic parameters were calculated DAS 2.0 (China State Drug Administration).

3 | RESULTS AND DISCUSSION

3.1 | Optimization of LC conditions

Chromatographic conditions were optimized to improve peak shape, increase sensitivity and shorten run time for simultaneous analysis of five flavonoids. At the onset of the experiments, different mobile phase compositions were tried to achieve good resolution and symmetric peak shapes for each analyte and the IS. The mobile phase had been tested with acetonitrile–water and methanol–water binary solvent system using different buffers such as ammonium formate and formic acid. Finally, the mobile phase consisted of methanol–water containing 0.1% formic acid with a gradient elution, which could improve the symmetry of peak shape and enhance the signal response.

3.2 | Optimization of MS conditions

In order to optimize Agilent Jet Steam (AJS) ESI determination conditions, we collected the mass spectra of five flavonoids and IS under both positive and negative ion detection mode. The results showed that the intensity of the most abundant molecule ion for each analyte produced in positive-ion mode was much stronger than that in negative-ion mode. Detection was finally operated in positive-ion mode in this study. Precursor ions, product ions, collision energy and the fragment voltage of each analyte were optimized by Optimizer software, which comes with the triple quad. The MS/MS transitions and energy parameters of the five compounds are shown in Table 1. Considering that other MS parameters also play an important role in the ion response of analytes, we performed some fine-tuning on the basis of autotuning. Finally, other parameters were set as follows: a sheath gas temperature of 300°C; a sheath gas flow of 12 L/min; a nozzle voltage of 2000 V; a nebulizer pressure of 45 psi; a capillary voltage 4000 V; a drying gas temperature of 200°C; and a drying gas flow of 5 L/min.

3.3 | Optimization of sample preparation

We compared protein precipitation with LLE during sample preparation. The former was discarded because of its high noise level and interference by endogenous substances. The latter offered a clean sample and high response. Ethyl acetate was used as the LLE solvent for sample preparation since these five flavonoids were extracted from the ethyl acetate part of *S. chamaejasme* L.

3.4 | Method validation

3.4.1 | Specificity

Typical chromatograms obtained from blank plasma, blank plasma spiked with six analytes and plasma samples from the rats after oral administration of ESL are shown in Figure 2. The retention times of stelleranol, chamaechromone, neochamaejasmin A, chamaejasmine, isochamaejasmin and IS were 2.4, 3.5, 3.9, 4.3, 5.1 and 2.9 min, respectively. All of the peaks of the analytes and IS were detected with excellent peak resolution (R > 2.0) as well as peak shapes, and no interference from the endogenous substances was observed at the retention times of the analytes and IS.

3.4.2 | Linearity, LLOQ and LLOD

The equations of linear regression and linearity range for the six analytes are shown in Table 2. The results showed good linearity and all correlation coefficients were >0.9950. The LLOD with a signal-to-noise ratio >3 ranged from 0.1700 to 0.2133 ng/mL, whereas the LLOQ with an signal-to-noise ratio >5 ranged from 0.5100 to 0.6400 ng/mL, which was sensitive enough for our pharmacokinetic studies using rat plasma.

3.4.3 | Accuracy and precision

The intra- and inter-day accuracy and precision data of five flavonoids in rat plasma are listed in Table 3. At each QC and LLOQ level, the

TABLE 1 Tandem mass spectrometric parameters of five flavionds and IS

Analytes	Precursor ion	Produce ion	Fragmentor (V)	CE (eV)
Stelleranol	559.2	153.0	125	24
Chamaechromone	543.3	198.9	125	4
Neochamaejasmin A	543.2	153.0	125	16
Chamaejasmine	543.1	152.9	125	16
Isochamaejasmin	543.2	153.0	125	16
Quercetin	303.1	153.1	125	35

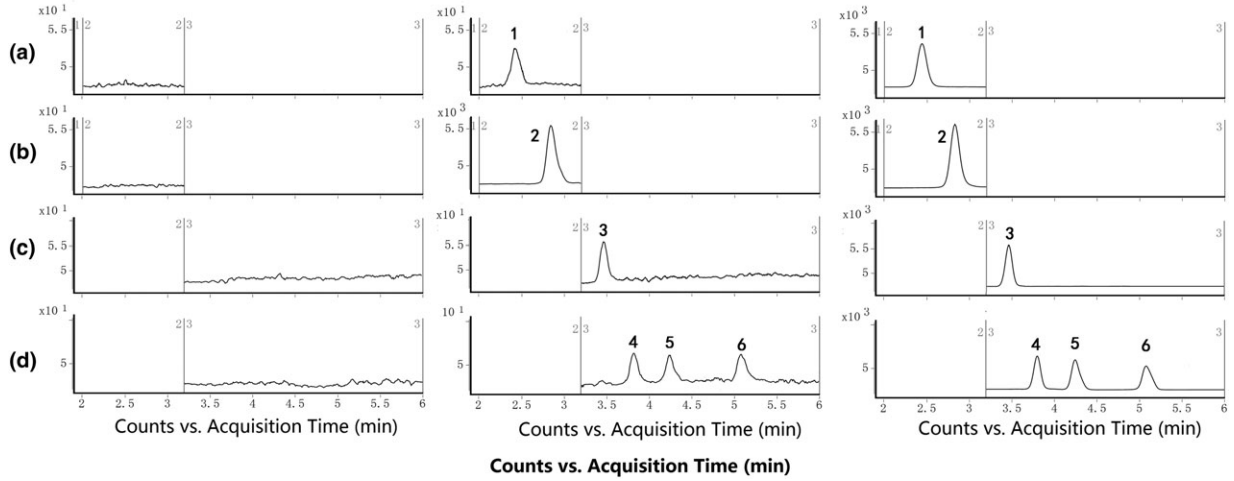

FIGURE 2 Multiple reaction monitoring chromatograms of five flavonoids and internal standard in (a) blank plasma, (b) blank plasma spiked with LLOQ samples, (c) plasma obtained from a rat 2 h after oral administration. 1, Stellerano; 2, quercetin (IS); 3, chamaechromone; 4, neochamaejasmin A; 5, chamaejasmine; 6, isochamaejasmin

TABLE 2 Regression equation, LOD and LOQ of five flavonoids in rat plasma

Analytes	Regression equation	Linear range (ng/mL)	r	LOQ (ng/mL)	LOD (ng/mL)
Stelleranol	$y = 0.0472x + 0.5680$	0.6400–1280	0.9967	0.6400	0.2133
Chamaechromone	$y = 0.0180x + 0.0789$	0.5300–1060	0.9990	0.5300	0.1767
Neochamaejasmin A	$y = 0.0581x + 0.2892$	0.5275–1055	0.9991	0.5275	0.1758
Chamaejasmine	$y = 0.0182x + 0.2660$	0.5800–1160	0.9956	0.5800	0.1933
Isochamaejasmin	$y = 0.0308x + 0.1657$	0.5100–1020	0.9994	0.5100	0.1700

TABLE 3 Summary of precision, accuracy, recovery and matrix effect in rat plasma (n = 5–6)

Analytes	Concentration (ng/mL)	Intra-day precision, RSD (%)	Inter-day precision, RSD (%)	Accuracy, RE (%)	Absolute recovery (%, mean ± SD)	Matrix effect (%, mean ± SD)
Stelleranol	0.640	6.4	8.2	6.21	–	–
	1.280	5.6	6.1	−2.31	84.65 ± 5.91	93.21 ± 7.79
	64.00	2.1	4.9	−9.44	97.32 ± 4.26	89.89 ± 6.25
	640.0	2.6	4.4	5.19	83.84 ± 3.56	80.48 ± 7.15
Chamaechromone	0.530	5.2	7.2	9.21	–	–
	1.060	0.3	5.5	−10.21	87.41 ± 3.17	95.35 ± 7.99
	53.00	3.9	4.8	−9.06	96.82 ± 7.6	83.09 ± 6.25
	530.0	4.9	4.6	3.28	98.93 ± 5.63	99.83 ± 3.09
Neochamaejasmin A	0.528	4.5	10.2	1.23	–	–
	1.055	3.1	9.3	0.29	97.14 ± 7.47	85.34 ± 6.13
	52.75	2.2	3.5	5.31	94.12 ± 3.32	97.66 ± 4.47
	527.5	7.4	4.8	−7.31	81.26 ± 5.68	85.94 ± 5.80
Chamaejasmine	0.580	6.4	3.4	4.13	–	–
	1.160	6.3	2.3	−11.79	88.44 ± 6.44	86.73 ± 5.21
	58.00	4.1	7.4	−9.02	92.74 ± 5.71	87.44 ± 5.98
	580.0	4.3	2.9	2.76	93.77 ± 3.44	84.65 ± 4.72
Isochamaejasmin	0.510	4.2	2.3	4.22	–	–
	1.020	3.2	3.0	3.99	80.16 ± 7.00	96.82 ± 5.40
	51.00	1.9	4.5	−9.39	94.15 ± 5.43	87.73 ± 3.24
	510.0	6.4	4.9	−9.14	83.74 ± 6.71	85.24 ± 7.08
Quercetin (IS)	94.20	–	–	–	95.34 ± 8.14	90.67 ± 4.65

inter-day and intra-day precisions (RSD) of the five flavonoids were 0.3–10.2% and the accuracy was −11.79–9.21%. The results for both intra- and inter-day were found to be within the acceptance criteria.

3.4.4 | Recovery and matrix effect

The data of recovery and matrix effect of the five flavonoids are summarized in Table 3. Mean absolute recoveries of stelleranol,

TABLE 4 Stability data of five flavonoids in rat plasma (n = 3)

Analytes	Concentration (ng/mL)	Accuracy (%) (mean ± SD)			
		Autoinjector	Short-term	Freeze–thaw	Long-term
Stelleranol	1.280	97.78 ±6.06	95.05 ±8.32	83.98 ±7.68	90.27 ± 10.4
	64.00	91.55 ± 9.80	91.56 ± 7.16	93.90 ± 6.96	96.85 ± 9.92
	640.0	96.06 ± 4.33	95.33 ± 9.21	92.47 ± 7.95	86.01 ± 9.49
Chamaechromone	1.060	92.40 ±8.47	98.74 ± 4.80	96.81 ± 4.03	87.16 ± 7.74
	53.00	90.28 ± 8.54	90.12 ± 3.31	98.18 ± 4.79	94.58 ± 9.73
	530.0	96.74 ± 5.84	93.66 ± 5.88	94.20 ± 8.40	91.67 ± 6.30
Neochamaejasmin A	1.055	90.33 ± 10.33	97.44 ± 7.24	85.70 ± 6.05	91.73 ± 10.26
	52.75	101.98 ± 3.25	91.31 ± 5.68	96.10 ± 8.73	85.35 ± 7.16
	527.5	94.25 ± 5.90	90.93 ± 7.06	98.68 ± 4.58	87.05 ± 8.22
Chamaejasmine	1.160	92.06 ±7.99	90.71 ± 5.99	93.48 ± 7.44	89.07 ± 5.13
	58.00	98.08 ± 8.17	99.50 ± 8.73	103.87 ±8.91	92.83 ± 7.64
	580.0	97.91± 6.01	92.14 ± 4.42	98.36 ± 6.02	91.74 ± 9.25
Isochamaejasmin	1.020	94.53 ±7.38	95.10 ± 7.69	97.54 ± 8.21	88.70 ± 5.71
	51.00	94.21 ± 6.46	92.33 ± 6.80	102.44 ± 6.40	96.50 ± 10.47
	510.0	90.15 ± 10.00	93.45± 8.51	94.89 ± 3.49	89.74 ± 4.18

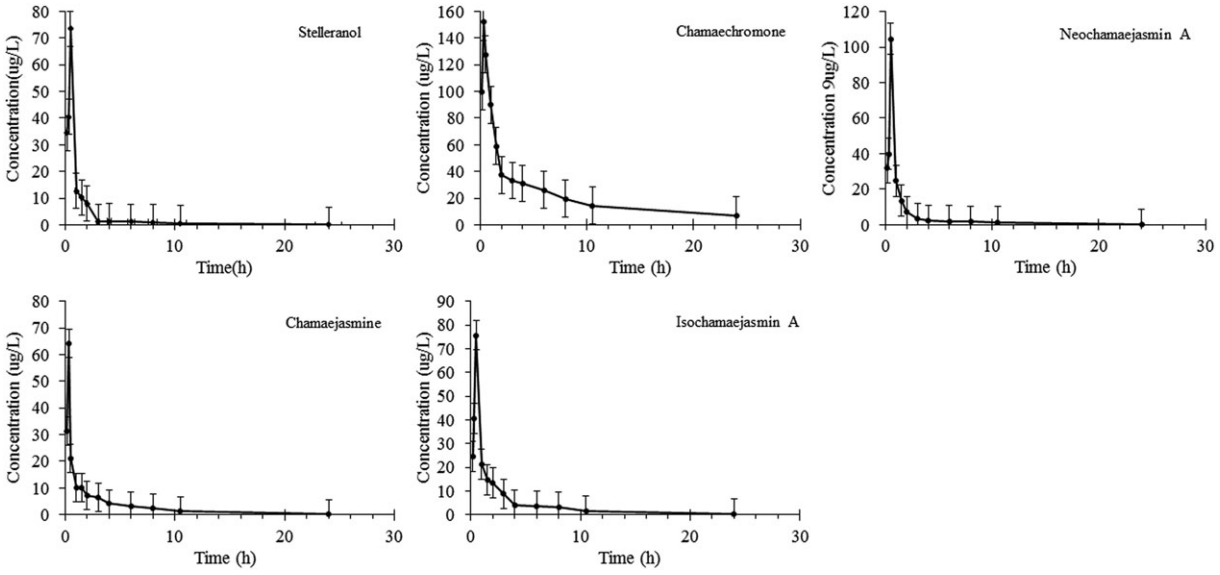

FIGURE 3 Mean (±SD) plasma concentration–time curves of five flavonoids in rats after oral administration of ESL (n = 6)

TABLE 5 Pharmacokinetic parameters of five flavonoids in rats after oral administration

Parameters	Stelleranol	Chamaechromone	Neochamaejasmin A	Chamaejasmine	Isochamaejasmin
C_{max} (μg/L)	73.47 ± 1.19	152.07 ± 3.62	104.56 ± 9.28	64.21 ± 7.92	75.73 ± 12.31
T_{max} (h)	0.51 ± 0.10	0.33 ± 0.18	0.55 ± 0.12	0.33 ± 0.18	0.50 ± 0.05
$t_{1/2}$ (h)	5.28 ± 0.34	5.28 ± 0.24	5.32 ± 0.91	5.42 ± 1.27	5.12 ± 1.38
AUC_{0-t} (h μg/L)	67.32 ± 11.03	525.51 ± 14.29	99.47 ± 2.33	81.56 ± 14.32	108.97 ± 21.49
$AUC_{0-\infty}$ (h μg/L)	68.11 ± 9.94	544.01 ± 11.35	101.29 ± 3.84	84.84 ± 16.27	111.49 ± 19.39
$AUMC_{0-\infty}$(h h μg/L)	176.41 ± 10.22	4062.04 ± 23.08	351.46 ± 26.75	483.11 ± 20.90	482.37 ± 30.24
$MRT_{0-\infty}$ (h)	2.24 ± 1.47	7.47 ± 1.02	3.47 ± 0.99	5.70 ± 1.62	4.33 ± 2.02

C_{max}, Peak concentration; T_{max}, time to C_{max}; $t_{1/2}$, elimination half-life time; AUC, area under the concentration–time curve; AUMC, area under the first moment of the plasma concentration-time curve; MRT, mean residence time.

chamaechromone, neochamaejasmin A, chamaejasmine and isochamaejasmin were 83.84–97.32, 87.41–98.93, 81.26–97.14, 88.44–93.77 and 80.16–94.15% at three QC levels. The results suggested that the sample preparation and extraction methods were effective and stabile. The matrix effects derived from QC samples were between 80.48 and 99.83%. These results confirmed that there were no significant matrix effects.

3.4.5 | Stability

The stability data of the five flavonoids in rat plasma including room temperature stability (storage for 6 h at ambient temperature), long-term stability (storage for 30 days at −40°C), freeze–thaw stability (three freeze at −40°C and thaw cycles) and post-preparation stability (storage for 12 h in the autosampler at 4°C) are shown in Table 4. The results indicate that all analytes were stable in rat plasma and processed samples under different storage conditions.

3.5 | Pharmacokinetic study

The newly developed method was sufficiently sensitive to determine the plasma concentration of stelleranol, chamaechromone, neochamaejasmin A, chamaejasmine and isochamaejasmin following a single oral administration of ESL. The concentration–time curves of the five flavonoids are shown in Figure 3 and its estimated pharmacokinetic parameters calculated by DAS 2.0 software are presented in Table 5. As shown in Table 5, the range of peak concentrations (C_{max}) of these five flavonoids was from 64.21 to 152.07 μg/L because the contents of five flavonoids varied greatly in the ESL. All five flavonoids achieved the maximum plasma concentration within 0.55 h after oral administration, while their elimination half-life times ($t_{1/2}$) were around 5 h, which suggests that these five flavonoids had similar elimination rates in rat plasma after oral administration of ESL.

In a previous study, only chamaechromone has been investigated in an *in vivo* analysis method (Lou et al., 2011), but its plasma concentration of LLOD was not low enough, and did not satisfy the requirements of our study. In the literature, a double-peak phenomenon has been found in the plasma concentration–time curve after oral administration of purified chamechromone, which was probably caused by enterohepatic circulation. However, no double-peak phenomenon was found in our study. The time to peak concentration (T_{max}) was about 11 h and $t_{1/2}$ was about 30 h, which was much later than reported in our results. The reason for this may be that we administered ESL orally to the rats rather than purified chamaechromone and drug–drug interaction would influence the absorption and elimination of flavonoids, which also requires further experiments for proof. These results should be useful for further studies on the pharmacokinetics, pharmaceutics and clinical practice of *S. chamaejasme* L.

4 | CONCLUSION

An UPLC-MS/MS method was developed and validated for the simultaneous determination of stelleranol, chamaechromone, neochamaejasmin A, chamaejasmine and isochamaejasmin in rat plasma. This is the first paper to simultaneously determinate these five flavonoids in rat plasma after oral administration of ESL. The advantages of this method include simple sample preparation, high sensitivity and short run time (7.0 min per sample). Moreover,this method was successfully applied to a pharmacokinetic study of ESL in rats. The results might be helpful for investigating the bioactivity mechanism and clinical application of *S. chamaejasme* L.

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